10e Colloque de la Société des Neurosciences
Marseille, 24-27 mai 2011

Résumés des communications affichées

P1.001

Mir-134 microRNA regulates LIMK1 expression in the rat spinal cord: possible implications in neuropathic pain processing

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Spinal cord lesions may induce severe chronic pain that develops in parallel with nociceptive circuitry remodeling and synaptic reorganization. Mechanisms of such remodeling remain unclear. The actin cytoskeleton is critically involved in morphology and motility of the dendritic arborization. Actin dynamics could therefore modulate morphological plasticity and act as a key player in neuropathic pain mechanisms.
Limk1 is a protein kinase responsible for the regulation of actin polymerization by cofilin (ADF) phosphorylation. The microRNA Mir-134 inhibits the translation of Limk1 mRNA. Mir-134 is considered as a negative regulator of dendritic spine volume. Accordingly, limk1 has been reported to promote actin polymerization in dendrites and spine enlargement. However in contrast, limk1 over expression results in axon growth defects.
Our objective is to investigate the effects of mir-134/Limk1 on the reorganization of pain circuits in spinal dorsal horn and on pain sensitization.
We first showed the variations in the expression of mir-134/limk1 in neuropathic animals when compared to shams. Von Frey test was used to evaluate pain behavior pre and post-surgery. Spinal cords were then processed for qrt-PCR analysis. Animals have also been subjected to intrathecal injection of mir-134 knockdown probes and functional consequences on pain behavior were also studied. Thereafter, we investigated the distribution of mir-134 in identified subcellular compartements: PFA fixed spinal cord of neuropathic as well as sham animals were then incubated with both mir-134 probes and different synaptic markers.

P1.002

Synchronous Plateau Assemblies are a prominent morpho-physiological stage for the development of GABAergic interneurons

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It is now well established that most developing neuronal networks display a variety of coordinated activity patterns that should provide essential signals for circuit maturation. Throughout brain development, the same structure will sequentially ground different forms of spontaneous patterned activity. We have recently described a common sequence for the maturation of population coherence

in neocortical and hippocampal networks. In these structures, population coherence emerges at birth in the form of Synchronous Plateau Assemblies (SPA) that synchronize small groups of neurons coupled by gap junctions before involving most neurons in the form of synapse-driven Giant Depolarizing Potentials (GDP). These patterns are mutually exclusive as GDPs actively shut off the production of SPAs. In order to assign a possible function to the SPA pattern, we have studied the fate and the morpho-physiological properties of single SPA-cells as a function of network maturation. We focused our analysis on hippocampal GABAergic neurons. To study individual cell fates we have designed an experimental approach that enables us to perform repeated imaging of the same neuronal populations on a daily basis. We show that cells involved in SPAs switch to a GDP pattern of activity within one day and that this transition is paralleled by a remarkable evolution of their morpho-physiological properties; SPA-interneurons present an immature firing pattern, receive exceptionally large miniature currents and display remarkable somatic filopodia that all disappear when these interneurons participate in GDPs. Therefore the involvement of interneurons in SPAs marks a remarkable step in their morpho- physiological development.

P1.003

Developmental plasticity of ventrolateral funiculus transmission to spinal lumbar motoneurons

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Although the plasticity in supraspinal structures such as hippocampus has been extensively studied, little is known about the synaptic plastic capabilities in the spinal cord and more precisely at the synapses between a major descending motor tract, the ventrolateral funiculus (VLF) and the lumbar Motoneurons (MNs). This study was undertaken to examine the activity-dependent plasticity (ADP) of VLF-motoneuron synapses and to determine whether ADP evolves with synaptic maturation. Experiments were performed in mouse spinal cord slices at two developmental stages: 1-4 and 9-12 postnatal days. Whole-cell voltage-clamp recordings were used to record lumbar MNs. Excitatory postsynaptic currents (EPSCs) were induced by ipsilateral VLF stimulation using tungsten microelectrodes. To characterize the receptors implicated in the EPSCs, AP5 and DNQX were applied in the bath. The results show that the EPSCs recorded from MNs at the two developmental stages are mainly mediated by AMPA/Kainate receptors activation. High frequency stimulation of VLF (50 Hz trains of 2 second duration, 2x threshold) was then performed in P2-P4 and P9-P12 old animals to test the developmental plasticity in the VLF-MNs synapses. The results show first, that descending locomotor inputs to spinal MNs can express ADP such as short-term potentiation, and/or short and long-term depression of the baseline EPSCs and second that the ADP observed at these synapses are not expressed in the same proportion according to the age of the animals. To access the characteristics of VLF-MNs synapses at the two developmental stages that could be involved in the different types of ADP observed, different stimulation paradigms were tested. Paired-pulse as well as trains of stimulation revealed any stricking differences between the two groups tested. Philantotoxin, a blocker of Ca^{2+}-permeable AMPA receptors, was also tested in order to determine whether these receptors underly the observed changes in synaptic coupling during synaptic maturation.

P1.004

Physiopathological link between epilepsy and periventricular nodular heterotopia due to FLNA mutations

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The malformations of the cerebral cortex represent a major cause of developmental disabilities, severe epilepsy and reproductive disadvantage. High-resolution imaging has facilitated the *in vivo* identification of a large group of cortical malformation phenotypes. Among those, periventricular nodular heterotopia (PNH) is caused by defective *neuronal migration that results in* nodules of neuron ectopically placed along the lateral ventricles walls. Clinical manifestations range from asymptomatic

to intractable epilepsy and mental retardation. Mutations in *FLNA*, on Xq28, were found in 100% of families with X-linked bilateral PNH and about 26% of sporadic patients. Limited studies on patient samples and the lack of *FlnA* animal models that mimic PNH have delayed our understanding of the underlying pathological mechanism. Although, the link between PNH and epilepsy has been well established, the physiopathological mechanisms responsible for epileptogenesis remain unknown. For this purpose, we have developed, by *in utero* RNA interference, the first animal model that reproduces a *FlnA* gene-driven PNH phenotype in rats (see. Poster Carabalona et al.). These animals are now available to investigate the physiopathological link between PNH and epileptogenesis. To address this question, we are currently investigating in our FlnA-RNAi rat model: i) the cellular composition of the nodule at several stages of post-natal development; ii) susceptibility to seizures by PTZ injection as well as the correlation between nodule's size and epilepsy; iii) frequency and intensity of spontaneous seizures using EEG telemetric recordings. This work will help us to understand the mechanism of epileptogenesis, in the view to find new approaches in the diagnosis and the treatment of PNH.

P1.005

Development of microglial cells in the maturating barrel cortex of the mouse

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Microglial cells (MGCs), the resident macrophages of the central nervous system (CNS), have been mostly studied for their roles in pathologies. Accumulative evidence indicates, however, that they also influence the normal development of CNS synapses. Yet, the mechanisms by which microglial cells invade the brain and target maturating synapses at early postnatal developmental stages remain unknown.
We addressed these issues in the whisker-related barrel field of the murine somatosensory cortex used as a model of cortical map maturation. In adult mice, MGCs are distributed homogenously throughout cortical layers and independently of the barrel field organization. However, during an early phase of development, until postnatal day (P) 5, MGCs precisely delineate the periphery of each individual barrel and adjust their distribution to new cortical maps modified by sensory deprivation. MGCs invasion of the barrel centers occurs in a second phase, after P5, when thalamo-cortical synapses located in the barrel centers undergo important functional maturation. Electrophysiological recordings showed that between P5 and P9 MGCs transiently acquire an activated phenotype characterized by the up-regulation of Kv1.3 potassium channels. Interestingly, inhibiting MGC activation with minocycline *in vivo* inhibited their entry into the barrel centers. We also tested for the functional expression of P2Y6 purinergic receptors which regulate the phagocytosis functions of these cells and found that MGCs located within the barrels centers expressed more often P2Y6 receptors than those located outside the barrels. Finally, analysis of CX3CR1 knockout mice indicates that timing of MGC activation and entry into the barrel centers were governed by the neuron-to-microglia fractalkine signaling pathway.
Our results indicate that maturating neurons guide MGCs to their final destination in the developing cortex, allowing them to reach their putative targets on time to play an active role in the functional maturation of neuronal circuits.

P1.006

Role of aberrant kainate receptors in the coding properties of dentate granule cells in a model of temporal lobe epilepsy

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The Dentate Gyrus plays a major role at the gate of the hippocampus, filtering incoming information from the entorhinal cortex. Dentate granule cells use a sparse and specific firing for efficient

discrimination of synaptic inputs. In temporal lobe epilepsies, the hippocampus displays important coding alterations that may play a role in cognitive impairments described in patients and animal models. However, the cellular mechanisms remain poorly understood. In animal models of temporal lobe epilepsies and human patients, neuronal tissue undergoes major reorganization; some neurons die whereas others, which are severed in their inputs or outputs, sprout and form novel aberrant connections. This phenomenon called reactive plasticity is well documented in the Dentate Gyrus where dentate granule cell axons (the so-called mossy fibers) sprout and create a powerful excitatory network between dentate granule cells. We recently showed that besides the axonal rewiring, recurrent mossy fibers convert the nature of glutamatergic transmission in the Dentate Gyrus because they operate via long-lasting kainate receptor-mediated excitatory post-synaptic potentials not present in the naïve condition. The aim of our present study is to investigate how aberrant kainate receptor-operated synapses affect the coding properties of dentate granule cells and interfere with the major Dentate Gyrus function of “translator” from neocortical to hippocampal “language”.

P1.007

Role of presynaptic release mechanisms in the refinement of the retinogeniculate connexions

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In the visual system, the development of retinotopic connections requires neural activity. However whether synaptic activity is required is still unclear, since neural activity can act directly via Ca2+/cAMP mediated signaling to control axon guidance and transcription. Moreover, initial stages of retinotopic wiring are not disturbed in munc-18 mice that entirely lack neurotransmitter release.
Here we examined the role of synaptic release during late stages of development, namely the activity-dependent refinement of retinal ganglion cells (RGCs) projections to the dorsolateral geniculate nucleus (dLGN). In mice, 5% of the RGCs project to the ipsilateral hemisphere, while the others project to the contralateral side. Eye-specific projections initially overlap but become segregated in the dLGN during postnatal life. Moreover, a precise topographic distribution of RGCs is established in the dLGN. Eye-specific segregation and retinotopy involve the elimination of imprecise connections and the strengthening of correctly targeted connections. Mouse models where spontaneous neural activity in the retina is blocked disrupt both segregation and retinotopy.
To distinguish between the synaptic (activity-dependent release of neurotransmitters) and non synaptic (activity along the axon) effects of activity blockade, we reduced specifically the retinogeniculate neurotransmission. This was obtained by a conditional invalidation (Cre/LoxP system) of *Rimα.* The removal of Rim proteins lead to a strong reduction of Ca2+-dependent neurotransmitter exocytosis without affecting the spontaneous release. We used the Sert-Cre mouse line to ablate Rims in RGCs. Using a LacZ reporter mouse line, we find that a large majority of RGCs show effective recombination. Tracing studies indicate that the recombined mice have defects in the eye-specific segregation of retinal inputs in the dLGN. To further analyze the retinotopic organization in the recombined context, we plan to perform focal injections of anterograde tracers in retinas. Future experiments will involve the specific reduction of neurotransmission in a group of RGCs using *in utero* electroporation to analyze whether synaptic release mechanisms play a role in the competitive axonal terminals in target fields.

P1.008

p21-activated kinase 3 regulates AMPA receptor-dependent synaptic transmission through its interaction with the Nck2/Grb4 adaptor

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Mutations in the p21-activated kinase 3 gene (*pak3*) are responsible for mental retardation and the PAK3 protein plays a major role in synaptic plasticity and in learning and memory. The molecular

mechanisms underlying PAK3 functions remain however unknown. PAK proteins are recruited to the membrane through the Nck1 and Nck2/Grb4 adaptors to be activated by Rac1 and Cdc42 GTPases. These adaptors containing one SH2 domain and three SH3 domains link membrane-localized phosphotyrosine residues to proline-rich domain containing proteins implicated in cytoskeleton regulation. Recent reports show that Nck1 and Nck2 play different roles in spinogenesis and synaptic plasticity. Thus the notion that interaction between PAK3 and Nck adaptors may play a role in synaptic signalling is particularly attractive.
We report here that PAK3 interacts preferentially with Nck2/Grb4 in brain extracts and in transfected cells. This interaction is independent of PAK3 kinase activity and of the phosphorylation state of the S20 residue. Selective uncoupling of the Nck2 interaction with PAK1 and PAK3 in acute cortical slices using an interfering peptide led to a rapid increase in glutamatergic transmission to pyramidal neurons. We show in transfected neuronal cultures that the PAK3-P12A mutant, which no longer interacts with Nck2 while continuing to interact with Nck1, has no effect on spine morphogenesis or synaptic density. The PAK3-P12A mutant did not affect synaptic transmission, whereas surprisingly, the expression of the wild-type PAK3 protein decreased the amplitude of miniature excitatory currents. Altogether, these data show that the PAK3/Nck2 complex down-regulates AMPA receptor-dependent synaptic transmission.

P1.009

Involvement of ARX (Aristaless related homeobox gene) in the normal and pathological development of cerebral cortex

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Mutations in *ARX* (Aristaless related homeobox gene) have been found in a large spectrum of diseases that includes mental retardation, epilepsy, and/or cortical malformations. *ARX* is a transcription factor that plays an important role in cortical development, specifically in cortical progenitor cells and interneurons. Although Arx expression in these cells is clearly recognized, its involvement in their differentiation and migration still remain unclear.
Given the interneuron defects observed in previous studies, we decided to clarify the effect of *ARX* inactivation on interneuron differentiation. Transfections of ShRNA on rat hippocampal cultures revealed that *ARX* inactivation results in a wider neuritic outgrowth and branching points on neurons GAD67 immunopositive while neurons immunonegative for this antibody failed to display any significant change. These results suggest that *ARX* is involved in the differentiation of interneurons specifically, but not in pyramidal cells.
Previous reports support the notion that *ARX* is required for a proper migration of interneurons and pyramidal cells. To test this hypothesis, we performed *in utero* electroporations of *ARX* altered forms on rat brain at E15:
1) Electroporations in ganglionic eminence, targeting interneurons progenitors, revealed that *ARX* is directly involved in interneurons migration.
2) Electroporations in cortical ventricular zone, targeting pyramidal cells precursors, confirm that *ARX* alterations lead to pyramidal cells migration defects.
Surprisingly, some electroporated cells (with *ARX* overexpression or *ARX* GCG7 mutation forms) reach the cortex displaying a tangential pattern of migration. Even if these neurons are pyramidal neuroblasts (no colocalization with GABA antibody), they behave like interneurons. This suggests that maintaining Arx expression results in changing the migration mode of pyramidal cells (radial migration becomes tangential).
Our data support the notion that *ARX* is involved on tangential and radial migration. Further studies are required to clarify these issues and evaluate the functional repercussions of these alterations on network operation and epileptogenesis.

P1.010

Regulation of axonal and blood vessel growth by Synectin

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The assembly of neural circuits requires the intricate orchestration of multiple molecular cues which guide axonal growth. Recent studies have demonstrated that developing blood vessels use some of these signals and surface receptors. One example is the secreted Semaphorin 3E (Sema3E) and its high affinity receptor PlexinD1, which direct repulsive guidance events during nerve and blood vessel navigation. Here we study the Sema3E-induced intracellular signalling events downstream of PlexinD1. In a yeast-two hybrid screen, we identified the single PDZ-domain scaffold protein Synectin (also known as GIPC1) as a cytoplasmic interactor of PlexinD1. This interaction occurs through the SEA PDZ-binding motif of PlexinD1. Synectin function has been mainly studied in the vascular system, where it regulates multiple biological processes, including endothelial cell migration. Here we show that exposure of human umbilical vascular endothelial cells (HUVECs) to Sema3E causes rapid collapse and detachment of the cells. Synectin is recruited from the cytoplasm to the plasma membrane and co-localizes with PlexinD1 receptor at sites of membrane retraction. In addition, we show that Synectin function is also important in axonal growth. In cortical neurons expressing PlexinD1, Sema3E exerts axon growth inhibition. This effect is blocked by knockdown of Synectin or expression of a PlexinD1 mutant deleted of the SEA PDZ-binding motif. We are now investigating the mechanism of action of Synectin and whether similar mechanisms are used in both neuronal and endothelial cells.

P1.011

Long term depression of neurotransmitter release at the cerebellar parallel fiber synapse

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The cerebellar granule cell to Purkinje cell glutamatergic synapse exhibits long-term plasticities which have been suggested to underlie motor learning. However, their experimental induction requires specific input patterns whose physiological relevance is unclear. *In vivo*, granule cells appear to fire with bursts at high frequency. Here, we show that in adult mouse cerebellar slices, such bursts induce a long term depression (LTD) which, in contrast to the previously reported LTD, is associated with increased paired-pulse facilitation and coefficient of variation of synaptic responses, implying its expression is presynaptic. We show that this presynaptic LTD is mediated by a retrograde signaling different from the endocannabinoid 2-arachidonoyl glycerol. The presynaptic LTD is abolished by phorbol 12,13-dibutyrate, suggesting mediation by presynaptic diacylglycerol signaling. In contrast to short-term endocannabinoid-mediated depression, the presynaptic LTD does not depend on the amount of glutamate spillover, supporting synapse-specificity and a role in information storage.

P1.012

Cellular distribution and molecular targets of MT5-MMP, a membrane type MMP expressed in the developing CNS and involved in physiological and reactive plasticity

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Matrix Metalloproteinases (MMPs) are major regulators of the pericellular environment. The MMP family comprises 24 members, subdivided in 5 sub-families depending on their substrates and structure i.e. secreted or membrane bound (membrane type MMPs, MT-MMP). Among the latter, MT5-MMP (MMP-24) is essentially specific of the nervous system and has been involved in reactive plasticity of Abeta fibres in the spinal cord, and allodynia following sciatic nerve injury *(Komori et al., 2004)*. Knock out (KO) mice for MT5-MMP are impaired in learning in an olfactory maze and show deficits in LTP which is diminished in amplitude compared to WT, indicating a potential role for MT5-MMP in brain plasticity. Expression of MT5-MMP mRNA is induced in situations of reactive plasticity such as axonal sprouting following induction of seizures and epilepsy. In vitro, neurite outgrowth is significantly slower in cultured cortical neurons from KO mice. Using plasmid constructs encoding MT5-MMP fused to GFP/RFP, we studied cellular distribution and trafficking of MT5-MMP and show that besides its vesicular distribution in the cytosol and localisation in the plasma membrane, MT5-MMP is also located in cell nuclei. Deletion of nuclear localisation signals present in the N-terminal portion of the molecule significantly diminishes nuclear distribution of MT5-MMP. In order to address at the molecular level the role of MT5-MMP we performed a proteomic analysis of KO vs WT mice brains, and identified a number of proteins that are putative substrates of MT5-MMP, including nuclear proteins. Altogether, our results indicate that MT5-MMP is involved in the regulation of a number of regulatory proteins, including in the nucleus, that impacts on neuronal development and physiological or reactive plasticity in brain.

P1.013

Long-term deleterious consequences of early life caffeine exposure

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Whether coffee consumption should be decreased/stopped during pregnancy in human remains debated. To address this issue, female mice were continuously exposed to caffeine in their drinking water (0.3 g/L - the equivalent of 3 espressos/day in human); the treatment starting two weeks before mating. Pups were thus exposed to caffeine in utero until weaning. At postnatal day 6, hippocampal somatostatin-containing interneurons displayed altered maturation, whilst principal cells remained unaffected, suggesting that caffeine treatment affects the development of the GABAergic circuitry. Adult offsprings displayed structural alterations, including changes in $GABA_A$, AMPA and NMDA receptor subunits. The kinetics of glutamate and $GABA_A$ receptor mediated currents were modified. *In vivo*, adult offsprings displayed decreased seizure thresholds and spatial memory deficits. Therefore, caffeine exposure during development has long-term deleterious consequences in adult animals. Coffee consumption during pregnancy should be questioned.

P1.014

d-IMP, the *Drosophila* ortholog of the mRNA transport factor ZBP1 controls axon growth and branching *in vivo*

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In vivo, developing neurons extend axonal processes through a complex environment to find their targets. Studies performed in cultured neurons have shown that recruitment of specific mRNAs to axon growth cones, and local axonal translation of these mRNAs is very important for the growth cone

to sense extracellular cues and translate them into polarized growth. Although it has been shown that the RNA-binding protein ZBP1, a conserved factor involved in mRNA targeting, is dynamically recruited to vertebrate axon growth cones, and that its binding to *b-actin* mRNA is required for axon turning *in vitro* (Leung et al. 2006; Yao et al. 2006), the biological role of this post-transcriptional regulatory process still remains to be tested *in vivo*. Furthermore, the molecular mechanisms controlling axonal mRNA transport and translation largely remain to be explored.
To address these questions, we are using a population of neurons located in the *Drosophila* brain as a genetically tractable model system. In this system, we have discovered that d-IMP, the *Drosophila* ortholog of ZBP1, is required for polarized axon growth and branching. Using a live-imaging protocol we have recently developed, we have further shown that d-IMP is actively transported to growing axons *in vivo*, consistent with a function in active transport of selected mRNAs. To identify d-IMP mRNA targets, we have combined candidate-based and genome-wide (RIP-chip) approaches, and have so far focused on a target found in axons and encoding a regulator of the actin cytoskeleton. We have shown that this regulator is essential for the polarized growth of axons, is required genetically downstream of d-IMP, and has a conserved 3'UTR bound by d-IMP. Results from experiments aiming at analyzing the distribution of this target using *in vivo* reporters will be presented.
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- Yao, J., Sasaki, Y., Wen, Z., Bassell, G.J., and Zheng, J.Q. 2006. *Nat Neurosci* **9**(10): 1265-1273.

P1.015

Presynaptic voltage facilitation of synaptic transmission between CA3-CA3 pyramidal neurons

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The spread of subthreshold somatic voltage fluctuations into the axon modulates spike-evoked synaptic transmission at cortical excitatory and cerebellar inhibitory synapses. In neocortical layer 5 pyramidal neurons, depolarization-induced facilitation of synaptic efficacy is thought to occur through the enlargement of presynaptic action potential (AP) driven by inactivation of axonal Kv1 potassium channels.
We report here in hippocampal slice cultures that synaptic transmission at excitatory CA3-CA3 synapses also depends on the membrane potential of the presynaptic neuron ($V_{m\text{-}pre}$). In synaptically connected cell-pairs, presynaptic action potentials elicited at low frequency (0.1 Hz) produced postsynaptic response recorded in voltage (EPSC) or current-clamp (EPSP) configuration. Synaptic transmission was tested when $V_{m\text{-}pre}$ was held continuously at rest (-61 mV), hyperpolarized (-77 mV) or depolarized potential (-48 mV). The presynaptic voltage facilitation (PVF) of synaptic transmission was quantified by normalizing the postsynaptic responses obtained at -48 mV to those measured at -77 mV. In these conditions, PVF amounted to 141 ± 18% (n =10) and was associated with a decrease in the paired-pulse ratio (from 117 ± 9% at -77 mV to 95 ± 7% at -48 mV). We found that PVF was totally occluded by bath application of the Kv1 channel blocker DTX. Time constant of PVF was determined by evoking single presynaptic APs at increasing delays (0.2, 3.2, 6.2 & 9.2 s) after the onset of a presynaptic depolarization. The measured time constant (2 s) was compatible with the time constant of the inactivation of D-type current carried by Kv1 channels. Using confocal laser scanning microscopy and Fluo-4 fluorescent calcium indicator, we measured calcium transients in axons of CA3 pyramidal neurons. Depolarization of the cell body from -65 to -50 mV enhanced spike-evoked axonal calcium transients by 18%. Notably, this facilitation followed the time course of the PVF.
We conclude that PVF is a short-term plasticity present at excitatory CA3-CA3 synapses resulting from the increase in spike-evoked calcium transients in the axon caused by voltage-inactivation of Kv1 channels.
Supported by Région PACA (APO-2009 Plexin).

P1.016

Slit1 enables Netrin1 attractive activity on rostral thalamocortical axons

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Brain functioning relies on the formation of stereotyped axonal connections that are established in response to multiple environmental guidance cues. How growing axons interpret *in vivo* the positional information encoded by a combination of guidance factors remains largely unknown. Here, we investigate this issue using the model of mouse thalamocortical axons, which is a major topographically organized projection of the mammalian brain. Thalamocortical axons convey the majority of sensory and motor information to the cerebral cortex and their initial topography is set at the level of an intermediate target, the ventral telencephalon. Using several *in vitro* and *in vivo* assays, we show that a spatially restricted domain that we previously identified as the “corridor” sets the initial topography of thalamocortical projections. At the molecular level, we show that corridor cells express a high rostral - low caudal pattern of *Slit1* and *Netrin1*. In *Slit1* mutant embryos, the corridor forms correctly but the pathfinding of thalamocortical axons to intermediate and rostral cortical areas is altered, suggesting that Slit/Robo signaling in the corridor is essential for correct axonal topography. *Ex vivo* co-culture assays using *Slit1* mutant and *Robo1;Robo2* double mutant mice show that Slit/Robo signaling repels intermediate axons, thereby preventing their rostral growth. Paradoxically, Slit1 has a similar strong repulsive effect on rostral thalamocortical axons in *vitro*, although these axons normally cross a high *Slit1*-expressing region of the corridor *in vivo*. Strikingly, Slit1 repulsion was switched to attraction when Netrin1 and Slit1 proteins were presented together to rostral axons, while Netrin1 alone had no effect. Furthermore, *ex vivo* experiments using single and double mutant mice for these molecules show that this attractive effect is necessary for establishing the path of rostral thalamocortical axons. Together, our study shows for the first time that the combination of two guidance cues can elicit a novel response, that none of the factors have alone. These results provide a new conceptual framework to study axon guidance in the complex environment of the developing brain.

P1.017

Effects of the reductant N-acetyl- L-cysteine on age-related deficits of D-serine-dependent hippocampal synaptic plasticity

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Oxidative stress (OS), resulting from an imbalance between antioxidant defenses and the intracellular accumulation of reactive oxygen species, contributes to age-associated memory deficits. Although an impaired induction of synaptic plasticity in hippocampal networks is proposed to support cognitive aging, the fine mechanisms targeted by OS remain elusive. In this study, we investigated the effects of the reductant N-acetyl-L-cysteine (L-NAC) on the activation of the N-methyl- d-aspartate receptor (NMDA-R) by its co-agonist d-serine, for which an alteration has been proposed to underlie deficits of

synaptic plasticity in aging. Our results showed that long-term dietary with L-NAC reversed the injury of redox status that occurred in hippocampal tissues of aged rats. Electrophysiological recordings in CA1 hippocampal slices indicated that NMDA-R-mediated synaptic potentials and theta-burst-induced long-term potentiation (LTP) were depressed in aged animals. These deficits were reversed by exogenous D-serine. Long term treatment with L-NAC, but not acute application of the reductant, restored the functional deficits of NMDA-R activation, of LTP induction and of sensitivity to exogenous D-serine. On the other hand, western blotting showed that the weaker expression in aged rats of the d-serine synthesizing enzyme serine racemase, that underlies the decreased NMDA-R activation by the amino acid, was prevented by long-term dietary with L-NAC.
These results indicate that preserving the redox status in advanced age prevents the injury of cellular mechanisms of learning and memory, at least by maintaining the regulation of NMDA-R function by the D-serine-dependent pathway.

P1.018

Primary afferent terminals acting as excitatory interneurons contribute to spontaneous motor activities in the immature spinal cord

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Patterned, spontaneous activity plays a critical role in the development of neuronal networks. A robust spontaneous activity is observed *in vitro* in spinal cord preparations isolated from immature rats. The rhythmic ventral root discharges rely mainly on the depolarizing/excitatory action of GABA and glycine early during development whereas at later stages glutamate drive is primarily responsible for the rhythmic activity and GABA is thought to play an inhibitory role. However, rhythmic discharges mediated by the activation of $GABA_A$ receptors are recorded from dorsal roots. In the present study we used the *in vitro* spinal cord preparation of neonatal rats to identify the relationship between discharges that are conducted antidromically along dorsal roots and the spontaneous activity recorded from lumbar motoneurons. We show that discharges occur earlier in dorsal than in ventral roots suggesting the existence of a causal link between dorsal root discharges and motor activities. The activity in both dorsal and ventral roots was transiently blocked by the $GABA_A$ receptor antagonist picrotoxin and increased by diazepam. High intracellular concentrations of chloride are maintained in primary afferent terminals by the sodium-potassium-chloride cotransporter NKCC1. Blocking these cotransporters by bumetanide decreased both dorsal and ventral root discharges. Importantly, we show that discharges recorded in dorsal roots are not an epiphenomenon due to the recording conditions (temperature, extracellular concentration of calcium, energy substrates supplementation). We conclude that primary afferent fibers act as excitatory interneurons and that GABA, through primary afferent depolarizations, is still playing a key role in promoting spontaneous activity in neonates.

P1.019

Synaptic plasticity in the hyperdirect pathway of the basal ganglia

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Basal ganglia, a network of sub-cortical nuclei, are involved in motor learning, related to the environment and motivation. Cortico-basal ganglia synaptic plasticity provides a fundamental mechanism for procedural learning and memory. Information from the cerebral cortex is processed by basal ganglia through three anatomo-functional pathways: (1) the hyperdirect (cortico-subthalmo-

nigral) (2) the direct (cortico-striato-nigral), and (3) the indirect (cortico-striato-pallido-subthalamo-nigral) pathway. Synaptic plasticity has been extensively investigated in the direct and indirect corticostriatal pathways but remains unexplored in the hyperdirect pathway. With 3D anatomical reconstruction, we established a rat brain slice of the hyperdirect pathway, preserving the connection between the cerebral cortex and the subthalamic nucleus (STN), and between the STN and the substantia nigra *pars reticulata* (SNr). First, we have investigated the plasticity at cortico-STN step with patch-clamp recordings. Electrical stimulation in the layer V of the motor cortex induced monosynaptic and glutamatergic EPSC in the STN. Hebbian high-, low-frequency stimulation or spike-timing-dependent plasticity (STDP) protocols induced a robust long-term depression (LTD) at cortico-STN synapses. A synaptic potentiation, lasting for 15 to 30 minutes, could be observed when brief presynaptic high-frequency stimulation preceded a short hyperpolarization of the STN neuron. In a second step, we investigated the plasticity at STN-SNr synapses. Similarly to cortico-STN synapses, a robust LTD was observed with Hebbian high-, low-frequency stimulation or STDP protocols. These results indicate a marked difference of plasticity rules between the corticostriatal direct-indirect pathways, whose display bidirectional plasticity, and the hyperdirect pathway, which appears less sensitive to bidirectional synaptic efficacy changes. This could be put in relation with the different properties of these neurons: striatal neurons act as coincidence detectors and are silent at rest while STN and SNr neurons are tonically active. Thus, different rules of plasticity are expected and our results bring new insights in the understanding of the cortical information processing through the hyperdirect pathway.

P1.020

The transcription factor Meis1 is required during the development of the sympathetic nervous system

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During development, peripheral sympathetic ganglia of the autonomic system arise from the neural crest. Concerted action of several transcription factors previously identified is required for its early formation. We found that the transcription factor Meis1 is expressed in the sympathetic nervous system during development. Meis1 belongs to the TALE homeobox family together with Meis2 and Meis3 and is the only member to be expressed in this structure. Using double *in situ* hybridization to better characterize its expression, we have shown that Meis1 is expressed by both noradrenergic neurons and cholinergic neurons which represent the two main sympathetic neurons populations. Moreover, its expression starts after the transcription factors implicated in early specification of sympathetic neurons such as Phox2a, Phox2b, Gata3 or dHand. Since the Meis1 deficient mice die at early embryonic stages and to determine the role of this factor in the specification of the sympathetic neurons, we undertake the analysis of a conditional knockout strain of mice in which Meis1 has been invalidated in the peripheral nervous system. Our results show that invalidating Meis1 expression in the peripheral nervous system using $HtPA^{CRE}/Meis1^{LoxP/LoxP}$ or $Wnt1^{CRE}/Meis1^{LoxP/LoxP}$ compound mice leads to perinatal death. Further investigations demonstrate that neurons forming the sympathetic ganglia are initially normally generated in space and time but degenerate at later embryonic stages, probably causing the perinatal death of homozygote newborn mice.
In order to dissect the mechanisms by which Meis1 regulates sympathetic neurons survival, we start *in vitro* studies using primary culture of embryonic sympathetic neurons and culture of the N2A neuroblastoma cell line. Moreover, we conducted a large scale screening by ChIP-seq to identify Meis1 target genes in these neurons.

P1.021

Corticogenesis from uniparental Embryonic Stem cells

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The cerebral cortex contains a myriad of cell types that are generated during development. The identification of mechanisms involved in corticogenesis is not only crucial for our understanding of diseases affecting human cerebral cortex structure and functions but also for the rational design of exogenous cortical cells for cell therapy.
In vitro corticogenesis is a simple model of corticogenesis based on Embryonic Stem cells (ESC) differentiation. *In vitro* corticogenesis reproduces the milestones of *in vivo* corticogenesis and leads to the generation of neurons displaying the molecular, electrical and connectivity properties of endogenous cortical neurons.
Uniparental, parthenogenetic (PG) or androgenetic (AG) embryos that carry 2 copies of maternal or paternal genomes fail to develop to term and form distinct structures. In chimera experiments PG and AG cells populate distinct brain regions (the cortex for PG and the hypothalamus for AG cells). This suggests that parental genomes support different developmental programs. It also demonstrates the existence of imprinted genes (IG), a subset of genes that have a monoallelic expression that depends on the parental origin of the inherited allele (while the majority of genes are biallelicaly expressed). For example *Igf2* and *H19* are only transcribed from the paternal or the maternal allele respectively.
There are growing experimental and clinical evidence that IG participate to brain function and disease; mutated *Ube3a* is linked to microcephaly and some imprinted loci are linked to Prader Willi and to autism spectrum disorders, two syndromes with altered cerebral functions.
Our *in silico* analysis reveals that at least 45 IGs are expressed during corticogenesis *in vivo*. qPCR experiments show that most IG transcripts are dynamically regulated during *in vitro* corticogenesis. IG expression thus substantiates a possible role in corticogenesis.
To get an insight into the role of IG and parental genomes in corticogenesis, AG and PG ESC are challenged with the protocol of *in vitro* corticogenesis. We have observed that uniparental ESC generate a progeny that display the molecular characteristics of cortical cells. Therefore both AG and PG cells may have the intrinsic capacity to form a cortex in the dish.

P1.022

Muscarinic and nicotinic modulation of long-term potentiation (LTP) and depression (LTD) in projections from the mediodorsal thalamus to the prefrontal cortex of rats in vivo

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The mediodorsal thalamic nucleus (MD) is a rich source of afferents to the medial prefrontal cortex (mPFC) and is involved in working memory and epilepsy. Considering the cholinergic role in cortical cognition and psychiatric disorders, our study aimed to investigate the cholinergic modulation of synaptic plasticity in the MD-mPFC pathway of urethanized rats. For that, we implanted a recording electrode in the mPFC, a stimulating electrode in the MD and a microinjection cannula above the lateral ventricle. Stimulation pulses were applied to the MD (0.05 Hz; 30 min) for baseline recordings of mPFC field post-synaptic potentials (fPSPs). Then, the rats received an icv microinjection of the muscarinic agonist pilocarpine (Pilo; 40 nmol/µL), or nicotine (Nic; 320 nmol/µL) or the vehicle (artificial cerebrospinal fluid, aCSF) before high-frequency (HFS; 10 trains of 50 pulses at 250 Hz) or low-frequency stimulation (LFS; 1,200 pulses at 2 Hz) in order to induce LTP and LTD, respectively

(groups: Pilo-HFS, Pilo-LFS, Nic-HFS, Nic-LFS, aCSF-HFS, aCSF-LFS). Control rats received Pilo/Nic/aCSF microinjection without HFS/LFS (Pilo-Ctrl, Nic-Ctrl, aCSF-Ctrl). Local field potentials (LFPs) were recorded in mPFC and MD during microinjections and fPSPs were evoked at 0.05 Hz and monitored for 4 h after HFS/LFS/Ctrl. Our results show that both Pilo and Nic induced cortical and thalamic desynchronization of LFPs, indicating cholinergic activation by the time of HFS/LFS/Ctrl. Although the HFS was unable to induce LTP in aCSF-HFS rats, it induced a delayed-onset form of LTP in Pilo-HFS and Nic-HFS rats. The LFS induced stable LTD in aCSF-LFS rats but impaired LTD in Pilo-LFS and Nic-LFS rats for at least 4 h. Pilo-Ctrl, Nic-Ctrl and aCSF-Ctrl group did not show changes in fPSP amplitudes. Therefore, our findings show that HFS under cholinergic activation induces a late-phase LTP in the MD-mPFC pathway, which is similar to our previous reports for the CA1-mPFC projections. On the other hand, Pilo and Nic suppressed LTD, which is the opposite of what occurs in CA1-mPFC. Such distinct modulatory influences of MD and CA1 on mPFC synaptic plasticity may shed some light on specific behavioral roles of limbic-prefrontal circuits both in normal conditions and in neurological disorders.

P1.023

Characterization of synaptic plasticity in corticostriatal transmission: role in Parkinson's disease

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Striatal Spiny Neurons receive cortical glutamatergic projections and nigral dopaminergic projections. The induction of long-term plasticity in the striatum involves both dopamine (DA) and glutamate receptors. Requirement of DA makes corticostriatal plasticity unique. The degeneration of the nigrostriatal pathway in Parkinson's disease (PD) leads to significant morphological and functional modifications in the striatal neuronal circuitry such as overactivity of the corticostriatal glutamatergic pathway, pruning of synaptic spines in striatal neurons and loss of synaptic plasticity. Current treatments of PD lead to motor complications due to maladaptive forms of corticostriatal plasticity. Therefore, understanding signaling pathways and interactions between glutamate and DA receptors in order to restore physiological plasticity could be an alternative strategy for new treatments. Metabotropic glutamate (mGlu5a) and DA (D1 or D2) receptors are co-expressed at postsynaptic sites of MSNs. We first looked at physical and functional crosstalk between these receptors in HEK cells. BRET experiments, indeed, emphasis the existence of mGlu5a-D1 and mGlu5a-D2 heterodimers. These physical associations lead to functional regulations, in particular mGlu5a hampers the potency of D2 agonist to decrease cAMP formation. To study these interactions and the mechanisms of plasticity related to PD in a more physiological environment, we set up and characterized an original model of corticostriatal primary cultures. We here present immunocytochemical and electrophysiological evidences of functional connections between cortical and striatal neurons in culture. We focused on the induction of long-term striatal plasticity in this model, using patch-clamp. Our preliminary results indeed exacerbate the need of glutamate and DA receptors co-stimulation to induce long-term plasticity. This coculture model seems therefore appropriate to dissect and identify the pathways involved to sustain long-term plasticity in a final attempt to reestablish the plasticity in DA deprived models of PD.

P1.024

Function of Agrin for synapse formation in adult neurogenesis

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The heparan sulfate proteoglycan Agrin is necessary for the formation, maintenance and regeneration of the neuromuscular junction. In the central nervous system, Agrin is widely expressed and

concentrated at interneuron synapses, but its function during synaptogenesis in the brain is largely unclear.
Using a comprehensive gene expression screen in combination with *in situ* hybridization and immunohistochemistry, we identified expression of Agrin in neuronal precursors in the Subventricular Zone - Rostral Migratory Stream - Olfactory Bulb System (SVZ-RMS-OB- System) of the adult brain. In this system, new neuronal precursors are generated throughout life and migrate from their place of birth, the SVZ of the lateral ventricles, into the OB where they differentiate into GABA and dopamine producing interneurons.
In order to investigate the role of Agrin during the integration of these new neurons into the pre-existing circuitry of the OB, we transplanted neurons derived from Agrin deficient mice into the brain of wild type hosts. We found that transplanted cells showed little synaptic integration and were selectively eliminated from the system over time.
Next, we asked if Agrin gain-of-function could be beneficial for synaptic integration. We used postnatal in vivo electroporation to overexpress a transmembrane form of Agrin in adult neuronal precursors. These experiments revealed that increased expression of transmembrane Agrin induced additional synapses in postnatal generated neuronal precursors. Thus, Agrin is necessary and sufficient for the induction of new synapses in postnatal and adult neurogenesis.
To further investigate this effect, we are currently analysing which specific isoforms of Agrin mediate the synapse inducing activity. In addition, we address the molecular interactions between Agrin and the recently discovered new Agrin receptor, the alpha Na-K-ATPase alpha and elucidate parts of the downstream signalling events.

P1.025

In utero FLNA knockdown leads to periventricular nodular heterotopia in rats

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Cortical malformations are important causes of mental retardation and account for 20-40% of drug-resistant epilepsy in childhood. Periventricular Nodular Heterotopia (PNH) is one of these cortical malformation caused by defective neuronal migration resulting in abnormal positioning of post-mitotic neurons. These ectopic neurons remain in the periventricular zone, close to their birth place, and form nodules of heterotopic grey matter. Clinical manifestations range from asymptomatic to intractable epilepsy and mental retardation. To date, two genes have been identified to cause PNH: FLNA (Xq28) and ARFGEF2 (20q13). Several others chromosome locations containing additional putative PH genes have been identified. Although the link between PNH and epilepsy has been well established, the physio-pathological mechanism responsible for epileptogenesis remains obscure. In addition, the limited availability of human samples and the lack of genetic animal models that mimic PNH have both delayed our understanding of the underlying pathological mechanism. Thus, It is unclear whether the epileptogenic foci involve ectopic neurons or normotopic cortex or both.

P1.026

Cellular changes underlying the normal postnatal development of the amygdala: a stereological study in monkeys

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Abnormal development of the amygdala has been linked to several neurodevelopmental disorders, including schizophrenia and autism. However, the postnatal development of the amygdala is not easily explored at the cellular level in humans. We therefore performed a stereological analysis of the monkey amygdala in order to characterize the cellular changes underlying its normal structural

development in primates. We counted the number of neurons, astrocytes and oligodendrocytes, measured the size of neuronal somas and the volume of the main amygdala nuclei at different postnatal ages. The lateral, basal and accessory basal nuclei exhibited the same developmental pattern, with a large increase in volume between birth and three months of age, followed by a slower growth until at least 5-9 years. In contrast, the central nucleus was already highly developed at birth and increased significantly in volume only between one year and 5-9 years of age; the medial nucleus was highly developed at birth and exhibited only a marginal increase in volume to reach adult levels. Quantitative analyses of different cell types revealed that neither neuronal soma size nor the numbers of neurons or astrocytes changed during postnatal development. In contrast, there was a large increase in oligodendrocyte number and myelination, which was associated to an increase in amygdala volume after one year of age. Interestingly, at birth, the paralaminar nucleus contained a large pool of immature neurons that developed gradually into mature neurons, leading to a late increase in volume of this nucleus. Our findings revealed that different amygdala nuclei have distinct developmental profiles and that the amygdala is not fully mature until young adulthood. We discuss how pathogenic factors at different postnatal ages might lead to the abnormal development of distinct amygdala circuits, thus contributing to different neurodevelopmental disorders affecting amygdala structure and functions in humans.

P1.027

Role of matrix metalloproteinases and their inhibitors in glial scar formation and axon-glia interactions *in vitro*

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In the injured central nervous system, the glial scar constitutes a true physicochemical barrier for axonal growth and functional recovery. The interactions between different glial cells, in particular astrocytes and microglia, may be determinant to understand both the genesis of the scar and the functional interplay of its components with lesioned axons. We and others have previously shown that high levels of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) are produced by glial cells following injury and that the MMP/TIMP system is involved in neurodegeneration and post-lesion axonal remodelling. We have used the scratch assay model of glial scar on mice mixed astrocyte/microglia cultures and also transwell chambers to investigate
i) the expression and activity of several MMPs and TIMPs presumably involved in cell motility;
ii) the role of MMPs in glial cell migration, and
iii) the interactions of axotomised post-natal DRG axons with astrocytes and microglia.
The scratch rapidly triggered individual migration of microglial cells into the scratched area, while astrocytes displayed a collective migration that maintained cell-cell contacts behind the migration front. Astrocyte migration was concomitant with cell shape polarisation and changes in the distribution and activity of MMPs. MMP inhibitors also differently affected the migration behaviour of astroglia and microglia, the first being more sensitive to MMP inhibition. Finally, although axotomised DRG explants found on astrocytes a permissive ground to regenerate, they were repelled by microglial cells invading the centre of the scar, but surprisingly, specific MMP inhibitors attenuated the axon repulsive behaviour of the migcroglia and promoted axonal growth though the isolated microglia. In summary, the present work provides evidence for the involvement of MMPs in the formation of the glial scar and highlights the prominent role these proteinases in axon-glia interactions within the scar.

P1.028

VEGFR2 (KDR/Flk1) signaling mediates axon growth in response to semaphorin 3E in the developing brain

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Common factors are thought to control vascular and neuronal patterning. Here we report an in vivo requirement for the vascular endothelial growth factor receptor type 2 (VEGFR2) in axon tract formation in the mouse brain. We show that VEGFR2 is expressed by neurons of the subiculum and mediates axonal elongation in response to the semaphorin (Sema) family molecule, Sema3E. We further show that VEGFR2 associates with the PlexinD1/Neuropilin-1 (Nrp1) receptor complex for Sema3E and becomes tyrosine-phosphorylated upon Sema3E stimulation. In subicular neurons, Sema3E triggers VEGFR2-dependent activation of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway that is required for the increase in axonal growth. These results implicate VEGFR2 in axonal wiring through a mechanism dependent on Sema3E and independent of vascular endothelial growth factor (VEGF) ligands. This mechanism provides an explanation as to how a semaphorin can activate an axon growth promoting response in developing neurons.

P1.029

Control of information flow through the hippocampus via opioid-mediated LTD at inhibitory synapses in CA2

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The hippocampus processes information through two parallel pathways. In the well-studied trisynaptic pathway, signals originating from the cortex sequentially pass through the dentate gyrus, CA3 and then CA1. In the disynaptic pathway, information from the cortex is transmitted to CA2 and then to CA1. Independence of these two pathways is likely maintained in basal conditions by strong feed-forward inhibition from CA3 to CA2, which prevents CA3 from evoking action potentials in CA2. Pharmacological block of this inhibition has been found to readily allow CA3 to drive firing in CA2. An important question that arises is how this gating of information between the two pathways is modulated in normal hippocampal function: what physiological conditions reduce inhibition sufficiently to allow CA3 to drive CA2? We have found that a high frequency tetanus stimulation classically used to evoke LTP at excitatory synapses evoked LTD at inhibitory synapses. Our results indicate that this LTD occurred presynaptically and was blocked by opioid antagonists. Furthermore, transient application of Delta opioid agonist evoked a lasting depression of inhibitory synapses that occluded subsequent depression by the tetanus. The tetanus did not evoke any lasting change at excitatory synapses when inhibition was blocked. However, when inhibition was intact, the same tetanus evoked an indirect LTP at excitatory synapses resulting from LTD at inhibitory synapses. Interestingly, the resulting increase in EPSP amplitude was sufficient to allow CA3 inputs to evoke action potentials in CA2, and thus remove the gate between CA3 and CA2. These results suggest that the independence between the di- and the trisynaptic pathways can be removed during physiological conditions to generate a new quadrisynaptic pathway between the cortex, CA3, CA2 and CA1. Furthermore, this change in information flow is likely relevant for information processing by the hippocampus not only during normal memory formation, but also in the context of pathological conditions, as a specific decrease in inhibition in CA2 is observed during schizophrenia.

P1.030

Connexin 43 tunes hippocampal synaptic transmission

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Astrocytes play active roles in brain physiology by dynamic interactions with neurons. One of their typical feature is to express high levels of connexin 43 (Cx43), the main gap junction channel and

hemichannel subunit. However, the specific role of Cx43 on synaptic activity is presently unknown. Here we show that astrocyte-directed deletion of Cx43 (GFAP-CreCx43$^{fl/fl}$ mice) alters hippocampal synaptic transmission in CA1 pyramidal cells. Although these mice display normal hippocampal morphology, their field excitatory postsynaptic potentials are reduced, compared to wild type animals. The decrease in excitatory transmission is not due to changes in pyramidal cells intrinsic properties, excitability or postsynaptic AMPA receptors density, since synaptic AMPA-NMDA current ratios, as well as AMPA currents induced by exogenous AMPA application are unchanged. However, it is mediated by a decrease in synaptic glutamate levels, as demonstrated by the increased inhibition of evoked AMPA EPSC by γ-DGG, a low affinity competitive AMPA receptor antagonist. Since no changes in astroglial glutamate transporter currents were detected in GFAP-CreCx43$^{fl/fl}$ mice, this suggests that presynaptic alterations account for the decreased glutamatergic transmission. This is further supported by the reduced frequency and amplitude of mEPSCs, as well as the increased paired-pulse facilitation of evoked AMPA EPSCs. Altogether, these data demonstrate that Cx43 is essential for normal excitatory synaptic transmission in the hippocampus.

P1.031

Semaphorin3a regulates local axonal branching of GABAergic interneurons through fine regulation of cGMP level

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Different classes of GABAergic interneurons impose strong electrical regulation of their target cells by developing specific local axonal branching. Yet, the molecular mechanisms triggering this branching activity remain unclear. In cerebellar cortex, Basket interneurons form exuberant axonal branches at the axon initial segment (AIS) of Purkinje neurons and supposedly regulate their firing output. By using both in vitro and in vivo approaches, we showed that a member of the semaphorin family, SEMA3A, secreted by Purkinje neurons during local circuit formation induced basket axon branching at AIS. SEMA3A through activation of its receptors, directly regulates the level of cGMP in Basket interneurons. Indeed, SEMA3A modulates soluble guanylate cyclase activity through tyrosine phosphorylation of the beta1 subunit.
We propose a new transduction pathway in which sema3A through direct regulation of cGMP level, mediates axonal branching during GABAergic local circuit formation.

P1.032

PAK3 and its splice variants form regulatory heterodimers with PAK1 in brain

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p21-activated kinase 3 (pak3) is an XLMR gene in which 5 mutations responsible for non-syndromic mental retardation have been identified. *pak3* knockout mice revealed LTP anomalies but also CREB pathway defects (Meng *et al*, 2005 *J Neurosci* 25, 6641-650). Mental retardation mutants of the PAK3 protein expressed in rat hippocampal neurons induced dendritic spine abnormalities (Boda *et al*, 2004 *J Neurosci* 24, 10816-825; Kreis *et al*., 2007 *J Biol Chem* 282, 21497-506). In these two examples, endogenous PAK1 and PAK2 proteins do not compensate for the observed defects suggesting that PAK3 has specific neuronal functions among the PAK family. In line with this, we recently identified in *pak3,* two highly conserved alternative exons called b and c (Rousseau *et al*., 2003 *J Biol Chem* 278,

3912-920; Kreis *et al*., 2008 *J Neurochem* 106, 1184-197). In addition to the classical PAK3a variant (without any alternative exon), the *pak3 gene* encodes 3 new splice variants PAK3b, PAK3c and PAK3cb which are constitutively active and insensitive to GTPase activation. Moreover, unlike PAK1 or PAK3a, their autoinhibitory domain is unable to inhibit a kinase domain. The aim of this project was to understand how PAK3 regulation occurs. Based on the crystal structure of PAK1, a model of regulation was proposed in which PAK1 forms homodimers that can be dissociated through GTPase binding, leading to kinase activation (Lei *et al*., 2000 *Cell 102, 387-397*, Parrini *et al*, 2002 *Mol Cell* 9, 73-83). Given these observations, we searched to identify PAK3 dimers and showed that PAK3a, b, c and cb form dimers with PAK3a in fibroblasts but preferentially form heterodimers with PAK1. In order to show that dimers exist in mouse brain, we first demonstrated that PAK3a, b and cb are co-expressed with PAK1 in cortical neurons by single cell RT-PCR and co-purify with the PSD fraction of mouse synaptosomes. We then showed that PAK1 coimmunoprecipitates with PAK3 in mouse brain extracts. We also demonstrated that the different heterodimers allow each monomer to regulate the kinase activity of its partner. Through this study, we propose a symmetric regulation model for PAK3a which heterodimerizes with PAK1 and a new asymmetric regulation model for splice variants, also based on heterodimerization with PAK1.

P1.034

Neural stem cell proliferation is regulated by the anti-coagulant factor protein s and its structural homolog Gas6 regulate in the subventricular zone

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The adult mammalian brain hosts neural stem cells (NSC) within the subventricular zone (SVZ). The potential use of neural stem cells to treat neurodegenerative disorders and the pivotal role of stem cells in cancer reasearch have lead to an increasing body of studies designed to discover molecules that, when exogenously provided, control neural stem cells activity. However, the endogenous mechanisms, especially the inhibitory ones, that control proliferation, self-renewal and neurogenic activity of NSC have only poorly been explored.
Vitamin K-dependent proteins (VKDP) are a family of mainly secreted proteins that share a post-translationnal modification catalyzed by an enzyme that requires vitamin K. VKDP are mostly known for their role in blood coagulation. VKDP production is blocked by warfarin, a vitamin K antagonist that is a widely used anticoagulant. Recently, two vitamin K-dependent proteins, Gas-6 and protein S, have identified as ligands for the TAM tyrosine kinase receptors (Tyro 3, Axl, Mer) which regulate normal development and tumorigenesis, especially in the brain. Given the emerging pleioptropic functions of VKDP and TAM receptors, we assessed possible regulatory roles of VKDP and TAM in neural stem cells biology.
We demonstrate that the suppression of functional VKDP production by warfarin leads to a substantial increase in SVZ neural stem cells proliferation both in vitro and in vivo. This effect is reversed by either vitamin K or endogenously produced VKDP, suggesting that VKDP constitutively inhibit neural stem cells proliferation. Protein S and Gas6 were identified as the two only VKDP produced by SVZ cells. In addition, SVZ cells express the TAM receptors. We demonstrate that the anti-coagulant factor protein S inhibits while neutralization of endogenously produced protein S enhances SVZ cells proliferation, suggesting that protein S is a constitutive inhibitor of SVZ cells proliferation. Further, in Gas6 knock-out mice, we observed fewer neurosphere forming cells and fewer neurogenesis in the olfactory bulb, suggesting that Gas6 maintains SVZ neural stem cells pool.
Our study opens new insights in the endogenous regulation of stem cells activity and suggests new perspectives for the pharmacological use of the anti-coagulant warfarin.

P1.035

Characterization of last-order premotor interneurons by transneuronal tracing with rabies virus in the neonatal mouse spinal cord

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We characterized the interneurons involved in the control of ankle extensor (*Triceps surae* [TS] muscles) motoneurons (MNs) in the lumbar enlargement of mouse neonates by retrograde transneuronal tracing using rabies virus (RV). Examination of the kinetics of the retrograde transneuronal transfer at sequential intervals post inoculation enabled to determine the time window during which only last-order interneurons (loINs) were RV-infected. Consistent with results observed in rat or monkey, in the mouse neonate, the infection of the network resulted exclusively from a retrograde transport of RV along the motor route. About 80% of the loINs were observed ipsilaterally to the injection. They were distributed all along the lumbar enlargement but the majority was observed in L4 and L5 where TS MNs were localized. Most loINs were distributed in laminae V-VII while the most superficial laminae were devoid of RV-infection. Contralaterally, commissural loINs were found essentially in lamina VIII of all lumbar segments. Groups of loINs were characterized by their chemical phenotypes using dual immunolabeling. Glycinergic neurons monosynaptically connected to TS MNs represented 50% of loINs ipsilaterally and 10% contralaterally. As expected, the ipsilateral glycinergic loINs included Renshaw cells, the most ventral neurons expressing calbindin. We also demonstrated the monosynaptic connection between a group of cholinergic interneurons and TS MNs. These ChAT-positive loINs were observed ipsilaterally in L3 and the rostral part of L4. To conclude, the transneuronal tracing with RV, combined with an immunohistochemical detection of neuronal determinants, enables a very specific mapping of motor networks involved in the control of single muscles.

P1.036

Hoxa2 activation in cranial neural crest interferes with head and brain development by targeting BMP signaling through Six gene down-regulation

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The neural crest is a transient structure of vertebrate embryo that develops from the lateral borders of the neural epithelium, and provides the developing embryo with a large variety of cell lineages. At cephalic level, NC cells participate in head morphogenesis by giving rise to most of its skeletal, dermal, and connective tissues. The emergence of the NC-derived mesenchyme is considered as an evolutionary innovation that has accompanied the development of the head and the expansion of the forebrain in Vertebrates.
Our present work aims at deciphering the molecular mechanisms whereby the cephalic NC cells coordinate the development of the face and the brain in embryo. The cranial NC (CNC), which is responsible for the formation of the craniofacial skeleton, arises from the anterior region devoid of Hox gene activation. When CNC cells are transfected with Hox genes their capacities to differentiate into skeletal tissues is inhibited. This condition results in craniofacial abnormalities as well as neural tube defects. In order to figure out the molecular mechanisms involved in these processes, we have unraveled the effects of ectopic Hox gene activation and focused our interest on the molecular interplay between Hoxa2 and its downstream target, Six2. Here we show that the down-regulation of Six2, either due to Hoxa2-forced expression of to Six2-silencing, entails the down-regulation of Bmp-antagonists, Noggin, Gremlin and Dan in CNC cells. The excess in Bmp activity resulting from the global reduction in Bmp inhibitors was detrimental to Fgf8 expression in the prosencephalic organizer and in the maxillo-mandibular region. These molecular changes accounted for severe defects in fore- and midbrain development: the dorso-lateral aspects of the optic tectum, thalamus, and pallium were

completely abolished, and the choroid plexuses failed to develop. In addition, several defects in facial chondrogenic development were observed.
Taken together, these results suggest that over vertebrate evolution, the emergence of an Hox-negative domain in Six-expressing CNC cells was critical to both head and brain ontogenesis.

P1.037
Endocannabinoid spike-timing-dependent plasticity underlies fast learning

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Synaptic plasticity is admitted to underlie learning and memory. The experimental protocols to induce plasticity imply a high number (i.e. hundreds) of stimulations. Recently, spike-timing-dependent plasticity (STDP) was used as a Hebbian synaptic learning rule using of the order of one hundred of paired stimulations (PS). These requirements seem at odds with the fact that one can learn and recall out of a few trials. Thus, we tested whether a STDP could be induced with very low number of PS. Our experimental system is the corticostriatal synapse because of its involvement in procedural learning and memory. We have previously reported a robust bidirectional STDP at corticostriatal synapses when induced with 100 PS. Here, leveraging mutual interactions between patch-clamp recordings in rat brain slices and a realistic biophysical synapse model, we report dramatic changes in STDP-timing rule when the PS number decreases: (1) 100 to 75 PS induced bidirectional STDP (post-pre PS induced LTP while pre-post PS triggered LTD), (2) 50 PS induced unidirectional STDP (no plasticity with post-pre PS, and LTD with pre-post PS) and (3) 10 to 5 PS induced an opposite unidirectional STDP (i.e. LTP induced by post-pre PS and no plasticity with pre-post PS). Note that no significant plasticity was observed for less than 5 PS, denoting a limit of the induction for STDP. Most notably, the biophysical synapse model predicted LTP at 10 to 5 PS to be endocannabinoid-mediated. Strikingly, this prediction was confirmed experimentally: LTP induced by 5-10 post-pre PS was not NMDA-receptor-mediated (unlike the LTP induced by 100 post-pre PS) but indeed endocannabinoid-mediated. We found that this LTP depended on activation of metabotropic glutamate receptor (mGluR5), voltage-sensitive calcium channels, phospholipase Cb, the diacyglycerol lipase and cannabinoid CB1 receptors. To test the genericity of this result, we questioned STDP at corticocortical synapses in the somatosensory cortex (between layers 2/3 and 5) and found that we could similarly induce an endocannabinoid-dependent LTP with low PS numbers. Endocannabinoid LTP may thus represent a common form in central structures, suggesting a crucial role for endocannabinoid-mediated STDP in fast learning.

P1.038
Reorganization of the respiratory bulbospinal pathways after an unilateral cervical spinal cord injury

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Spinal cord injury can have irreversible consequences on the body functions, but recent studies have shown that after a partial lesion, plasticity processes may lead to some degree of functional restoration.

The aim of the study was to analyze the reorganization of the bulbospinal respiratory network in adult rats following a partial lateral lesion of the cervical spinal cord (C2 level), that leads to an interruption of most respiratory bulbospinal axons.
In these animals, we have previously shown that respiratory recovery appears gradually after the injury depending on "new" functional spinal pathways located in the medial part of the spinal cord. Here, we analyzed the cellular reorganization of the respiratory bulbospinal network after a chronic lesion (3-6 months post-injury) using anterograde (Fluororuby, FR) and retrograde (Fluorogold, FG) neuronal fluoro-tracers. After a chronic lateral injury, we observed an increased number of respiratory bulbospinal neurons and C1 propriospinal neurons projecting to phrenic motoneurons (at C4 level). Rostrally to the lateral injury, we observe a higher proportion of bulbospinal fibers in the medial versus lateral white matter area (in the ventral spinal cord); and a higher number of axotomised fibers entering the ventral grey matter in 3 months post-injury rats compared to 7 days post-injury rats.
In conclusion, after a chronic unilateral cervical injury, we observe a reorganization in the distribution of bulbospinal fibers innervating phrenic motoneurons that is correlated with the functional recovery previously shown.

P1.039

Regulation of Pax6 expression by micro-RNAs in the mouse forebrain

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Different progenitor populations localized in the postnatal and adult periventricular region (PVR) generate different types of neurons for the olfactory bulb. The molecular mechanisms underlying the functional regionalization of the different progenitor cells are poorly understood. The transcription factor Pax6 is expressed in the dorsal wall of the lateral ventricle, and is necessary for the production of dopaminergic periglomerular interneurons. We show that forced expression of Pax6 specifically in lateral progenitors is sufficient to change the fate of their offspring towards TH expressing neurons. Interestingly, while the Pax6 protein is strictly confined to the dorsal SVZ, expression of the promoter and localization of Pax6 transcripts extend far into the lateral aspect of the wall. We investigated the molecular basis for this discrepancy between transcript and protein distribution. We found that sequences that prevent the expression of Pax6 protein laterally are contained in the 3'UTR of the mRNA. Therefore, Pax6 transcripts lacking the UTR get translated regardless of their dorso-ventral postion. Furthermore, linking the 3'UTR of Pax6 to a Myc-tag encoding transcript is sufficient to confer dorsal restriction of the resulting protein in vivo. Altogether this behavior is suggestive of an involvement of miRNAs in the regulation of Pax6 expression. Bioinformatical analysis of the Pax6-3'UTR identified several potential mi-RNA binding sites. Deep sequencing based expression analysis and RT-qPCR showed that two of these candidate miRNAs were differentially expressed along the ventro-dorsal axis of the SVZ lining the lateral ventricle in a gradient opposing Pax6 transcripts. Both miRNAs were able to down regulate Pax6 transcript activity in the dorsal aspect of the (PVR) in vivo. Altogether, this data provides evidence that defined miRNAs targeting the Pax6-3'UTR are expressed in the lateral wall of the lateral ventricle to restrict Pax6 protein exclusively to dorsal progenitors.

P1.040

Cortico-striatal synaptic plasticity is altered in a new mouse model of gene dosage for the telomeric part of the human chromosome 21

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Down syndrome (DS) is a genetic disorder characterized by the presence of an extra copy of human chromosomes 21 (Hsa21). This pathology has a prevalence of 1:800 live births and is the most common genetic cause of memory and learning defects. The regions on Hsa21 are conserved with

three regions of synteny located on mouse chromosome 16(Mmu16), Mmu17 and Mmu10.To better understand the genotype and phenotype relationships several mouse models have been created such as TC1 or Ts65Dn. In this report we explored the contribution of a new monosomic mouse model, Ms4Yah. It carries a deletion of 47 genes (2.2 Mb) located in the most telomeric part of Hsa21 corresponding to the mouse chromosome 10 (Mmu10) included in the *Cstb-Prmt2* genetic interval (Duchon et al. 2011).We have explored the transmission between cortex and striatal medium-sized spiny neurons (MSNs) and their capability to develop activity-dependent long-term plasticity. Striatum, the main input nucleus of basal ganglia, is involved in memory and learning processes. Using a corticostriatal slice preserving the connections between the somatosensory cortex and the target striatal cells, long term potentiation (LTP) was induced by a High Frequency Protocol (HFS: 3x100Hz; 1 sec), performed in the cortex with a bipolar electrode placed in the layer V. Excitatory postsynaptic currents (EPSC) were recorded in MSNs with the whole-cell configuration of the patch clamp technique. We investigated first the EPSC amplitude stability during 10 min recordings by applying a 0.1 Hz stimulation. There was no significant variation of EPSC amplitudes along time. Results showed that HFS protocol induced LTP in the EPSC amplitude in euploid mice (115 ± 9%). In contrast, for Ms4Yah HFS induced a significant decrease in EPSC amplitude (65 ± 11%, WT n=9; Ms4Yah n=6, $p< 0.02$ Mann Whitney Test). Results suggest that the portion of mouse chromosome 10 deleted in Ms4yah model (Cstb-Prmt2) is playing a role in cortico-striatal synaptic plasticity and may be essential for cognitive deficit found in DS.

Duchon et al. (2011) Thetelomeric part of the human chromosome 21 from Cstb to Prmt2 is not necessary for the locomotor and short-term memory deficits observed in the TC1 mouse model of Down Syndrome. Behav. Brain Res. 217 :271-281.

P1.041

Reliability of spike timing in CA1 hippocampal pyramidal neurons is modulated by endocannabinoids

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Hippocampal pyramidal neurons constantly receive spontaneous GABAergic activity which regulates their firing behaviour. Many studies in the last ten years showed a short-term plasticity of GABAergic activity mediated by endocannabinoids. This phenomenon, called DSI (Depolarization-induced Suppression of Inhibition), has been described in different structures in the brain such as cerebellum, cortex and hippocampus. Our study evaluates how GABAergic activity and endocannabinoids can control reliability of spike timing in CA1 pyramidal neurons. Indeed, spontaneous background GABAergic activity can modulate both the excitability and the capacity of a neuron to reproduce a same pattern of firing for a given stimulation. Following a sustained discharge of a CA1 pyramidal neuron, we show that its fidelity is increased coincidently with the reduction of spontaneous GABAergic activity received by this cell. When $GABA_A$ or CB1 receptors are blocked by application of Picrotoxin or AM251, respectively, discharge fidelity was no longer modified, suggesting that CA1 pyramidal neurons can modulate their own fidelity by retrograde endocannabinoid-mediated control of spontaneous GABAergic activity. Moreover, Given the fact that *in vivo* recorded patterns of place cell discharges during spatial exploration were able to modulate both GABAergic activity and the fidelity of neuronal discharge, we conclude that DSI is a genuine plastic phenomenon that can occur *in vivo* and let the place cells the ability to tune both their excitability and temporal fidelity.

P1.042

Calcium and the development of electrophysiological properties of dopaminergic neurons of the substantia nigra pars compacta

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The importance of calcium during the development of the nervous system is now well established. Calcium has been shown to be involved in axon motility, synapse development and stabilization. In

addition, spontaneous patterns of activity generating specific calcium dynamics have been observed in different neuronal types during early developmental stages.
We studied the evolution of the electrophysiological properties of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc) during early post-natal development (between P6 and P21) and demonstrated that there were significant differences between these two developmental stages that may be related to changes in calcium dynamics. While P21 DA neurons are essentially tonic pacemakers (75%) when recorded in acute slices, a significant proportion (30%) of P6 DA neurons display bursting activity. This observation is consistent with previous studies suggesting that there is strong spontaneous activity of low-threshold calcium currents in DA neurons in juvenile rats. DA neurons are separated into 2 different subpopulations based on the expression of the calcium-binding protein calbindin (CB). While CB-negative cells are predominant in the adult (and also at P21), we demonstrated that CB-positive cells are predominant at P6, suggesting that important changes in calcium metabolism occur between the first and the third postnatal weeks. Experiments performed in the lab have shown that the A-type and H-type currents have correlated voltage-dependences in SNc DA neurons in P21 animals, and that this correlation is dependent on cytosolic calcium buffering. We performed measurements in P6 animals, and found that the properties of these currents indicate a free calcium concentration higher at P6 than at P21.
Altogether, our data indicate that i) CB expression is higher at P6 than at P21 and that ii) electrophysiological properties indicate a higher cytosolic calcium concentration at P6 than P21. Although these findings seem paradoxical, published results and consideration of technical parameters strongly suggest that cytosolic calcium might be higher in P6 animals and critically influence the specific electrophysiological behavior recorded in juvenile DA neurons.

P1.043

Altered subthalamo-nigral synaptic plasticity in a rat model of Parkinson's disease

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GABAergic neurons of the substantia nigra pars reticulata (SNr) form the main output of the basal ganglia network in rodents and exert an inhibitory regulation on the motor thalamus, thus controlling voluntary movement execution. They receive both inhibitory inputs from the striatum and the globus pallidus and excitatory inputs from the subthalamic nucleus (STN), as well as a dopaminergic innervation from the substantia nigra pars compacta. Particularly, the subthalamo-nigral glutamatergic synapse is believed to be a critical connection in sculpting the activity patterns of SNr neurons and to be involved in the occurrence of their pathological activity in Parkinson's disease (PD). Yet, the properties of this synapse remain poorly understood. Our goal was to perform a comparative characterization of the plastic properties of STN-SNr synapses in rat brain slices, both in a physiological and pathological context. Excitatory postsynaptic currents (EPSCs) were evoked onto SNr GABAergic neurons by minimal bipolar electrode stimulation of subthalamic afferents and recorded in whole-cell patch-clamp in the presence of $GABA_{A/B}$ receptor blockers (SR95531, CGP55845). A pharmacological analysis revealed that these EPSCs rely mainly on AMPA receptors and are facilitated when challenged with paired-pulse stimuli. Using 6-OHDA-lesioned rats as an animal model of PD, we observed that paired-pulse facilitation is enhanced in a pathological context, suggesting a presynaptic impact of dopamine (DA) depletion. We found that the efficacy of STN-SNr synapses can be down-regulated in response to tetanic stimulation through a calcium-, NMDA-dependent form of long-term depression (LTD) with a postsynaptic origin confirmed by paired-pulse ratio analysis of evoked EPSCs. Consistently, LTD does not involve metabotropic glutamate receptors. However, activation of D2 DA receptors by DA or quinpirole significantly attenuated LTD, suggesting a modulatory role of endogenous DA on the plastic properties of these synapses. Moreover, tetanic stimulation failed to induce LTD in 6-OHDA-treated animals, revealing a change in synaptic plasticity in a pathological context. Together, our data suggest that the ability of STN-SNr synapses to undergo LTD is conditioned by the presence or lack of DA.

P1.044

Klf9 is necessary and sufficient for Purkinje cell survival in organotypic culture

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During development, neurons pass through a phase of programmed developmental cell death (DCD) during which trophic factors released by their targets are essential for their survival. After this period, neurons can survive without targets. The molecular mechanisms closing this phase remain unknown. In mouse cerebellum, the DCD of Purkinje cells finishes at the end of the first postnatal week. Based on a temporal correlation between the disappearance of Purkinje DCD and the increase in expression of Krüppel like factor 9 (Klf9), we selected Klf9 as a candidate gene for turning off the Purkinje DCD. We showed that the number of Purkinje cells in organotypic culture from newborn mice drops between the 3rd and 7th day of culture and remains stable (40% survival) at least until the 14th day. On this new model of Purkinje DCD, we tested the function of Klf9 using lentiviral vector-mediated manipulation of Klf9 expression. Klf9 overexpression multiplies the survival rate of the Purkinje cells by a factor of 2.2 whereas its depletion divides it by 2.6. Klf9$^{-/-}$ Purkinje survival rate is reduced to 23%. The known Purkinje cell trophic factors IGF-1 (insulin growth factor-1) and NT3 (neurotrophin3) were able to rescue these neurons from Klf9 depletion. Altogether, these results show that Klf9 is necessary and sufficient for Purkinje cell survival, suggesting that Klf9 is indeed involved in closing the developmental phase of cell death.

P1.045

Modifications of neural cell proliferation in the vestibular nuclei and the subgranular zone after vestibular damages in the adult cat

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After unilateral vestibular nerve section (UVN) in adult cats, our previous studies have demonstrated a strong reactive neurogenesis in a new zone: the deafferented vestibular nuclei of the brainstem. Interestingly, the newborn neurons were mostly GABAergic, survived up to two months and functionally contributed to fine posturo-locomotor functions recovery impaired by the lesion. However, we did not know whether a bilateral vestibular nerve section (BVN) would induce a reactive neurogenesis in the vestibular nuclei. Further, since the hippocampus uses vestibular inputs to build spatial maps and is well known to produce ongoing neurogenesis, we examined whether changes in the number of newborn cells stained with bromodeoxyurudine (BrdU) occur in the subgranular zone (SGZ) of the hippocampus after vestibular damages. We found that compared to the UVN group, BVN induced a two-fold increase of BrdU-Immunoreactive(-Ir) cells 3 days after the lesion in all the vestibular nuclei on both sides. Some of these cells colocalized BrdU/GAD67 stainings at 3 days post-BVN, suggesting that newborn cells differentiated faster after BVN. Furthermore, a high percentage of BrdU-Ir newborn cells survived and differentiated into GABAergic neurons and astrocytes 30 days after BVN. In the SGZ, 3 days after BVN the rate of cell proliferation was similar to controls and was

decreased at 30 days. In contrast, 3 days post-UVN in the SGZ, the BrdU-Ir cells decreased strongly and then reached progressively control values one month later. The neural cell proliferation occurring bilaterally in the vestibular nuclei could result from the disturbed microcellular homeostasis generated by the complete and sudden bilateral vestibular loss. Three days after lesion, no BrdU-Ir changes were detected in the SGZ of the BVN group when vestibular nuclei were silent on both sides. At this same postlesional delay however, the decreased number of BrdU-Ir cells observed in the SGZ after UVN was probably due to the asymmetric electrical activity between homologous vestibular nuclei. These results show that the suppression of vestibular information alters differentially the hippocampal neural cell proliferation depending on the nature of the vestibular damage.

P1.046

Consequence of diet lacking methyl donors on the expression of protein StAR (Steroidogenic Acute Regulatory Protein) in the brain of rat

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An animal model developed in the laboratory showed that 21-days-old rats early exposed to a deficient diet in methyl donors (folate, vitamin B12 and choline) display severe cognitive impairment associated with mechanisms of apoptosis in neurons of the hippocampus and cerebellum. These effects could contribute to impaired synaptic plasticity and neurogenesis.
It has recently been established that the brain contains the enzymes involved in the synthesis of neurosteroids which play a key role in neuroplasticity, modulation of neurotransmission and memory performance in rats.
The major cholesterol transfer protein, Steroidogenic Acute Regulatory protein (StAR) constitute the rate-limiting step for steroid biosynthesis by transferring cholesterol into the mitochondrial inner membrane.
It is widely distributed in the brain with highest levels in the neocortex, the hippocampus and the cerebellar Purkinje cells as well as in the neurogenic areas of the adult brain thus confering to this protein a critical role in neurogenesis.
Despite the crucial role of steroidogenesis in various cerebral functions and brain plasticity, nothing is known about the consequences of perinatal alterations of the one-carbon metabolism and the related changes in Hcy amounts on neurosteroidogenesis. We therefore measured brain expression levels of StAR in young rats born to dams subjected to a methyl donor deficient diet during the gestational and lactating period.
The 21-days-old rats early exposed to the deficient diet showed a significant decrease of StAR expression levels associated with hyperhomocysteinemia, as measured by immunohistochemistry, western immunoblot and q-PCR analysis. These alterations were more specifically measured in the regions of neurogenesis and synaptic plasticity (olfactory bulbs, hippocampus) as well as those involved in brain development (cerebellum). The most significant effects were observed in female rats, suggesting a sexual dimorphism in the response to a methyl-donor deficiency.
In conclusion, our results provide the first evidence of the deleterious effects of a gestational methyl donors deficiency on the expression levels of the cholesterol transfer protein StAR during the postnatal period, thus suggesting a consecutive impairment of neurosteroidogenesis

P1.047

Ephrin/Eph modulation after facial nerve axotomy

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We evaluate the effect of peripheral nerve lesion on expression of ephrin/Eph using the facial nerve transection model. The left facial nerves of adults rats were transected at the level of the stylomastoid foramen and changes in ephrin family were studied by RT-PCR and immunofluorescence studies in

both facial nuclei and vibrissae muscles from day 1 to day 60 after axotomy. Our results shown that transcripts and protein expression of EphA4 were down regulated in the facial nucleus, 8 days after the lesion with a return to normal value after 30 days. Similarly, axotomy caused a significant decrease in EphA4 expression at day post lesion 8. These data suggest that EphA4 should be considered as a potential cue for axonal regeneration and guidance after facial nerve axotomy

P1.048

Key role of miRNAs expression in bone cancer pain model

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Bone cancer pain is frequent among cancer patients and can have a devastating effect on their quality of life. Such pain occurs in patients with primary bone cancer and in patients with cancer that has metastasized to bone from distant sites such as the breast, prostate, ovary and lung. In recent years, the newly discovered microRNAs (miRNAs), a class of non-coding small RNA molecules, have been shown to be tightly linked to cancer progression. They have a role in suppressing the translation of targeted genes into proteins through binding to the complementary region in the 3'UnTranslated Region of mRNAs.
Because long-lasting changes in cancer pain sensitivity are accompanied by altered gene regulation, miRNAs expressed in nociceptive pathways may influence the development and maintenance of pain. In order to determine how miRNAs influence cancer pain, we first examined variations in their expression levels. We used a murine model of bone cancer, which shares similarities with human cancer-induced bone pain. C3H/HE normal mice, 5-6 weeks old, were injected with osteolytic sarcoma cells (NCTC 2472) into the medullary cavity of the right distal femur. The animal behavior indicative of pain has been studied using Dynamic Weight Bearing (DWB) and Plantar tests before and after the operation (days 0, 3, 7, 14, 21). DWB results showed sharp decrease in the weight bearing of the right paw at day 14 that persisted until mouse sacrifice (day 21). Plantar test results correlated to those of DWB, with a decrease in the withdrawal latency of the right paw that has been noticed since day 14. We characterized the extent of cancer-induced bone destruction by using X-rays and gross anatomy. In addition, gene expression profiling based on micro-array has been used to identify candidate genes which expression is changed in response to tumor induction. These changes in the expression level of miRNAs and target mRNAs were further confirmed using quantitative RT-PCR.
The change of miRNAs expression in the spinal cord of bone cancer has never been studied in pain context. Here, we show that miRNAs are likely to play a major role in cancer pain, and might represent a potential therapeutic target.

P1.049

Effects of lead exposure on the development of lumbar spinal networks

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Heavy metals including Lead (Pb) found in drinking water represent a significant public health problem in some countries like Morocco. The lead poisoning can lead to diseases affecting the central nervous system development. Glial and neuronal changes have been described during development after chronic lead poisoning. Animals exposed to Pb have higher rates of monoamines (dopamine, norepinephrine, serotonin) (Antonio et al. 1996). Monoamines involved in the maturation of motor

networks in particular lumbar spinal networks. We studied the effect of lead exposure on spontaneous activity as well as fictive locomotion recorded at the spinal level on in vitro preparations. The newborn rats whose mothers were exposed to lead have episodes of spontaneous activity more frequent and fictive locomotion faster. Our results suggest that lead exposure affects the expression of co-tansporteurs KCC2, important for the development of inhibitory transmission and the development of lumbar locomotor networks.
Key words: lead, spinal cord, fictive locomotion, spontaneous activity,

Acknowledgements: GDRI, action CNRS/CNRST Sc. Vie 05/10, PROTARS

P1.050

Tmprss3, a transmembrane serine protease deficient in human DFNB8/10 deafness, is critical for cochlear hair cell survival at the onset of hearing

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Mutations in the type II transmembrane serine protease 3 (TMPRSS3) gene cause non-syndromic autosomal recessive deafness DFNB8/10. This deafness is characterized by congenital or childhood onset bilateral profound hearing loss.TMPRSS3 is a transmembrane protein containing three extracellular domains. The LDLRA domain binds calcium and lipoprotein, the SRCR domain is involved in protein-protein interaction and the serine protease domain is the catalytic one.
In order to explore the role of TMPRSS3 in cochlear physiology, we have generated an ENU-induced mutant mouse in which the tyrosine 260 was changed into a STOP codon (Y260X), thus deleting the major part of the serine protease domain. Auditory brainstem responses revealed that wild type and heterozygous mice have normal hearing thresholds out to 5 months of age, whereas Tmprss3Y260X homozygous mice are completely deaf. Histological investigations revealed that both type of cochlear hair cell develop normally until hearing onset (postnatal day 12) and then began to degenerate in the basal turn, reaching complete degeneration in entire cochlea within 2 days. This is the first model showing a so drastic and rapid degeneration of both types of cochlear hair cells at the onset of hearing.
Given that auditory and vestibular deficits often co-exist, we evaluated the balancing abilities of Tmprss3Y260X homozygous mice by using rotating rod and vestibular-specific behavioural tests. We have shown that adult Tmprss3Y260X homozygous mice effectively displayed mild vestibular syndrome that correlated histologically with a slow degeneration of only saccular hair cells.
In situ hybridization in the developing inner ear showed that *Tmprss3* mRNA is localized in sensory hair cells in the cochlea and the vestibule. These data are in accordance with histological consequences of Tmprss3 deficiency.
Our results show that TMPRSS3 acts as a permissive factor for cochlear hair cells survival at the onset of hearing and is required for saccular hair cell survival. This mouse model will allow us to decipher the molecular mechanisms underlying DFNB8/10 deafness and cochlea function.

P1.051

Zebrafish Fidgetin-like 1 controls spinal motor axon guidance through the regulation of microtubule dynamics

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Fidgetin-like 1 is a newly identified member of the AAA (ATPase Associated with diverse cellular Activities) protein subfamily 7, which includes the three microtubule-severing enzymes Spastin, p60-Katanin and Fidgetin. In the zebrafish, Fidgetin-like 1 is the closest protein to Spastin, a protein frequently altered in hereditary spastic paraplegia. We have performed the first functional study of Fidgetin-like 1 *in vivo* using loss- and gain-of-function analyses during development of the zebrafish. We here show that zebrafish Fidgetin-like 1 is highly expressed in numerous populations of axons both in the developing brain and spinal cord. A lack of Fidgetin-like 1 induces a drastic decrease in larval mobility associated with obvious defects of spinal motor axon pathfinding. Using videomicroscopy *in vivo* and primary cultures of spinal neurons, we demonstrate that Fidgetin-like 1 affects spinal motor axon guidance and growth cone morphology by altering microtubule organisation and orientation. Finally, we show that Fidgetin-like 1 is enriched in the axon shaft of cultured spinal neurons where it binds to microtubule cytoskeleton. Our study thus provides compelling evidences for a role of Fidgetin-like 1 in the regulation of microtubule dynamics during spinal motor neuron outgrowth and further confirms the importance of the microtubule-associated proteins in axon guidance processes.

P1.052

Integrative silencing of Cav1.2 calcium channel subunits by microRNA miR-103: involvement in chronic pain sensitization

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In chronic pain states, up-regulation of Cav1.2 comprising L-type calcium channel (Cav1.2-LTC) contributes to long term sensitization. Consequently, it regulates gene expression underlying long-term plastic changes. The aim of this study is to determine whether variations of Cav1.2-LTC expression are regulated by microRNAs (miRNAs) and whether miRNAs play a major role in chronic pain mechanisms.
MiRNAs are short non-coding transcripts targeting mRNA 3' UnTranslated Region (3'UTR) for cleavage or translation inhibition. Because perfect matching to target sequence is not required for inhibition, miRNAs are likely to regulate simultaneously several mRNAs. Here we show, using a luciferase assay, that a single miRNA (miR-103) is able to silence simultaneously the three subunits of Cav1.2-LTC through binding their 3'UTR. To assess the physiological relevance of such an integrative regulation, we imaged calcium transients elicited by KCl-induced depolarization in primary cultures of spinal cord neurons transfected with miR-103, mutated miR-103, or empty vector. Results indicate that the integrative silencing of Cav1.2-LTC by miR-103 dynamically regulates neuron excitability *in vitro*.
In an animal model of neuropathic pain, we demonstrate that miR-103 is down-regulated, leading to an up-regulation of Cav1.2-LTC subunits. Moreover, we show that miR-103 intrathecal applications repress Cav1.2-LTC up-regulation and consequently relieve pain, demonstrating a causal role of miRNA in the maintenance of pain sensitization.
Previous demonstrations of a concerted control by miRNAs were related to several components of a signaling pathway. Here, we show for the first time, that a single miRNA may exert integrative silencing of all constitutive subunits of a given macromolecular complex. This novel mechanism could shed light on complex co-modulations of multiple mRNAs within a cell, in either normal or pathological conditions. Moreover, we demonstrate that impairment of this miRNA-induced integrative regulation in spinal cord is a major factor of chronic pain sensitization and, thus, deserves exploration as a therapeutic target.

P1.053

Serotoninergic modulations of the anxiolytic/anxiogenic effects of cannabinoid receptor stimulation by CP 55,940 in rats

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We have studied the possible modulations by serotoninergic neurotransmission of the effects of the cannabinoid receptor agonist, CP 55,940, on anxiety-related responses in the open field test, a validated behavioural paradigm in rats. Under acute treatment conditions, a low dose of CP 55,940 (10 µg/kg but neither 20 µg/kg nor 40 µg/kg) induced anxiolytic-like effects, whereas high doses (0.2-0.4 and 0.6 mg/kg) of this CB receptor agonist evoked anxiogenic-like responses. The 5-HT1A receptor agonist, ipsapirone (6 mg/kg), when administered alone, induced a marked increase in the number of central squares visited in the open field test, in line with its well established anxiolytic-like properties. Surprisingly, the ipsapirone effect was antagonized by acute as well as subchronic pretreatment with CP55,940 (0.4 mg/kg/day/11 days). Acute administration of the 5-HT reuptake inhibitor fluoxetine (3 mg/kg) alone had no observable effect on behavioural parameters considered, but after subchronic pretreatment by CP55,940, acute fluoxetine significantly decreased rearing scores and the number of central squares visited, indicated an anxiogenic-like action.These results provide further support to the idea that cannabinoid systems are involved in anxiety-like responses in rats. In addition, they strongly suggest that complex interactions between cannabinoidergic and serotoninergic mechanisms participate in the control of emotion-driven neuronal networks.
Keywords: Cannabinoid receptor agonist, 5-HT1A receptor agonist, fluoxetine, anxiety, emotional behaviour

P1.054

The 5-HT$_{1A}$ receptor is targeted into the dendrites by an original intracellular pathway

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The 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) is a G Protein-Coupled Receptor, localized at the plasma membrane of somas and dendrites of well identified neurons in the brain where it plays an important role in the control of their firing rate. Previous studies identified Yif1B as a membrane-bound protein whose interaction with the 5-HT$_{1A}$R C-tail is required for the distal dendritic targeting of this receptor in brain neurons (Carrel *et al.*, 2008).
In the current study, surface plasmon resonance (SPR) experiments showed that the interaction between Yif1B and the 5-HT$_{1A}$R is direct and characterized by a high affinity (KD≈40nM). By combining site directed mutagenesis, GST Pull down and SPR, we identified the specific amino acid residues implicated in the 5-HT$_{1A}$R/Yif1B binding.
Validated proteomic approaches have allowed us to identify three new proteins interacting with the 5-HT$_{1A}$R C-tail in the rat brain: tubulin, a Yip member and a Rab protein, all being involved in intracellular traffic. Interestingly, the mutated form of 5-HT$_{1A}$R which lost the capacity to bind Yif1B, interacted with none of these three new partners. Further SPR analyses demonstrated direct interaction between Yif1B and the Yip member (KD≈1µM) or the Rab protein (KD≈0.3µM), strongly suggesting that Yif1B could play the role of a scaffold protein between 5-HT$_{1A}$R and the other partner proteins.

Furthermore, we investigated the implication of these interactions in the 5-HT_{1A}R traffic in hippocampal neurons in primary cultures. Immunofluorescence experiments showed co-localization of the receptor and its different partners in somatic and dendritic vesicles. Treatment with nocodazole, a drug depolymerizing the microtubules, disturbed the subcellular distribution of both the receptor and its partners. Moreover, as previously found for Yif1B, siRNA-mediated inhibition of endogenous expression of either the Yip member or the Rab protein prevented the distal dendritic localization of the 5-HT_{1A}R.
Finally, live imaging experiments showed vesicles containing the 5-HT_{1A}R and/or the partner proteins transported from the soma to the dendrites.
Altogether, these results demonstrate the crucial role of the Yip member and the Rab protein in the distal dendritic targeting of the 5-HT_{1A}R along the microtubule network.

P1.055

Differential distribution of Nav channel beta subunits in axon initial segments and nodes of Ranvier in the adult mouse

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Axon initial segments (AISs) and nodes of Ranvier are characterized by a high density of voltage-gated sodium (Nav) channels, essential respectively for the generation and propagation of action potentials. Nine genes encoding the pore-forming alpha subunits exist, three of them (Nav1.1, Nav1.2 and Nav1.6) are expressed in the adult central nervous system. Recently, it was demonstrated that AISs can be divided into sub-compartments, expressing different Nav alpha subunits: Nav1.6 in the distal AIS and Nav1.1 or Nav1.2 in the proximal AIS. Nodes of Ranvier have also been shown to be heterogeneous, expressing Nav1.6 and/or Nav1.1. Given the biophysical property differences between Nav alpha subunits, this AIS subdivision and this nodal heterogeneity likely play important roles in action potential initiation and propagation properties, respectively. Yet, the mechanisms responsible for segregating Nav alpha subunits into different nodes or AIS sub-compartments are so far unknown. These must imply another binding partner than Ankyrin G, the major Nav-anchoring protein, since Ankyrin G is expressed homogeneously in all AISs and nodes of Ranvier.
Nav alpha subunits are associated with one or two auxiliary beta subunits, encoded by four different genes (beta 1-4). These beta subunits have been shown to regulate alpha subunit cell surface expression, possibly through their ability to interact with cell adhesion molecules present in AISs and nodes of Ranvier, as well as components of the extracellular matrix. In addition, beta subunits are able to modulate the biophysical properties of alpha subunits and have been suggested to contribute to the generation of persistent and resurgent currents. Nav beta subunits are thus good candidates that could contribute to the differential distribution and function of alpha subunits among AISs and nodes of Ranvier. However, given the difficulty to obtain reliable antibodies against the different beta subunits, almost nothing is known about their distribution in AISs and nodes. We thus investigated the distribution of Nav beta 1 to 4 in the adult mouse central nervous system, with a particular focus on the AIS and nodes of Ranvier of specific neuronal populations.

P1.056

Ephrin-B1 controls non-spatial memory via its role on radial glia morphology

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Eph receptors and ephrins have been widely implicated in brain development and plasticity yet we are only beginning to understand their role in cognition. EfnB1 is an X-linked gene whose mutation leads to CranioFrontoNasal Syndrome in humans. Herein, we show that EfnB1 mutant mice present a deficit in non-spatial learning and memory but have intact spatial learning and memory. Investigation of the adult EfnB1 mutant mouse cortex revealed a disorganization of cortical lamina and an increased neuronal density reminiscent of neurodevelopmental disorders. Accordingly, we demonstrate that the lamination defect observed in EfnB1 mutants stems from changes in radial glial cell morphology during development which impairs neuronal migration. We further show that ephrinB1 cell-autonomously controls radial glial cell morphology by regulating ARF6 activity. Our results provide a mechanistic link between cognition deficits and malformation of the radial glia scaffold and suggest that CranioFrontonasal Syndrome may be a neurodevelopmental disorder.

P1.057

The three cellular partners of the neuromuscular junction are affected in the *med* mutant

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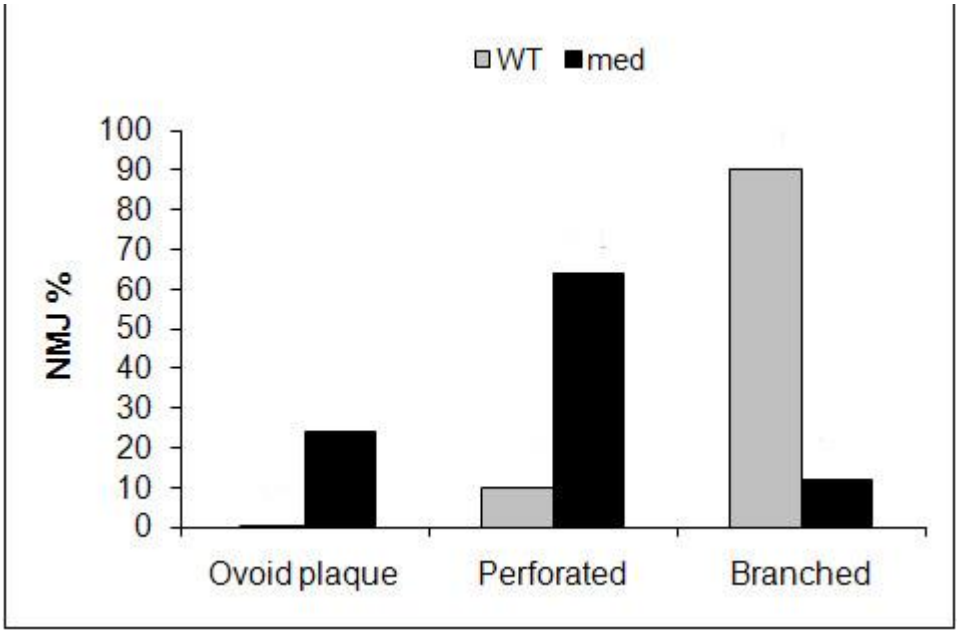
Med (motor endplate disease) mice display a functional neuromuscular denervation. They are voltage-gated sodium channel Nav1.6 null mutants, affected by a progressive paralysis and a juvenal lethality. We previously observed (Musarella et al., 2006)) that in wild-type (WT) mice, Nav1.6 is not only expressed at nodes of Ranvier but also in terminal Schwann cells (TSCs), the glial cells that wrap around the nerve terminal at the neuromuscular junction (NMJ). Moreover, the TSC number per NMJ is dramatically decreased at terminal stage of *med* pathology.
In this study we show by confocal microscopy, 3-D reconstruction and quantification how the postnatal development of the three partners of the NMJ is affected in the *med* phenotype : the process of synapse elimination is impeded (Table), the maturation of the post-synaptic structure stopped (Fig) and TSC apoptosis induced from the onset of the phenotype.
These results suggest that the *med* mice could be a pathology of the TSCs and more largely of the NMJ development.

NMJs identified by α–bungarotoxin staining		*WT*		*med*	
		number	*%*	*number*	*%*
Neurofilament co-staining	Multi-innervation	8/196	4	46/326	16
	Mono-innervation	188/196	96	280/326	84

[Table:The difference in multi-to mono-innervation]

Table:The difference in multi-to mono-innervation between *med* and WT mice at postnatal (P) day 19 is significant



[Fig: Percentage of immature (ovoid plaque + perfor]

Fig: Percentage of immature (ovoid plaque + perforated types) and mature (branched type) motor endplates from P19 muscles.
Grant sponsor : Association Française contre les Myopathies (AFM), n° 11352

P1.058

A vimentin-Tubulin Binding Site peptide (Vim-TBS.58-81) is internalized by human glioma cells and can deliver a functional pro-apoptogenic peptide cargo

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Cell Penetrating Peptides (CPP) are promising as vectors to deliver molecules into different cell compartments. One such peptide, Tat.48-60, is derived from the HIV-1 Tat protein. It translocates through the plasma membrane via an energy-dependant process and in several cell lines it subsequently accumulates in nuclei. Several CPPs including Penetratin from Antennapedia (Derossi et al., 1994), VP22 from Herpes Simplex Virus (Elliott and Ohare, 1997), or chimeric CPP like polyarginine (Futaki et al., 2001) have been described. Amongst these vectors, several have been used successfully to deliver various products including oligonucleotides (Morris et al., 1997), liposomes (Torchilin et al., 2003) and proteins (Snyder et al., 2004) to different cellular compartments.
We recently showed that intermediate filaments serve as a reservoir of unpolymerized tubulin. By peptide-array analysis we identified their tubulin binding sequences that we named Tubulin-Binding Sites (TBS). We next showed that peptides corresponding to these sequences affect the *in vitro* polymerization of tubulin and that some are internalized by cells where they can inhibit microtubule assembly and cell division (Bocquet et al., 2009).
Here we describe further properties of one of such peptide, Vim-TBS.58-81, derived from the intermediate filament protein, vimentin. We show that it enters T98G human glioblastoma cells via an energy dependent endocytosis pathway but, unlike TBS from neurofilaments, it does not disrupt their microtubule cytoskeleton. When internalized, it distributes throughout the cytoplasm but progressively accumulates in the nucleus. Further, when coupled to the pro-apoptogenic P10 peptide, it maintains its capacity to be internalized and translocate to the nucleus leading to a sharp limitation in the capacity of the T98G cell population to expand. In contrast, a Tat.48-60-P10 fusion peptide, while

internalized, has no similar effect. These observations demonstrate that Vim-TBS.58-81 can serve as a novel vector capable of delivering functional cargos to the cytoplasmic and nuclear compartments of T98G glioblastoma cells.
This work was supported by ARC and AFM to J. Eyer.

P1.059

Physiological spontaneous calcium activity in SNr astrocytes: autonomous and evoked parts

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Basal ganglia, a group of interconnected subcortical nuclei, are involved in learning of contextual cognitive and motor sequences related to environmental stimuli. The final stage of information processing within basal ganglia takes place in the substantia nigra pars reticulata (SNr) that may be considered as a gate in the transmission of information to the motor and cognitive systems. The neuronal network and signal processing of the basal ganglia and particularly the SNr are relatively well known but the role of glial cells has never been studied. We showed that astrocytes represent the main cellular population within SNr and we wonder about their influence on neuronal transmission. Using sagital brain slices containing the subthalamo-pallido-nigral loop, we showed that 30% of SNr astrocytes elicit spontaneous calcium activities in basal condition. Half part of this activity is autonomous (*i.e.* independent on any synaptic activity) while the other half is dependent on spontaneous glutamate and GABA releases probably under the control of the subthalamo-pallido-nigral loop pace-maker activity. Modification of the activity of the loop (through STN-HFS for example) impacted this astrocytic calcium activity. These data potentially reflects a role for astrocytes in modulating the SNr output activity involving probably a bilateral communication between astrocytes and neurons.

P1.060

Mechanisms of Golgi fragmentation in progressive motor neuronopathy (*pmn*) mice

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Pathology of the Golgi apparatus represents one of the earliest features of degenerating motor neurons in amyotrophic lateral sclerosis (ALS) but its mechanisms remain so far unclear. To address this issue, we investigated *pmn* (progressive motor neuronopathy) mice mutated in TBCE, a tubulin-specific chaperone protein expressed at the Golgi apparatus. Here we show that lumbar motor neurons of *pmn* mice displayed progressive Golgi fragmentation and atrophy characterized by transformation of stacked Golgi cisternae into small vesicles and massive accumulation of Golgi-specific vesicular v-SNARE proteins. These Golgi abnormalities were completely rescued by transgenic complementation with wild-type TBCE but not induced by sciatic nerve axotomy indicating defective TBCE expression as their specific cause. In vitro, TBCE-depletion caused Golgi abnormalities through decreased microtubule polymerization at Golgi membranes and ensuing defects in vesicle transport and v-SNARE-mediated vesicle docking and fusion. To our knowledge these findings provide the first mechanistic explanation for Golgi pathology in motor neuron disease.

P1.061

GSK3β negatively regulate axonal branching via MAP1B phosphorylation during axonal regeneration

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A fundamental question in studies on axonal regeneration concerns the molecular mechanisms that guide axons to their appropriate synaptic targets after a nervous system lesion. Thus, integration of environmental signals by regenerating axons leads to morphological and functional changes, highly depending on a reorganization of the cytoskeleton. In this context, we focused on MAP1B (Microtubule-Associated Protein 1B), for which have demonstrated a role in guidance, branching and retraction of regenerating axons had been demonstrated. These functions are regulated by phosphorylation. However, the specific signaling pathways underlying MAP1B phosphorylation during axonal regeneration are not yet fully elucidated. We have recently demonstrated that signaling by individual JNKs is differentially implicated in axon regeneration and JNK1 and JNK2 regulate MAP1B phosphorylation state. Moreover, lack of MAP1B prevents neurite retraction induced by JNK inhibition (Barnat et al. 2010). Here we have pursued our study by investigating the role of GSK3ß (Glycogen-synthase kinase 3ß) in MAP1B phosphorylation and axonal regeneration from adult DRG neurons, combining pharmacological GSK3ß inhibition (by SB216763) and use of MAP1B deficient mice. We demonstrated that GSK3ß regulates neurite branching through MAP1B phosphorylation. The function of MAP1B phosphorylation by GSK3ß in the regulation of microtubule (MT) dynamics was then analyzed in the cell line COS7. We showed that MAP1B expression in these cells leads to increased MT maturation, while MAP1B-P preferentially preserves labile MTs from drug-induced depolymerisation. Taken together, our data demonstrate the involvement of GSK3ß in controlling cytoskeleton reorganization, notably by regulating the function of MAP1B. Our study therefore provides new insight into how extracellular cues regulate branching of neurites through orchestrated rearrangement of the cytoskeleton, triggered by specific intracellular signaling cascades.

P1.062

Neuronal mitochondrial dynamics and oxidative metabolism

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In the past few years, multiple findings have suggested that disruptions of mitochondrial functions and dynamics contribute to neurodegenerative diseases. Mitochondrial functions in neurons include regulation of calcium and redox signaling, developmental and synaptic plasticity and the arbitration of cell survival and death. Mitochondrial dynamics controls the organelle's morphology *via* a delicate balance of two opposing forces: mitochondrial fusion and fission that are regulated by large dynamin-related GTPases evolutionary conserved from yeast to human. We have previously shown that the fusion protein OPA1 loss or mutations led to mitochondrial inner membrane dysfunctions and apoptosis of particular importance in optic nerve pathologies like ADOA1 (autosomal dominant optic atrophy). While a link emerges between defects in mitochondrial fusion and neurodegeneration, the processes involved are still largely unknown.

To understand the mechanisms by which alterations of mitochondrial dynamics could contribute to mitochondria dysfunction, eventually leading to neurodegeneration, we undertook OPA1 loss of function experiments in neurons *ex vivo*. In rat cortical neurons in primary culture, RNA interference of the fusion protein OPA1 led to mitochondrial fragmentation without altering neither mitochondrial distribution nor neuronal death. While there was no incidence on dendrites and axon size, synaptophysin protein quantity was reduced. In these conditions, the redox state of OPA1 depleted-neurons was impaired and specific respiratory complex proteins quantities were decreased. Therefore, our data demonstrate a link between mitochondrial dynamics and oxidative metabolism in neurons that may influence neuronal physiology, further stressing the necessity of mitochondrial morphology integrity for proper oxidative metabolism.
In conclusion, our data may offer new insights not only into mitochondrial dynamics-linked neurodegenerative diseases like ADOA1 but to other neurodegenerative pathologies correlated with oxidative metabolism including Huntington's, Parkinson's and Alzheimer's diseases.

P1.063

Unveiling an exceptional zymogen: the single-chain form of tPA is a selective activator of nmdar signaling

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Objectives: Tissue plasminogen activator (tPA) is an ubiquitous and exceptional serine protease in that it displays low zymogenicity. Indeed, the single-chain tPA (sc-tPA) and two-chain tPA (tc-tPA) display similar activity in the presence of blood templates such as fibrin. Interestingly, tPA was also highlighted in the brain to control neuronal migration, synaptic plasticity, learning and memory, through modulation of the N-methyl-D-aspartate receptor (NMDAR) signaling. Up to now, no difference has been underlined between the two forms of tPA regarding its neuromodulation. In the present study, we wonder whether sc-tPA and tc-tPA behave equally toward a non-fibrin substrate, the NMDAR, in the central nervous system.
Methods: tc-tPA was prepared by plasmin treatment from the commercial preparation of sc-tPA and conditioned in 0.5M bicarbonate ammonium buffer. Both forms were tested toward their modulation of NMDA-neurotoxicity and NMDAR signaling *in vitro* on cortical neurons and *in vivo* in the striatum and the hippocampus.
Results: Sc-tPA enhances NMDAR-mediated calcium influx (+34 % vs. tc-tPA), promotes NMDA Erk(½) activation (+19 % vs. tc-tPA) and NMDA neurotoxicity in cortical neurons (+51 % vs. tc-tPA) and in the striatum (+100 % vs. tc-tPA). We demonstrated that tPA mediates NMDA neurotoxicity through a plasminogen independent mechanism that requires its proteolytic activity. In the hippocampus only the sc-tPA is able to promote NMDAR-dependent long-term potentiation (LTP) in the CA1 network (+16 % vs. control), whereas tc-tPA does not. Moreover, only the sc-tPA can reverse a mild long-term depression (LTD) into LTP (excitatory post-synaptic potential slopes increased from 86 % of the baseline to 107 % in the presence of sc-tPA vs. 81 % in the presence of tc-tPA).
Conclusions: We have demonstrated, both *in vitro* and *in vivo*, the first differential function between sc- and tc-tPA, for that sc-tPA is the selective modulator of NMDAR signaling, via its proteolytic activity and through a plasminogen-independent mechanism. This finding opens a new area of investigations into plasminogen-independent functions of tPA in the brain, including mechanisms controlling its expression, its secretion and its proteolytic processing.

P1.064

Characterization of new orthosteric agonists of subtype-4 metabotropic glutamate receptor (mGluR4) in the rodent cerebellar cortex

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mGluRs are the products of a family of eight G-protein-coupled receptor genes divided into three groups (I, II, III) according to their sequence homology, pharmacological profile and signal transduction mechanisms. The mGluR4 subtype belongs to the presynaptic group III mGluRs along with mGluR6, 7 and 8. The most compelling demonstration for a physiological function of presynaptic group III mGluRs is found at the glutamatergic synapses between parallel fibers and Purkinje cells in the rodent cerebellar cortex. At these synapses, pharmacological activation of mGluR4, the only member of the group III mGluR functional (Abitbol et al., 2008), depresses excitatory synaptic transmission. It has also recently been demonstrated that these receptors are also functional at parallel-fiber-molecular layer interneurons (Stellate cells and Basket cells) synapses (Zhang et al., 2009). L-AP4 is generally used to activate mGluR4, but this drug is a broad-spectrum group III mGluRs agonist. The purpose of this study is to characterize new potent orthosteric agonists more selective for mGluR4: LSP1-2111 and LSP4-2022 developed by Francine Acher's team. These novel pharmacological tools have been studied with conventional whole-cell patch-clamp and presynaptic calcium dynamic measurement approaches, applied to synaptic transmission in cerebellar brain slices.
This work was supported by the “Fondation pour la Recherche Médicale” (INE20050303431), by the “Association Nationale pour la Recherche” (ANR-07-NEURO-047-01), the Era-net NEURON programme (ANR-08-NEUR-006-02), the CNRS (DIRE) and by the “Ministère de l´Education Nationale, de la Recherche et de la Technologie”
Abitbol K., Acher F., Daniel H. (2008) Depression of excitatory transmission at PF-PC synapse by group III metabotropic glutamate receptors is provided exclusively by mGluR4 in the rodent cerebellar cortex. *Journal of Neurochemistry 105:2069-2079*
Zhang W., Linden D.J. (2009) Neuromodulation at Single Presynaptic Boutons of Cerebellar Parallel Fibers Is Determined by Bouton Size and Basal Action Potential-Evoked Ca Transient Amplitude. *Journal of Neuroscience 29:15586-15594*

P1.065

Rise in endogenous morphine in parkinsonism is not corrected by levodopa

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Morphine is endogenously synthesized in the central nervous system and endogenous dopamine (DA) is thought to be necessary to endogenous morphine (eM) formation. As Parkinson's disease (PD) results from the loss of DA and is associated to L-dopa-resistant pain, we wondered how eM is regulated in the untreated and L-dopa-treated parkinsonian brains.
As the cellular origin and overall distribution of eM remains however obscure in both the normal and pathological adult brain, we first characterized the eM-like-immunoreactive cell distribution in the rat

striatum and then studied the changes in the eM-like-immunoreactivity of the medium spiny neurons (MSNs) in normal, PD-like and L-dopa-treated PD-like conditions in experimental (rat and monkey) and human PD. Our results unravel an unexpected dramatic upregulation of neuronal and glial eM-like immunoreactivity and levels in experimental and human PD, only partially normalized by L-dopa treatment.
Interpretation: Our data suggest that eM formation is more complex than originally proposed and that the parkinsonian brain experience a dramatic upregulation of eM immunoreactivity. The functional consequences of such eM upregulation are yet unknown but, based upon current knowledge of morphine signalling, we posit it is involved in fatigue, depression and pain symptoms experienced by PD patients.

P1.066
N-Methyl-D-Aspartate receptor subtypes in the cerebellar molecular layer

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The biophysical properties of NMDA receptors make them key actors in synaptic physiology. Besides their well known function as postsynaptic detectors of simultaneous pre- and postsynaptic activities, there is accumulating evidence for NMDA receptors presynaptically located.
In the cerebellar molecular layer of juvenile rats, NMDA receptors are involved in the induction of Long-Term Depression (LTD) at granule cells to Purkinje cells synapses. We had previously shown that NMDA receptors involved in LTD contain NR2A (but not NR2B) subunits and we brought together evidences for their presynaptic location on parallel fibers, the granule cell axons (Casado et al., 2002; Bidoret et al., 2009). However, this last point is still debated, and it has been proposed that NMDA receptors involved in LTD are located on molecular layer interneuron axons (Shin & Linden, 2007). The characterization of NMDA receptor subtypes expressed in the molecular layer should discriminate between these possibilities. We had previously shown by immunohistochemistry that NR2A-containing NMDA receptors are expressed on parallel fiber boutons. This is in agreement with the molecular profile of LTD. Here, we characterized the subunit composition of NMDA receptors expressed by the molecular layer interneurons. First, by the use of specific pharmacological tools discriminating between NR2A and NR2B-containing NMDA receptors (zinc and Ro256981). Second, by performing immunohistochemistry and electron microscopy, with subunit specific antibodies. This set of experiments shows that molecular layer interneurons express mainly NR2B receptors. This result discards the involvement of NMDA receptors from interneurons in LTD induction at granule cells to Purkinje cells synapses.

P1.067
Mir 125 promotes early neural specification of human pluripotent stem cells

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The role of micro-RNAs as coordinator of stem cells fate decision has emerged during the last decade. Here, we have used human embryonic stem cells to identify micro-RNAs involved in their commitment toward the neural lineage. Several candidate micro-RNAs shown to be expressed in the foetal brain where activated as the neural differentiation proceeded, but only the two isoforms of miR-125 were

detected in a time window compatible with a role in neural commitment. Functional analysis using antago- or pre-miR complexes indicated that miR-125 isoforms were actively involved in the promotion of pluripotent cells conversion into SOX1 positive neural precursors. MiR-125 promotes neural conversion by avoiding the persistence of non-differentiated stem cells and repressing alternative fate choices. This is associated with the regulation by miR-125 of Smad-4, a central actor of Smad-dependant pathway and a key regulator of pluripotent stem cells lineage-commitment. Activation of miR-125 was directly responsive to the levels of Activin and BMP present in the cellular environment, placing miR-125 at the core of the mechanisms that lead to irreversible commitment of pluripotent stem cells into the neural lineage in response to external stimuli.

P1.068

Somatic depolarization enhances GABA release in cerebellar interneurons via a calcium/protein kinase C pathway

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In cortical and hippocampal neurons, tonic somatic depolarization is partially transmitted to synaptic terminals, where it enhances transmitter release. It is not known to what extent such 'analog signaling' applies to other mammalian neurons, and available evidence concerning underlying mechanisms is fragmentary and partially controversial. In this work we investigate the presence of analog signaling in molecular layer interneurons of the rat cerebellum. GABA release was estimated by measuring autoreceptor currents in single recordings, or postsynaptic currrents in paired recordings of synaptically connected neurons. We find with both assays that moderate subthreshold somatic depolarization results in enhanced GABA release. In addition, changes in the calcium concentration were investigated in the axon compartment using the calcium-sensitive dye OGB-1. Following a step somatic depolarization, the axonal calcium concentration and the GABA release probability rise with a common slow time course. However, the amount of calcium entry that is associated to one action potential is not affected. The slow increase in calcium concentration is inhibited by the P/Q calcium channel blocker w-agatoxin-IVA. The protein kinase C inhibitor Ro 31-8220 did not affect the calcium concentration changes but it blocked the increase in GABA release. EGTA was a weak blocker of analog signaling, implicating a close association of protein kinase C to the site of calcium entry. We conclude that analog signaling is prominent in cerebellar interneurons, and that it is triggered by a pathway involving activation of axonal P/Q channels, followed by calcium entry and local activation of protein kinase C.

P1.069

Regulatory effects of a novel sodium channel ancillary subunit on sodium currents are modulated by alternative splicing in the insect *Periplaneta americana*

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Insect voltage-gated sodium channel (Na_v) is formed by the association of a pore-forming subunit (α-subunit) encoded by *para*-related gene and one or several regulatory subunit(s) (β subunits). The *Drosophila melanogaster* genome contains five different genes of β-subunits, named DmTipE and DmTEH1-4, which modulate both channel expression and gating properties of Na^+ currents elicited by *para*-genes expressed in *Xenopus* oocytes (Feng *et al*, 1995, Cell; Derst *et al*, 2006, Biochem Biophys

Res Commun). Whereas in insects Na_v β-subunits contain two transmembrane segments, in mammals they contain a single transmembrane segment. These β-subunits share little homology with mammals β-subunit of BKCa channel (Derst *et al*, 2006, Biochem Biophys Res Commun). DmTipE, the first regulatory subunit highlighted, has been routinely used for heterologous expression experiments of insects Na_v channels in *Xenopus* oocytes. The aim of our study is to investigate the physiological and molecular roles of these ancillary subunits in the nervous system of *Periplaneta americana*. First, using RT-PCR, we cloned two-full length cDNA encoding PaTEH1 in the nerve chord (PaTEH1.1 and PaTEH1.2) and one full-length cDNA encoding PaTEH1 in pace-maker octopaminergic DUM neurons (for Dorsal Unpaired Median neurons). Analysis of PaTEH1 expression in various tissues showed PaTEH1 was expressed in nerve chord, head and ganglions. Then, we coexpressed $DmNa_v1.1$ (Olson *et al*, 2008, Insect Biochem Mol Biol) with PaTEH1.1 and PaTEH1.2 in *Xenopus* oocytes in order to characterize electrophysiological properties of Na_v channel. These co-expression experiments showed a strong (6-fold, $DmNa_v1.1$ + PaTEH1.1) and no ($DmNa_v1.1$ + PaTEH1.2) increase in Na^+ current amplitude in comparison to $DmNa_v1.1$ and DmTipE coexpression. In addition, we performed a detailed phylogenetic study in order to characterize the evolutionary links among auxiliary sodium channel subunits of insects. This study allowed us to know whether BKCa subunit is phylogenetically close to Na_v channel β-subunits. In conclusion, our preliminary aimed to demonstrate the importance of the nature of the ancillary subunits in the regulation of insect Na_v channels expression.

P1.070

Inhibition of autophagy induces neuroprotection in a model of excitotoxicity in newborn mouse brain

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Perinatal brain lesions have a significant socioeconomic impact and therapeutics are still limited. Prematurity is a common cause of these lesions; the main mechanism involved glutamate release and excitotoxicity through its NMDA receptors. Excitotoxicity induces neuronal death; if apoptosis and necrosis are clearly implicated in this cell death, the involvement of autophagy is still uncharacterized.Autophagy is the major cellular pathway for the recycling of long-lived proteins and defective organelles. During this housekeeping process, the intra-cytoplasmic material is wrapped by double-membranes to form autophagosomes and which subsequently fused to lysosomes for degradation. Autophagy is also an adaptative response under nutrient stress and contributes to cellular homeostasis as a result of quality control of cytoplasmic components. Autophagy is also involved in programmed cell death (autophagic cell death).The aim of this work is to characterize the apoptotic and necrotic effect of autophagy modulation under excitotoxicity condition. The study was performed on cortical slices from neonate mice (post-natal day 2) which present development similarity with premature child. The autophagic activity was assessed by immunostaining of protein LC3, a marker of this process and the apoptotic activity by immunostaining of cleaved caspase-3. Apoptosis and necrosis were respectively measured by caspase-3 activity and LDH activity. In this model, the effective concentrations of an activator of autophagy (rapamycin 200nM) and an inhibitor (3-methyladenine 30 mM) were determined.These modulators did not affect necrotic death. Rapamycin induces an increase in caspase-3 activity mainly in the neocortex, whereas 3-methyladenine decreases caspase-3 activity. Importantly, the addition of 3-methyladenine tends to increase the anti-apoptotic effect of the NMDA and allows to reverse the pro-apoptotic effect of the dizocilpine, an antagonist of NMDA receptor, without modifying its anti-necrotic effects. These results suggest that inhibition of the autophagic process could be an important target in the context of excitotoxicity in the newborn. *This work was supported by grants from the University of Rouen, Inserm, The Region of Haute-Normandie, ELA and FEDER.*

P1.071

Functional urotensin II receptors are expressed by ependymal cells in adult rat brain

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The urotensin II family is composed of two peptides, Urotensin II (UII) and Urotensin II-related peptides (URP) which exert peripheral and central effects. Both UII and URP act via a G protein-coupled receptor, the UTR protein. Using autoradiographic experiments performed on brain slices, we established the first complete distribution of ^{125}I-UII and ^{125}I-URP binding sites in the adult rat brain. In contrast to the UTR gene expression pattern (Jegou et al., 2006), it appeared that UII- and URP-binding sites have a narrow distribution, identical for the two peptides. In addition to few regions already described by others, we detected labeling in two other areas which are the sphenoid nucleus, located in the midbrain, and the wall of the fourth ventricle. Intracerebro-ventricular injection of each peptide caused c-fos activation in these two regions as revealed by immunohistochemistry experiments, showing thus the functionality of these receptors. In fish, both UII and URP were shown to be expressed by cerebrospinal fluid-contacting neurons (CSF-contacting neurons) lining the central canal of spinal cord and the fourth ventricle (Yulis and Lederis, 1988). Hence, we hypothesized that UTR, like UII and URP in fish, could be expressed in CSF-contacting neurons in rat brain. However, double-labeling performed with c-fos and NeuN antibodies ruled out this possibility. In contrast, c-fos positive cells were stained by an S-100 β antibody, a marker of ependymal cells, and also by vimentin and nestin antibodies. In conclusion, we provide evidence for the existence of two additional regions containing functional UII and URP receptors in rat brain. The presence of UTR in a subpopulation of ependymal cells suggests a distant action of these peptides in the brain, *via* the CSF.

P1.072

Striatal integration of dopamine and glutamate signals by MAPKs in response to cocaine

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Long term behavioural adaptations elicited by cocaine are dependant on the activation of Extracellular Regulated Protein Kinase (ERK). Cocaine-induced ERK activation occurs in Dopamine Receptor 1 (D1R)-expressing medium spiny neurons of the striatum and requires the stimulation of both the D1R and glutamate NMDAR. We recently showed that integration of dopamine and glutamate signals onto ERK involves a D1R-mediated potentiation of calcium influx through NR2B-containing NMDAR, which triggers cocaine-induced ERK activation. Beside this signalling crosstalk to activate ERK, D1R forms a heteromeric complex with the NR1 subunit of NMDAR in the striatal post-synaptic density (PSD) through direct c-terminal interactions. D1R/NR1 heterodimers are constitutive, sequester both receptors at the membrane and cause a mutual potentiation of signalling cascades. Their association is down regulated by the presence of PSD- 95, a scaffold protein enriched at the synapse that is down regulated by chronic cocaine treatment. In the current work we study the role D1R/NR1 heterodimers in signalling pathways converging onto ERK and behavioural adaptations induced by cocaine. We designed TAT-coupled interfering peptides to perturb the receptor association. These peptides correspond to C-terminus end of D1R or NR1. We first analysed their effects *in vitro* on primary striatal culture in a model ERK activation induced by a co-stimulation of D1R and NMDAR, which mimics cocaine-induced ERK activation *in vivo*. We found that a peptide encoding the NR1 C-terminal domain (NR1cT) significantly blocks the activation of ERK induced by a low dose of glutamate together with a D1R agonist. Blockade of ERK phosphorylation occurs with a low dose of NR1cT peptide (5μM),

which does not interfere with ERK activation induced by D1R-independent stimulations. We also show that disrupting D1R:NR1 association blocks D1R-mediated potentiation of Ca2+ influx through NMDAR, responsible for ERK activation. We are currently investigating the impact of D1R/NMDAR heterodimers on striatal signalling and behaviour in mice treated acutely or chronically with cocaine. Overall, this NR1cT peptide provides a specific tool to further elucidate the underlying mechanisms of cocaine induced changes in synaptic plasticity.

P1.073

Interactions between dopaminergic and glutamatergic networks in oxidative stress vulnerability

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Environmental oxidative stress generators like the pesticides paraquat (PQ) and rotenone are suspected to be potential factors of dopaminergic neurodegeneration in Parkinson's disease (PD). The mechanisms of action of PQ at the cellular level are not well known. In *Drosophila*, previous evidence suggested that dopamine (DA) level modulates resistance against PQ poisoning. We found that increasing DA biosynthesis in CNS neurons during fly development is protective against PQ toxicity. This appears to be linked to the down-regulation of a D1-like dopaminergic receptor. Targeted inactivation of this receptor in glutamatergic motor neurons, but not in other neuronal subtypes, increases resistance of *Drosophila* under PQ-mediated oxidative stress. These results suggest that responses to PQ toxicity involve interactions between dopaminergic and glutamatergic systems in the *Drosophila* CNS. Comparable cellular interactions could be implicated in the compensatory reactions to pathological factors in PD.

P1.074

Reelin controls progenitor cell migration in the healthy and pathological adult brain

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Neural progenitor cell migration in the adult brain is a restricted event, however it is involved in crucial physiological and pathological processes. Therefore, understanding the signals that control migration is a major challenge since it may open up new therapeutic perspectives. We have uncovered new functions of Reelin, a factor responsible for correct cell positioning during brain development, in neural stem/progenitor cell migration in the healthy and pathological adult brain. First, we show that Reelin is upregulated in the injured brain around ischemic and demyelinated lesions. Second, experimentally increasing Reelin expression levels in healthy mouse brain leads to a change in the migratory behavior of subventricular zone-derived progenitors, triggering them to leave the rostral migratory stream to which they are normally restricted during their migration to the olfactory bulb. Third, both our *in vivo* and *in vitro* data show that Reelin increases endogenous neural stem progenitor cell dispersal in periventricular white matter fiber tracts independently of any chemoattraction but via cell detachment and chemokinetic action, and thereby potentiates spontaneous cell recruitment to demyelination lesion sites. Conversely, animals lacking Reelin signaling exhibit reduced endogenous

progenitor recruitment at the lesion site. Altogether, these results reveal that beyond its known role during brain development, Reelin is a key player in post-lesional cell migration in the adult brain. Finally our findings provide proof of concept that allowing cells to escape from the RMS may provide a potential therapeutic approach to promote myelin repair.

P1.075

Modulation of T-type / Cav3 calcium channels by endogenous signalling lipids

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T-type calcium channels (T-channels) have important roles in cell excitability and calcium signalling, contributing to a wide variety of physiological functions. Despite their importance in neurophysiology, their regulation by neurotransmitters and hormones only begins to emerge. We report here that many endogenous lipids are direct inhibitors of T-currents, including polyunsaturated fatty acids, endocannabinoids and lipoamino acids. We also investigated the properties of lipoxygenase, cyclooxygenase and P450 products, and identified 5,6 EET as a potent T-channel blocker. Using radioactive ligand for T-channels, we identified a putative binding site on the Cav3 protein for these lipids, which could mediate their effects. Collectively, our data reveal T-channels as the target of many signalling molecules, suggesting multiple roles in cell functions and diseases.

P1.076

Vasopressin induces changes in synaptic activity in the hippocampus

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Previously, vasopressin (VP) was only known as a hormone. Subsequently, VP has been identified as a neurotransmitter, released in the central nervous system and involved the regulation of social behavior. The hippocampus represents a major experimental system for studying synaptic plasticity in the context of information-storage mechanisms. This plasticity is thought to rely on long-term potentiation (LTP) of excitatory glutamatergic synaptic transmission. Here, we have studied pyramidal cells in the CA2 area, a region of the hippocampus where it has recently been demonstrated that VP_{1b}-R, one of VP receptors subtypes, is highly expressed. In order to determine the influence of VP on synaptic transmission, we have stimulated entorhinal cortex (EC) fibres and recorded postsynaptic potentials from CA2 neurons, in acute sagittal mice brain slices. In most cells but not all, high-frequency stimulation (HFS) triggered a LTP. VP (10^{-8} M) decreased transiently the amplitude of the excitatory post synaptic potential (EPSP) without modifying the inhibitory post synaptic potential (IPSP). Interestingly, this decrease occurred only after effective LTP-induction. By contrast, VP had no effect in pyramidal CA2 cells which either were not subjected to HFS or did not express LTP in response to HFS. Altogether, these data provide the first demonstration of the role of vasopressin in the modulation of excitatory cortico-CA2 synapses, a circuit recently characterized (Chevaleyre and Siegelbaum, 2010; Neuron 66, 560-572) and supposed to play a role in the synchronisation and modulation of electrical activities throughout the hippocampus. This modulation may support some of the new roles of VP in the regulation of social behaviour. Supported by l'Agence Nationale de la Recherche.

P1.077

Optogenetic stimulation of Purkinje cells *in vitro* and *in vivo* on a new strain of transgenic mice

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The cerebellar system is organized in a series of parallel anatomical modules which govern its functional interactions with the rest of the sensori-motor system. However, very little effort has been made so far to study coordination of individual cerebellar modules. We set out to develop a strain of mice in which optogenetic methods would allow a selective stimulation of Purkinje cells, the sole output of modules of the cerebellar cortex. Because viral infection of Purkinje cells is hardly possible, we developed a new strain of transgenic mice carrying a ChR2-YFP cassette. This genetically-targeted optical stimulation technique will be used to modulate the patterns of activity in the cerebellar microcircuits and evaluate the effects of these precise spatio-temporal alterations on cerebellar output channels during simple motor tasks.
A BAC driver containing the L7 gene was used for Purkinje cell specific expression of the ChR2-YFP or ChR2-mCherry cassettes. For the ChR2-mCherry construct, we have obtained one founder line in which the transgene was specifically expressed in 50% Purkinje cells. However, immunohistochemistry revealed a high level of protein localization in endoplasmic reticulum and Golgi compartments leading to poor expression at the membrane as previously shown with other constructs containing mCherry. Finally, photocurrent recorded in positive Purkinje cells of acute cerebellar slices was modest, although action potential could be elicited in cell-attached mode.
For the second cassette - L7-ChR2-YFP - one founder line gave a high expression level of ChR2 in almost 100% of Purkinje cells and no evidence of protein retention in the endoplasmic reticulum. We have tested the effect of optical stimulation both *in vitro* and *in vivo*. We demonstrate that photocurrent of several nanoamps can be elicited in whole cell mode by using simple blue LED. Flashes of 2 ms length elicit action potential both in cell-attached and current-clamp mode. We show that this strain is suitable for a selective stimulation of individual Purkinje cells both *in vitro* and *in vivo*.

P1.078

Cellular distribution and subcellular localization of spatacsin and spastizin, proteins involved in hereditary spastic paraplegia

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The hereditary spastic paraplegias (HSP) are clinically and genetically heterogeneous inherited neurological disorders mainly characterized by progressive spasticity. Neuropathological studies have shown retrograde degeneration (dying back) in the long descending and ascending tracts of the spinal cord, particularly the pyramidal tract and the dorsal columns, especially in their terminal portions. The symptoms can be associated, in complicated forms of HSP, with various neurological and extraneurological signs. More than 46 genetic loci (SPG) have been identified, but only 21 genes have

been cloned. The putative roles of the encoded proteins suggest that mitochondrial function, protein folding and intracellular trafficking are implicated in the dying back of pyramidal tract axons in these disorders.
A common form of autosomal recessive HSP associates spastic paraplegia, mental retardation or cognitive deficits and thin *corpus callosum.* Most families (~60-80%) are linked to SPG11, while ~11% of the families are linked to SPG15. *SPG11* and *SPG15* encode spatacsin and spastizin, respectively, two proteins of unknown function that are present in a same protein complex. So far, all but one of the mutations found in SPG11 and SPG15 patients results in abnormally truncated proteins, suggesting loss of function. We explored the intracellular and tissue localisations of these proteins with specific polyclonal anti-spatacsin *(SPG11)* and anti-spastizin *(SPG15)* antisera that we developed. We observed expression of both proteins in human and rat central nervous system, particularly strong in cortical and spinal motor neurons as well as in retina. Both proteins were also expressed ubiquitously and strongly in embryos. In cultured cells, these two proteins had similar diffuse punctate cytoplasmic distributions. They partially co-localized with multiple organelles, particularly with protein-trafficking vesicles, endoplasmic reticulum, microtubules and mitochondria. This first study of the endogenous expression of spatacsin and spastizin shows similarities in their expression patterns that could account for their overlapping clinical phenotypes and cooperation into a common protein complex.

P1.079

Development of peptide-vectors for CNS drug delivery across the blood brain barrier

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The blood-brain barrier (BBB) is a major obstacle in the treatment of diseases of the CNS because it prevents the entry of many drugs in the brain. Some receptors expressed at the BBB are able to perform transcytosis, i.e., to bring their natural ligand from the apical pole (lumen side of the blood vessel) to the basal pole (nervous parenchyma side) of endothelial cells. We have set up a technological platform to identify molecules that bind some of these receptors and to develop these peptides vectors as peptide-vectors that cross the BBB. Screening complex peptide libraries on cloned BBB receptors enabled us to characterize a family of peptides capable of carrying loads (S-Tag peptide, antibody) within cells and across the BBB *in vitro* and *in vivo*. Chemical optimization (truncation, Ala-scan, D-scan, etc.) of our peptide-vectors was undertaken to reduce their size, to improve receptor affinity, plasma stability and bioavailability. The most successful peptide-vector was coupled via different linkers to an analgesic peptide that does not pass the BBB. After radiolabelling and *in situ* brain perfusion experiments, we obtained our first *in vivo* proof of concept on the relevance of vectorization via BBB drug targeting peptide-vectors.

P1.080

TWEAK modulates blood brain barrier properties during central nervous system inflammation

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TWEAK (TNF weakly inducer of apoptosis) is a type II-transmembrane protein, member of the TNF ligand superfamily that can be cleaved to function as a soluble cytokine. TWEAK triggers multiple cellular responses depending on cellular type and biological context when it binds its main receptor, Fn 14, a TNF receptor superfamily member. The importance of TWEAK in CNS pathologies is evidenced by data proving that blocking the effects of TWEAK with blocking antibodies or Fn14 soluble decoy receptors is efficient in animal models. We have recently shown that circulating monocytes express membrane TWEAK during multiple sclerosis, suggesting that TWEAK could

contribute to blood brain barrier (BBB) leakage and monocyte diapedesis. In order to further characterize the mechanisms modulated by TWEAK in the BBB, we proposed to evaluate the consequences of TWEAK exposure on the expression of cell adhesion, tight junction molecules and metalloproteinases by brain microvascular endothelial cells (BMECs) forming an *in vitro* model of the BBB. We have chosen to work on hMEC/D3, a human BMEC line established by P.O. Couraud (Institut cochin, Paris). We have shown that hMEC/D3 cells do not express membrane or soluble TWEAK but constitutively expressed Fn14 on their surface and that TWEAK/Fn14 stimulation up-regulated pro-inflammatory cytokines in these cells. TWEAK stimulation increased ICAM-1 but not E-selectin membrane levels on hcMEC/D3. We observed that TWEAK exposure during 24h led to a significant increase of the BBB permeability assessed by passage of Lucifer yellow. In this context, we found that TWEAK reduced significantly the levels of ZO-1, a tight junction marker in hcMEC/D3. Finally, we observed an increased gelatinolytic activity in cerebral endothelial cells after TWEAK exposure associated with increased cellular MMP-9. Our data support the contention that TWEAK plays a role in BBB demise. We are now exploring whether other proteinases are involved in the up-regulation of BBB proteolytic activity. We are also characterizing the transcriptome of hMEC/D3 stimulated by TWEAK, compared to cells exposed to rhTNF, to assess whether the biological effects elicited by TWEAK involve signal transduction pathways distinct from those activated by TNF.

P1.081

Central effects of spadin on NTSR3 and TREK-1 trafficking and on brain neurogenesis

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Spadin, a peptide corresponding to Ala12-Arg28 of the propeptide released from the maturation of the neurotensin (NT) receptor-3 (NTSR3)/sortilin displays strong antidepressant (AD) properties when injected in mice (Mazella et al, PLoS Biol 2010). Its AD actions are the consequence of its ability to block the electrical activity of the two pores potassium channel TREK-1. Preliminary experiments showed that 125-radiolabeled-spadin specifically binds to TREK-1 and triggers its internalization. The objectives were
1) to study the cellular trafficking of TREK-1 and NTSR3, also recognized by spadin, upon their activation by the peptide and
2) to examine the effects of spadin on neuronal markers of neurogenesis.
We first observed that spadin incubation on neurones from WT and TREK-1 KO mice induced the phosphorylation of Akt but with different kinetics of activation indicating that both NTSR3 and TREK-1 contribute to this stimulation. By using biotinylated cell surface proteins and immunoprecipitation, we demonstrated that although spadin induced the sequestration of TREK-1 and NTSR3, the amount of both proteins at the plasma membrane was increased following incubation with 0.1 µM spadin. This indicates the presence of spare receptors and channels that are adressed to the cell surface when the process of internalization is activated.
This regulatory mechanism of the expression of TREK-1 at the neuronal cell surface is crucial to understand how to inhibit TREK-1 activity, a process responsible for the depression state.
In the second part of the work, we observed that spadin is able to regulate the expression of Psd95 and synapsin, two markers of synaptogenesis. These results will be discussed in relation to the role of spadin in neurogenesis in mice.

P1.082

Cocaine activates salt-inducible kinase 1, as attested by TORC1/3 shuttling, and induces MEF2C transcription factor in rat striatum

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Distinct forms of MEF2 transcription factor act as positive or negative regulators of dendritic spine formation, with MEF2C playing a key regulator role in synapse plasticity. We report here a novel

transduction pathway whereby acute or chronic cocaine treatment induced the expression of MEF2C in rat striatum. The mechanism by which MEF2C was induced involves the subsequent activation of the salt-inducible kinase SIK1 and the phosphorylation of HDAC5, a member of the class IIa of HDACs. Cocaine activated SIK1 by phosphorylation on Thr-182 residue, which was accompanied by the nuclear import of the kinase. In the nuclear compartment, SIK1 then phosphorylated HDAC5 causing the shuttling of its phospho-form from the nucleus to the cytoplasm of striatal neurons. Activation of SIK1 by cocaine was further validated by the phosphorylation of TORC1/3, which was followed by the shuttling of TORC proteins from the nucleus to the cytoplasm. Activation of MEF2C was assessed by measuring the expression of the *MEF2C* gene itself, since the gene is known to be under the control of its product. Given the importance of MEF2C in memory/learning processes, this pathway is probably involved in long-term plasticity mechanisms triggered by cocaine, some of them leading to drug dependence.

P1.083

Systematic mapping of integral membrane protein interactions using the membrane split-ubiquitin system (M-SUS)

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Integral membrane proteins and membrane-associated proteins represent approximately 30% of the entire human proteome and constitute important drug targets. Yet, very little information is available about interactions involving membrane proteins, mainly due to a lack of suitable methods. Here, we present a simple and powerful method to screen membrane proteins for interaction partners, the membrane split-ubiquitin system (M-SUS). M-SUS is a yeast-based genetic selection system which identifies protein interactions directly at the membrane. Full-length integral membrane proteins are fused to two small reporter modules. An interaction between the two proteins results in reporter module reconstitution and subsequent transcriptional activation of genomic reporter genes. Thus, a protein interaction at the membrane is converted into a genetic readout, selecting all cells expressing an interacting protein pair. Substitution of one defined partner by a pool of candidates expressed from a cDNA library of choice allows simultaneous screening of millions of potential interactors and the discovery of novel interaction partners for a membrane protein of interest.
We demonstrate the versatility of the system by mapping the subunit topology of the heterotrimeric sodium channel ENaC and by screening the beta-ENaC subunit against a human lung cDNA library to identify novel interaction partners. Follow-up assays verify the identified interactors and also demonstrate a role for several interactors in regulating ENaC activity.
In summary, M-SUS is a powerful tool to map protein interactions involving integral membrane proteins and can also be used at high throughput to systematically map interactions of different membrane protein classes, such as GPCRs, RTKs or ion channels.

P1.084

Allosteric mechanisms in $mGlu_2$: a time-resolved FRET study

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Allostery is central to the activation mechanism of neurotransmitter receptors and to their physiological regulation. Moreover, it offers the opportunity for the development of modulators that enhance or attenuate the response of a specific receptor to its natural agonist. For example, metabotropic glutamate receptor subtype 2 ($mGlu_2$) is under intense investigation to find allosteric enhancers with antipsychotic activity.

As all 8 mGlu receptors (mGluRs), $mGlu_2$ belongs to the superfamily of 7 transmembrane receptors and is a covalent homodimer. Both subunits contain an extracellular domain (ECD) responsible for glutamate and orthosteric ligand binding; and a transmembrane domain (TMD) responsible for downstream signaling and allosteric modulation. However, how these two domains communicate in a dimeric receptor is poorly understood. We thus developed a powerful FRET-based technique to monitor the conformational states of full-length $mGlu_2$ at the surface of living cells. This method combines time-resolved FRET (trFRET) and SNAP-tag technologies and is performed in microplates. While previous crystallographic studies suggested that the response to orthosteric agonists was mediated by a significant dimeric rearrangement of the ECD, there was no experimental evidence for such a conformational switch under physiological conditions. Here, we demonstrate for the first time that orthosteric agonist-induced activation is associated with a significant rearrangement of the ECD in the context of full-length $mGlu_2$. Moreover, we describe mutations that freeze the ECD in its active or resting conformation. Then, we show that positive and negative allosteric modulators, which bind into the TMD, can distantly potentiate or attenuate the effect of orthosteric agonists on the ECD conformation. Surprisingly, a mutation in the TMD that functionally uncouples $mGlu_2$ from G protein, also potentiates the effect of orthosteric agonists. Finally, we evaluate how the G protein state and other factors may influence the conformational switch of the ECD of $mGlu_2$.
Taken together, our results bring to light new aspects of the allosteric functioning of $mGlu_2$ and demonstrate the advantage of trFRET for drug discovery and for structure-function studies of neurotransmitter receptors.

P1.085

V-ATPase membrane sector associates with synaptobrevin to modulate neurotransmitter release

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Acidification of synaptic vesicles by the vacuolar proton ATPase (V-ATPase) is essential for loading with neurotransmitter. We identified a direct interaction between the c-subunit of the V-ATPase membrane domain V0 and the v-SNARE VAMP2. Interaction domains were mapped to the membrane-proximal domain of VAMP2 and the cytosolic 3.4 loop of c-subunit. Acute perturbation of this interaction with c-subunit 3.4 loop peptides did not affect synaptic vesicle proton pump activity but induced a substantial decrease in release probability, inhibiting glutamatergic as well as cholinergic transmission in mammalian cortical slices and cultured sympathetic neurons respectively. Our data identify a molecular link between V-ATPase and SNARE-mediated fusion and suggest that V-ATPase V0 sector ensures two independent functions: proton transport by a fully assembled V-ATPase and a role in SNARE-mediated neurotransmitter rerlease.

P1.086

Identification of the acylguanidine MRT-83 as a novel potent Smoothened antagonist

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The small-molecule inhibitor GDC-0449 targets the Smoothened (Smo) receptor and holds great promise for treating medulloblastoma driven by mutations in the Hedgehog (Hh) pathway as well as several Hh-dependent cancers (Scales and de Sauvage, 2009). However, a Smo mutation arising within the sixth putative transmembrane domain of Smo and disrupting the ability of GDC-0449 to bind

Smo, was found in the tumor of a medulloblastoma patient who had relapsed after an initial response to the drug (Rudin et al., 2009; Von Hoff et al., 2009). A Smo mutation occurring at a homologous position in mouse Smo was also observed in a GDC-0449-resistant mouse model of medulloblastoma (Yauch et al., 2009). Therefore, the development and characterization of novel Smo antagonists have valuable therapeutic interests.
Using a pharmacophoric model-based virtual screening strategy, we have recently reported the identification of the acylthiourea MRT-10 and the acylurea MRT-14 as members of novel families of Smo antagonists (Manetti et al., 2010). These new leads have rapidly allowed us to develop a new series of Smo inhibitors with high potency (Roudaut et al., in press) to which MRT-83 belongs. MRT-83 perfectly fits with the proposed pharmacophoric model for Smo antagonists that we have recently proposed. In respect to MRT-10 or MRT-14, MRT-83 is featuring the following structural differences:
(i) a guanidine is replacing the thiourea or urea functions;
(ii) a methyl residue is introduced in the central phenyl ring;
(iii) the lateral amide is formed with biphenylcarboxylic acid.
These discrete structural differences may account for the increased potency of MRT-83 observed in various *in vitro* Hh-based assays.
MRT-83 inhibits Bodipy-cyclopamine binding to human Smo and ShhN-mediated proliferation of rat granule cell proliferation with a nanomolar potency similar to GDC-0449. Our experiments demonstrate that MRT-83 did not display significant agonist or antagonist Wnt signaling activity evaluated by a Tcf/Lef-dependent luciferase assay. These data indicate that MRT-83 and its derivatives that are under development should be valuable tools for investigating the mode of action of Smo inhibitors and for characterizing Smo functions both *in vitro* and *in vivo*.

P1.087

Convulsant doses of a dopamine D1 receptor agonist result in ERK-dependent increases in Zif268 and Arc/Arg3.1 expression in mouse dentate gyrus

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Activation of dopamine D1 receptors (D1Rs) has been shown to induce epileptiform activity. In this study we have characterized the molecular changes occurring in the mouse hippocampus in response to the administration of SKF81297, a D1-like family agonist. Systemic administration of SKF81297 (5.0 mg/kg, i.p) induced behavioural seizures-like events and transient electrophysiological discharges in the dentate gyrus (DG), which are prevented by the D1-type receptor antagonist, SCH23390. The effect of SKF81297 was accompanied by increased phosphorylation of the extracellular signal-regulated protein kinases 1 and 2 (ERK) selectively observed in the granule cells layer of the DG. SKF81297-induced ERK activation was totally abolished pharmacological of genetic inactivation of D1Rs. In addition, SKF81297 increased phosphorylation of the ribosomal protein S6 and histone H3, two downstream targets of ERK. These effects were prevented by genetic inactivation of D1Rs, or by pharmacological blockade of MEK, the upstream kinase of ERK. SKF81297 was also able to induce plasticity-associated genes (Zif268 and Arc/Arg3.1) in an ERK-dependent manner. Interestingly, the CB1 receptor agonist, CP55,940, prevented acute seizures induced by SKF81297 and strongly reduced ERK activation. We hypothesized that the presence of CB1 receptors in glutamatergic hippocampal neurons could provide protection against SKF81297-induced seizure-like events. We are currently assessing the involvement of the glutamatergic transmission in the regulation of ERK induced by SKF81297.

P1.088

Parvalbumin-positive interneurons contribute to inhibitory and dis-inhibitory feedforward circuits int the lateral amygdala

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Plasticity of neuronal circuits in the amygdala is thought to play a central role in auditory fear conditioning, a simple form of associative learning. Integration and plasticity of excitatory sensory inputs onto lateral amygdala (LA) projection neurons (PNs) is critically involved in the initial stages of

associative learning. However, little is known about the local inhibitory circuits. We have investigated the functional properties of inhibitory circuits containing Parvalbumin-positive interneurons (PV+ INs), which represent 25% of GABAergic interneurons in the LA. Using multiple whole-cell recordings in brain slices obtained from mice expressing EGFP in PV+ INs, we found that the LA contains two distinct subpopulations of PV+ INs: About 80% of PV+ INs are highly connected to local PNs (>60% connectivity) and receive strong extrinsic input from cortical and thalamic afferents. This subpopulation mediates classical feedforward inhibition onto local PNs. In contrast, about 20% of PV+ INs are not connected to local PNs (but to other INs) and receive much weaker extrinsic sensory inputs. We propose that this subpopulation of PV+ INs contributes to feedforward dis-inhibition and thus likely represents an important regulatory component in the LA circuitry.

P1.089

TRPC6 channels: an unsuspected zinc entry pathway in cortical neurons?

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Recent data showed that some mammalian TRP channels (like TRPA1, TRPML1, TRPM7 or TRPV6) can function as physiological metal-conducting pathways. For instance, TRPC6, a diacylglycerol-sensitive cation channel, can favour the entry of Fe (biochem J, 2004, 378:975). This result prompted us to verify whether the over-expression of TRPC6 channels could alter the cellular content of Fe. In fact HEK-293 cells stably over-expressing TRPC6 channels have the same Fe content as HEK-293 cells. However, they are specifically enriched in Zn and S but impoverished in Cu. Experiments conducted with the specific fluorescent Zn indicator FluoZin-3 showed that the over-expression of TRPC6 channels enhances the size of the pool of chelatable Zn. Moreover, activating TRPC6 channels by means of DAG analogues triggers the entry of Zn. In addition, the over-expression of TRPC6 channels enhances the sensitivity of cells to an oxidative stress. Experiments were conducted on cortical neurons kept in primary cultures which express DAG-sensitive channels (J Neurochem, 2009 108:126). Neuronal DAG-sensitive channels permit the entry of Zn even in the presence of an exceeding concentration of Ca (2mM Ca and 2µM Zn). Using hyperforin as an activator of TRPC6, electrophysiological experiments showed that these channels can transport zinc in cortical neurons. In these cells, hyperforin-sensitive channels co-exist with voltage-gated channels, AMPA and NMDA receptors, which are all known to transport Zn. The ability of these channels to regulate the size of the mobilizable pools of Zn was compared. The main intracellular pools of Zn in neurons are metallothioneins, an important family of Zn-binding proteins which reversibly bind this metal, mitochondria and synaptic vesicles. The data collected indicate that the entry of Zn through TRPC6 channels can influence the size of the mobilizable pools of Zn without affecting the mitochondrial pool of zinc. By showing that TRPC6 channels constitute an unsuspected Zn entry pathway, our study reveals an unanticipated role of TRPC6 channels in zinc homeostasis.

P1.090

Monocyte chemoattractant protein-1 (MCP-1/CCL2) secreted by injured hippocampus is a chemotactic factor for human nasal olfactory stem cells

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Stem cell-based therapy has been proposed as a potential means of treatment for a variety of brain disorders including strokes and brain trauma. Among various candidates, we focused our attention on

adult stem cells from the human olfactory lamina propria, located in the nose. Previously, we showed that these stem cells, grafted in a rodent amnesic model, survive in injured brain areas and promote recovery of learning and memory abilities. Interestingly, olfactory stem cells migrate to lesioned hippocampi after transplantation in the cerebrospinal fluid or the blood, even when the graft is performed one month post-lesion, a delay required for raising high numbers of stem cells from a patient with acute brain injury. The aim of this study was to better understand the molecular mechanisms underlying stem cell-targeted migration towards lesioned hippocampus. For this purpose, a comparative transcriptome study of healthy and damaged hippocampi, using whole-genome mouse DNA microarray, was performed. We report that, one month post-lesion, some immune and inflammatory molecules were still over-expressed and, among these proteins, three cytokine candidates - MCP-1/CCL2, SPP1 and CXCL10 - known for their chemotactic properties, were selected. Using immunofluorescence microscopy, we observed that olfactory stem cells expressed the main receptors for these cytokines. However, *in vitro* chemotaxis assays, based on modified Boyden chambers, revealed that only human or mouse MCP-1/CCL2 induced a strong chemotactic effect on human olfactory stem cells. In further studies, we will assess the *in vivo* stem cell attractiveness of MCP-1/CCL2 by infusing this chemokine in unlesioned hippocampus.

P1.092

Manic-like response to amphetamine or sleep deprivation in rats is decreased by protein kinase C inhibition

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Protein kinase C (PKC) has emerged as a novel molecular target in neurological and psychiatric disorders. Recent clinical trials show that tamoxifen, an antiestrogenic drug which also exhibits PKC inhibitory properties, induces a significant improvement of mania in bipolar disorder patients[1,2].
In order to further define the role of PKC in the development of mania, we investigated the potential efficacy of tamoxifen and the selective PKC inhibitor chelerythrine to decrease manic-like behaviors triggered by amphetamine administration or REM-sleep deprivation, two putative animal models of the manic state of bipolar disorder.
Our results showed that amphetamine-induced hyperlocomotion was prevented by a single administration of either tamoxifen (80 mg/kg, i.p.) or chelerythrine (3 mg/kg, s.c.), as previously reported for the mood stabilizer lithium and other anti-manic agents[3,4]. We demonstrated that a 72-hour REM-sleep deprivation produced a striking enhancement of locomotor activity and an increased latency to sleep. These manic-like behaviors were reduced by lithium and aripiprazole, an atypical antipsychotic clinically efficient in acute mania. Interestingly, PKC inhibitor chelerythrine (3 mg/kg, s.c.) did not affect sleep latency but reduced the augmentation of locomotor activity induced by sleep deprivation. Our results further support a role for the PKC signaling system in the pathophysiology of the manic phase of bipolar disorder, and provide new insights on the use of PKC inhibitors as possible novel treatments for mood disorders.
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P1.093

Thalamocortical projections to the premotor cortical areas following unilateral lesion of primary motor cortex (M1) in macaque monkeys in presence or absence of anti-Nogo-A antibody treatment

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The premotor cortical area (PM) contributes to the preparation and programming of skilled movements. While the thalamocortical projections to M1, the dorsal premotor cortex (PMd) and the ventral premotor cortex (PMv) have been extensively described in intact macaques, almost nothing is known about the patterns of thalamocortical projections to PM after unilateral lesion of M1, representing an important issue as PM was shown to contribute to the incomplete functional recovery. The goal of the present study was to investigate the thalamocortical projections to PM in macaque monkeys after unilateral lesion of M1 and to assess the influence of anti-Nogo-A antibody treatment. The origin of the thalamic projections to PM was derived from injections of the retrograde neuroanatomical tracer BDA into PM in ten adult monkeys. Three monkeys were intact and seven monkeys were subjected to a unilateral cortical lesion produced by microinfusion of ibotenic acid in the hand area of M1. In the lesioned monkeys, three monkeys were treated with the anti-Nogo-A antibody and four monkeys were untreated. The anti-Nogo-A antibody treatment was delivered immediately after the lesion during 4 weeks. Following hand dexterity recovery, BDA was injected in PM on the lesioned hemisphere. The distributions of BDA-labelled neurons in the thalamus was plotted and then superimposed to photomicrographs obtained from the corresponding Nissl and/or SMI-32 stained sections. The number of retrogradely BDA-labeled thalamocortical neurons was normalized based on the volume of the BDA injection sites in PM. The normalized number of labeled thalamocortical neurons was significantly enhanced in the monkeys subjected to M1 lesion, as compared to the intact monkeys. However, there was no difference between the untreated and the anti-Nogo-A antibody treated monkeys. The distribution of labeled neurons across the thalamic nuclei of origin was comparable in the 3 subgroups of monkeys, with a predominance in the ventral thalamic nuclei, mainly VL. The present data suggest that, as a result of lesion in M1, some thalamic inputs originally sent to M1 may be redirected to PM.

P1.094

IFNγ triggers a LIGHT-dependent selective death of motoneurons contributing to the non-cell-autonomous effects of mutant SOD1

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Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease that primarily affects motoneurons in the brain and spinal cord. Dominant mutations in superoxide dismutase-1 (SOD1) cause a familial form of ALS. Mutant SOD1-damaged glial cells contribute to ALS pathogenesis by releasing neurotoxic factors, but the mechanistic basis of the motoneuron-specific elimination is poorly understood. Here, we describe a motoneuron-selective death pathway triggered by activation of Lymphotoxin-β receptor (LT-βR) by LIGHT, and operating by a novel signaling scheme. We show that astrocytes expressing mutant SOD1 mediate the selective death of motoneurons through the proinflammatory cytokine interferon-γ (IFNγ), which activates the LIGHT-LT-βR death pathway. The expression of LIGHT and LT-βR by motoneurons in vivo correlates with the preferential expression of IFNγ by motoneurons and astrocytes at disease onset and symptomatic stage in ALS mice. Importantly, the genetic ablation of Light in an ALS mouse model retards progression, but not onset, of the disease and increases lifespan. We propose that IFNγ contributes to a cross-talk between motoneurons and astrocytes causing the selective loss of some motoneurons following activation of the LIGHT-induced death pathway.

P1.095

Chronic hyperammonemia alters the circadian rhythms of corticosteroid hormone levels and of motor activityin rats

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Patients with liver cirrhosis may present hepatic encephalopathywith a wide range of neurological disturbancesand alterations in sleep quality and in the sleepwakecircadian rhythm. Hyperammonemia is a maincontributor to the neurological alterations in hepaticencephalopathy. We have assessed, in an animal modelof chronic hyperammonemia without liver failure, theeffects of hyperammonemia per se on the circadianrhythms of motor activity, temperature, and plasma levelsof adrenal corticosteroid hormones. Chronic hyperammonemiaalters the circadian rhythms of locomotoractivity and of cortisol and corticosterone levels in blood.Different types of motor activity are affected differentially.Hyperammonemia significantly alters the rhythm ofspontaneous ambulatory activity, reducing strongly ambulatorycounts and slightly average velocity during thenight (the active phase) but not during the day, resultingin altered circadian rhythms. In contrast, hyperammonemiadid not affect wheel running at all, indicating that itaffects spontaneous but not voluntary activity. Verticalactivity was affected only very slightly, indicating thathyperammonemia does not induce anxiety. Hyperammonemiaabolished completely the circadian rhythm of corticosteroidhormones in plasma, completely eliminatingthe peaks of cortisol and corticosterone present in controlrats at the start of the dark period. The data reportedshow that chronic hyperammonemia, similar to that presentin patients with liver cirrhosis, alters the circadianrhythms of corticosteroid hormones and of motor activity.This suggests that hyperammonemia would be a relevantcontributor to the alterations in corticosteroid hormonesand in circadian rhythms in patients with liver cirrhosis.
Key words: hepatic encephalopathy; body temperature;plasma corticosterone; locomotor activity; wheel running;cosinorhosis.

P1.096

Frataxin depletion in zebrafish embryo, towards a novel animal model for Friedreich ataxia

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Friedreich ataxia (FRDA), the most common form of hereditary ataxia is an autosomal recessive neurodegenerative disorder affecting the central and peripheral nervous system. FRDA patients also frequently display cardiomyopathy. FRDA is caused by a trinucleotide (GAA) repeat expansion in the first intron of the nuclear *frataxin* (*FRX*) gene leading to markedly reduced synthesis of *FRX* mRNA and thus, reduced accumulation of the encoded mitochondria targeted protein, frataxin. Although several animal models of the disease have provided insights into the physiological role of frataxin as well as its requirement for the disease process, the precise role of the protein remains largely unknown.
A faithful FRDA animal model must display reduced, albeit not full, frataxin depletion to reproduce the situation observed in patients (5 to 15% compared to wild-type levels) and avoid the embryonic lethality induced by full depletion of the protein. Here we report immunocytochemical and biochemical analyses of zebrafish embryos showing severe frataxin depletion as the result of morpholino-oligonucleotide-mediated depletion of the zebrafish *FRX* gene.

We observed that while complete, or nearly complete, frataxin depletion impairs development and embryo viability, as previously described for other frataxin deficient animal models, severe, albeit not full, frataxin inactivation affects several organs or tissues, including the ear, and of particular interest, spinal motor neuron axons, cerebellum and heart. These embryos also show motility defects, as the likely result of abnormal motorneuron axon growth and guidance and decreased number of cerebellar Purkinje and granule cells. We will also describe the mitochondrial respiration and oxydative stress response in these zebrafish embryos.

P1.097

Antiparkinsonian action of a selective group III metabotropic glutamate receptor agonist is associated with reversal of subthalamonigral overactivity

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% of the population over 50 year old. The loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) results in DA depletion at striatal projections and overactive glutamate activity in the basal ganglia (BG). Metabotropic glutamate receptors (mGluRs) are G-protein-coupled glutamate receptors that are abundantly expressed in the BG.
Group I (mGlu1/5),
group II (mGlu2/3) and
group III (mGlu4/7/8) receptors are located at key synapses of the BG and represent ideal targets to regulate abnormal glutamate and GABA transmission in PD.
We provided evidence that, in a rat model of early parkinsonism, nigrostriatal dopamine denervation produces akinetic deficits (delayed reaction times in operant responding) that can be alleviated by mGlu5 receptor blockade (Breysse et al. 2002) and mGlu4 or 7 receptor activation (Lopez et al. 2007; Greco et al. 2010). The intensity of the akinetic deficits were correlated to an increase of the neuronal metabolic activity of the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr) assessed by an increased gene expression of cytochrome oxidase subunit I (CoI) (Breysse et al. 2003, Oueslati et al. 2005). No change in the pallidal neuronal metabolic activity was found. The present results show that systemic administration over 2-3 weeks of daily treatment with MPEP, mGlu5 receptor antagonist or ACPT-1, a selective mGlu4,7,8 receptor agonist, alleviates the akinetic symptoms of 6-OHDA-lesioned rats by normalizing the enhanced metabolic activity of the STN and to a lesser extent the SNr. Paradoxically, ACPT-1 treatment also impaired behavioral performance of sham-operated rats that could be correlated with an increased expression of CoI activity in the lateral part of the SNr. These results show that selective ligands of mGlu4, 5, 7 or 8 receptors regulate the overactive subthalamo-nigral connection in Parkinson's disease and may therefore represent a pharmacological tool alternative to the dopatherapy.
Supported by CNRS, EranetNeuron program, ANR and Fondation de France

P1.098

Serotonin 4 receptors triggers adaptive mechanisms to limit cocaine reward

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Anorexia induced by activation of serotonin (5-HT, 5-hydroxytryptamine) receptors 4 (5-HTR_4) is mediated by addictive components, in the nucleus accumbens, a brain structure involved in reward.

"Ecstasy" triggers activation of an identical 5-HTR_4 pathway to reduce appetite. Here, cocaine was also found to require 5-HTR_4 to reduce appetite in food-deprived mice. Subsequently, we thought that an absence of 5-HTR_4 interrupts adaptive processes that "slow down" motivation for cocaine and food. The phosphorylated cAMP-reponsive element binding protein (pCREB), in the nucleus accumbens (NAc), is central in regulating motivation. Cocaine-induced increases in the levels of pCREB, in the NAc, was reduced in mice lacking 5-HTR_4 or treated with a 5-HTR_4 antagonist (RS39604). In an operant intravenous self-administration model under a progressive ratio schedule of reinforcement, the 5-HTR_4 KO mice exhibited elevated levels of lever pressing for cocaine injections of 0.5 $mg.kg^{-1}.injection^{-1}$. Impaired pCREB is then concordantly associated with a higher motivation to self-administer a low dose of cocaine in the absence of 5-HTR_4. It was however transient (for the first 4 consecutive days of training), suggesting that dependence to cocaine in null mice decreased to reach a wild-type level. FosB and ΔFosB enhanced cocaine dependence. Injecting acutely cocaine in KO mice was not sufficient to maintain an increase in the levels of FosB mRNA, in the NAc, for 3 hours. Increased in the truncated form of FosB, ΔFosB mRNA expression, in the NAc, following cocaine, was reduced by 60% in the absence of 5-HTR_4. KO mice were not more motivated for a higher amount of food, suggesting that pathological motivation for food and cocaine may use different process. Together with previous studies, findings suggest that activation of 5-HTR_4, in the NAc, may represent an adaptive process that challenges the rewarding effect of cocaine, but a "mistaken message" whereby the brain misevaluates the physiological need to eat and, perhaps trigger depressive-like signs.

P1.099

Identification of a 23aa peptide inhibitory of polyglutamine aggregation and pathogenesis in a *Drosophila* model of Huntington's disease

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Huntington's disease (HD) is caused by expansion of the polyglutamine (polyQ) tract in the human Huntingtin (hHtt) protein (polyQ-hHtt). Although this mutation behaves dominantly, *htt* loss of function may also contribute to HD pathogenesis. In particular N-terminal fragments of both *Drosophila* and Human wild-type Htt have been previously shown to specifically protect polyQ-hHtt-induced cellular defects (Mugat *et al.*, 2008, *Human Molecular Genetics*). A screen in mammalian culture cells allowed the identification of a 23aa peptide (pep42) lying within these normal Htt fragments that is able to rescue polyQ-hHtt aggregation. Using a *Drosophila* model of Huntington's disease, we confirmed the inhibitory abilities of pep42 on different polyQ-hHtt induced phenotypes, such as aggregation, eye degeneration, axonal trafficking of vesicles and larval locomotion, therefore demonstrating its therapeutic effect *in vivo*. We found that pep42 does not specifically target the polyQ, but directly interacts with the N-terminal part of Huntingtin. This allowed to design the molecular mechanism whereby pep42 is acting to prevent polyQ-hHtt induced phenotypes and so potentially prevent Huntington's disease.

P1.100

Vulnerability of Substantia nigra dopaminergic neurons to the reverse functioning of glutamate transporters: a new model of Parkinson's disease?

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The main neuropathological feature of Parkinson's disease (PD) is the progressive degeneration of dopaminergic (DA) neurons of substantia nigra *pars compacta* (SNc). Glutamate-mediated mechanisms are thought to contribute to the progression of the neuropathological process via excitotoxicity. Our working hypothesis is that altered functioning of excitatory amino acid transporters (EAAT) might contribute to the cell death process. Indeed, in PD state, DA neurons are submitted to intense and repetitive stimulation from overactive glutamate terminals, a condition known to favor the reverse functioning of EAAT. We previously showed using cultured mesencephalic neurons that DA neurons are preferentially vulnerable to EAAT reverse functioning mimicked by the substrate inhibitor PDC. In those neurons, oxidative stress increases the sensitivity to NMDA receptor-mediated excitotoxicity. Here we provide in vivo evidence that an acute intranigral PDC injection (300nmoles) could represent a new PD model reproducing cardinal cellular features of the pathology.
As soon as 4 days after its injection, PDC leads to a **specific loss** of SNc DA neurons sparing GABA neurons of SN reticulata. Second, the degeneration progresses over time (14 to 55% cell loss between 4 to 60 days post-injection), and exhibits a specific **topography,** with a preferential vulnerability of the posterior vs anterior part of SNc, reminiscent of that seen in PD. Neuronal loss is associated with microglial and astroglial reactivity up to 30 days post-injection, but thereafter, it continues to progress after extinction of glial responses (between 30 and 60 days).
Despite the neuronal loss in SNc, no loss in tyrosine hydroxylase immunostaining was measured in the striatum and no significant motor deficit was observed up to 60 days after PDC injection. All these features are consistent with presymptomatic PD model, in which compensatory processes at DA terminal levels might mask the primary cellular loss. Current work investigates whether this model can spontaneously evolve towards symptomatic phase at longer post-injection time and examines the contribution of NMDA receptors and oxidative stress in the PDC-induced neurodegeneration.
This work was supported by CNRS, Univ de la Méditerranée and France Parkinson

P1.101

Age-dependent upregulation of specific matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) in the 5xFAD mice model of Alzheimer's disease

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We have previously demonstrated that MMPs and TIMPs are pleiotropic proteins involved in nervous system degeneration, plasticity and inflammation. MMPs can also degrade Abeta and possibly process the amyloid precursor protein (APP). All this may affect the APP/Abeta metabolism with uncertain consequences for the pathological outcome. Surprisingly, the role of MMPs and TIMPs in the pathogenesis of Alzheimer's disease has been relatively overlooked, despite their potential in regulating the metabolism of APP/Aβ, synaptic function, and neuroinflammation, a major risk factor of the disease. We hypothesize that the MMP/TIMP balance affects these parameters and therefore influences the outcome of the pathology. In order to test this hypothesis, we have characterised the expression and activity of MMPs and TIMPs across age in the 5xFAD transgenic mice model, using quantitative real time PCR (qPCR), immunoblotting, zymography and immunohistochemistry techniques. We show upregulated levels of TIMP-1 and MMP-12, in the brain of AD transgenic mice, along with inflammation markers at early stages of the disease, preceding behavioural impairment and conspicuous Aβ deposition. In older animals (4 and 6 months), further increase of TIMP-1 and MMP-12 levels was found, along with significant MMP-2 elevation of mRNA and protein levels. Increases in MMPs take place principally in reactive glial cells in the vicinity of amyloid deposits in hippocampus, deep cortical layers, thalamus and entorhinal cortex. The spatio-temporal pattern of expression of MMPs and TIMPs suggests that the MMP/TIMP system may contribute to early events of the disease such as inflammation, progressively leading to synaptotoxicity and neurodegeneration, in pace with Abeta deposition resulting from perturbed APP/Abeta metabolism.

P1.102

Tryptophan/kynurenin pathway modifications and behavioural alterations induced by unpredictable chronic mild stress (UCMS) in mice are reversed by fluoxetine and indole dioxygenase inhibitor 1methyl tryptophan

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Background: The amino-acid tryptophan (TRP) is metabolized in two main pathways both involved in mood regulation and in stress-iduced neuropathology: serotonin and kynurenin (KYN). The metabolism of KYN is activated by pro-inflammatory cytokines, mediating the inflamatory pathway of depression and also by cortisol, mediating the activation of hypothalamic-pituytary axis by stress. It was shown that KYN pathway (KP) is altered in depressive patients an we previously showed that KP is also altered in rodents having been submitted to unpredictable chronic mild stress (UCMS) procedure (Laugeray et al. 2010). UCMS is considered as a valid model of chronic stress, inducing behavioural abnormalities which are reversed by antidepressant treatments.
Aim of the study: We investigated here wether inhibition the kynurenin pathway (KP) was able to block biochemical alterations of the kynurenin pathway (KP) and physio-behavioural abnormalities both induced by UCMS procedure in mice.
Method: UCMS mice vs controls were chronically treated with an inhibitor (1-methyltryptophan, 1MT) of the enzyme indoleamine-2,3-dioxygenase (IDO), which is one of the two enzymes degradating tryptophan (TRP) into kynurenine (KYN). A chronic treatment by the antidepressant fluoxetine was used as a reference.
Results: Both 1MT and FLX block UCMS-induced alterations of the KP (peripherally and centrally). 1MT is as effective as FLX in reversing UCMS-induced behavioural changes. The levels of some cerebral KP metabolites are correlated to the degree of behavioural alterations induced by UCMS while those of 5-HT metabolites (5-HT, 5-HIAA) are not.
Conclusion: Our results suggest that inhibiting the KP by 1MT might be a relevant mechanism for an antidepressant effect. Secondly, our data provide evidence that 1MT exhibit antidepressant-like effects similarly to fluoxetine in various behavioural tets. They also indicate that accumulation of some KP metabolites into corticolimbic structures is likely to play a key role in the pathophysiology of depressive disorders. We confirm that kunurenin pathway could play a central role in the physiopathology of mood disorders.

P1.103

Alterations of striatal proteasome subunit expression and function after D1R dopamine receptor stimulation in models of Parkinson´s disease

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The proteasome is a protein-destroying apparatus that regulates several cellular functions. In fact, the proteasome plays a central role in modulating neuronal response through regulation of neurotransmitter receptor intraneuronal fate. We have recently shown a relationship between dopamine (DA) receptors and especially the D1 receptor and the proteasome in the context of Parkinson´s disease and L-dopa-induce dyskinesia. While we showed alterations in proteasome catalytic activity in response to chronic L-dopa exposure through involvement of the D1 but not D2

dopamine receptor, the detailed study of the interaction between striatal proteasome subunits and D1 dopamine receptor in response to dopaminergic drugs activation (e.g. levodopa and dopamine agonists) has not been explored so far.
In this context, we studied the effect of D1R stimulation on different striatal proteasome subunits, using "*in vitro*" and "*in vivo*" models of PD. We used a range of models that includes *in vitro* rat primary striatal cell culture*,* mouse striatal slices culture and the reserpine mouse model of PD. At the *in vitro* level, four experimental conditions were performed: DMSO as control, Bortezomib (a proteasome inhibitor), a mixed Bortezomib/D1R agonist SKF 82958 and D1R agonist SKF 82958. Four experimental conditions were modelled *in vivo*: normal, parkinsonian, normal acutely treated with D1R agonist SKF 82958 and parkinsonian acutely treated with D1R agonist SKF 82958.
We report the immunohistochemical characterization of the spatial distribution of different proteasome subunits (alpha5, alpha2, Core 20S and beta5), whose immunoreactivity was confined to cytoplasm and neurites. Our data reveals profound reorganisation of the different proteasome subunits in response to dopaminergic challenges in a consistent manner across models. Our data highlight the intimate interplay between dopamine receptor and proteasome activity in a non-degenerative context.

P1.104

Activation of group II metabotropic glutamate receptors attenuates neurodegeneration in the experimental animal model of amyotrophic lateral sclerosis

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Dual mGlu2/3 receptor agonists have shown clinical efficacy in anxiety and schizophrenia and have a potential neuroprotective effect in animal models. We have showed that activation of mGlu3 receptors mediates neuroprotection through the production of neurotrophic factors, such as transforming growth factor-beta and glial cell line-derived neurotrophic factor (GDNF). GDNF is a potent neurotrophic factor with prominent survival-promoting and restorative effects in neurons and attenuates neurodegeneration in mouse models of amyotrophic lateral sclerosis (ALS). In mouse primary cultures of spinal cord motor neurons exposed to the excitotoxin kainic acid (25 mM for 24 hours), application of the dual mGlu2/3 receptor agonist, LY379268 (10 mM), significantly attenuated motor neuron death as assessed by immunohistochemistry for the neuronal marker SMI-32. Moreover, application of LY379268 in spinal cord motor neurons increased GDNF protein levels as assessed by immunoblotting. Chronic treatment of C57Black mice with LY379268 (0.5, 1 or 5 mg/kg/day for 28 days, by means of subcutaneously implanted osmotic minipumps), increased GDNF levels in the spinal cord, striatum and cerebral cortex at all doses used, as assessed by immunoblotting. Immunohistochemical analysis of GDNF in the spinal cord showed that LY379268 increased the number of GDNF-positive cells in the cervical, thoracic and lumbar spinal cord. Double immunohistochemistry for GDNF and GFAP or NeuN or SMI-32 showed that GDNF expression is localized in GFAP-positive cells in the cerebral cortex and spinal cord.
We extended the study to G93A mice, which over-express the mutated form of superoxide dismutase-1 (SOD1) and are considered as an animal model of ALS. Chronic treatment with LY379268 (1 or 5 mg/kg) increased GDNF immunoreactivity in the spinal cord. Chronic treatment with LY379268 (0.5, 1, 5 mg/kg/day, until death) induced a significant improvement of motor behavior and neurological symptoms in the last phase of disease at the doses of 1 and 5 mg/kg. Thus, in vitro and in vivo data converge in showing that administration of LY379268 may be neuroprotective and suggest that selective agonists of mGlu2/3 receptor may be new potential pharmacological tools for the experimental therapy of ALS.

P1.105

Impact of a serotoninergic lesion on motor and non-motor symptoms in MPTP-intoxicated monkeys

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A substantial body of data points to serotoninergic involvement in both motor and non-motor symptoms associated with Parkinsonism. By biochemical and positron emission tomography (PET) imaging approaches, correlations have been found between the alteration of serotoninergic system in some cerebral structures and the expression, or even severity, of some motor (tremor and dyskinesia) or non-motor (fatigue and depression) symptoms. Studies of animal models of Parkinsonism have also uncovered some changes in forebrain serotoninergic system. Our team has evidenced a striatal decrease or increase of serotoninergic fibers in the MPTP-intoxicated monkeys expressing stable motor symptoms or fully recovering from these symptoms, respectively, suggesting a beneficial role for serotonin in motor compensatory mechanisms. On the contrary, other data suggest a deleterious role for serotonin in motor complications following L-Dopa treatment. Regarding non-motor symptoms, very few studies have addressed the role of serotonin in the field of PD. It remains therefore crucial to investigate on monkey the precise implication of serotonin in PD. To this end, we used MDMA (also known as Ecstasy) as a tool to destroy specifically the serotoninergic fibers secondary to MPTP intoxication and subsequent motor recovery, and we characterized the impact of such a MDMA lesion on the expression of PD symptoms. Despite previous DA depletion, the MDMA treatment successfully induced a drastic lesion of serotoninergic fibers in the brain, attested by PET imaging using the ^{11}C-DASB, a ligand of the serotoninergic transporter, and by immunohistochemical tools on post-mortem tissues. Regarding the expression of symptoms, our preliminary data obtained on three monkeys indicate that the lesion of serotoninergic fibers does not lead to the reappearance of parkinsonian motor symptoms; refuting the hypothesis that serotonin would be involved in compensatory mechanisms. The effects on the expression of non-motor symptoms, such as attention or motivation deficits, are currently under investigation.

P1.106

Molecular mechanisms underlying T cell trafficking into the brain in a mouse model of Parkinson´s disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the ventral mesencephalon (VM) (i.e. the substantia nigra (SN)). Accumulating evidence suggests that non-cell autonomous mechanisms are involved in neurodegeneration in PD. In line with this, we have recently shown that peripheral T CD4+ cells, which invade the brain in PD patients and in MPTP-treated mice (an experimental model of PD), actively participate to neuronal cell death suggesting that therapeutic strategies aimed at preventing lymphocyte extravasation may be of interest. Yet, the molecular mechanisms underlying this infiltration are still largely unknown.
Our major aim is to unravel these mechanisms, in particular, the adhesion and chemotactic factors involved in these processes. Since infiltration of CD4+ T cells reached a pick at 2 and 7 days after MPTP, we first investigated the expression of chemokines and adhesion molecules by TaqMan® Low Density Arrays in the VM of mice at these time points. Then, to confront our data with the real pathology, we analyzed the expression of the most promising candidates by real-time RT-PCR on post-mortem specimen of human SN from control and PD individuals. Then, the impact of corresponding chemokine receptor deficiency on T lymphocyte infiltration and dopaminergic cell death following MPTP challenge was tested using knock-out animals.

Our results indicate that chemokines known to stimulate T cell tactism, in particular CCL3/4/5 and CXCL10, are overexpressed in the VM after MPTP treatment. Interestingly there was a tendency for an increase in CXCL10 but not in CCL5 mRNA expression in the SN of PD patients compared to control subjects. Yet, mice deficient either for CCR5 or CXCR3, two major chemokine receptors involved in T cell trafficking, were found to display as much dopaminergic cell loss and cerebral T cell infiltrates as their wild-type littermates after MPTP exposure. Although one can not exclude the possibility that CCR5 and CXCR3 are dispensable for T cell brain extravasation in the MPTP mouse model, our data could also suggest that compensatory and/or redundant mechanisms may underline these negative results. Further investigations are therefore needed to test such hypothesis.

P1.107

A novel metabotropic glutamate receptor 4 positive allosteric modulator for the treatment of Parkinson´s disease

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We had previously demonstrated the efficacy of mGlu4 receptor orthosteric agonists in alleviating PD symptoms when injected in the striatum or the globus pallidus, where they inhibit synaptic transmission (1, 2). mGlu4 receptor belongs to group III metabotropic glutamate (mGlu) receptors (3) which are located at key BG synapses (4) becoming hyperactive in PD, i.e., cortico-striatal, striato-pallidal and subthalamo-nigral pathway. Here we used a novel mGlu4 receptor positive allosteric modulator (PAM) discovered by Lundbeck Research USA, which crosses the blood-brain barrier (BBB). We utilized hemiparkinsonian rats (unilateral 6-OHDA injection in the substantia nigra pars compacta) and the cylinder test to evaluate akinesia. Preliminary results show that PAM or L-DOPA alone administered i.p. for a 2-week period had no effect on akinesia, while co-treatment with PAM + L-DOPA alleviated it suggesting a synergy between the neuropharmacological action of the two compounds. Here we will present and discuss future results of ongoing experiments in which we test this synergy hypothesis by treating hemiparkinsonian rats with a combination of PAM + low L-DOPA doses that are not effective per se on akinesia. We aim at demonstrating that targeting mGlu4 receptor with a PAM crossing the BBB could provide a solution for treating PD in combination with reduced L-DOPA doses, which could decrease its long-term side-effects (levodopa-induced dyskinesia).
This work has been supported by ANR, CNRS, Lundbeck Research USA, and Aix-Marseille Université.
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2) Beurrier et al, FASEB J 2009
3) Gubellini et al, Prog Neurobiol 2004
4) Conn et al, Nat Rev Neurosci 2005

P1.108

Disruption of acetylcholine synthesis in mice motor neurons: a new model to investigate the long-term impact of neuromuscular transmission defects

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Motor neuron diseases, including amyotrophic lateral sclerosis or spinal muscular atrophy, are etiologically heterogeneous disorders characterized by progressive motor neuron degeneration, muscle denervation, skeletal muscle atrophy and profound muscular weakness. Neuromuscular defects occurring in these disorders are generally attributed to axonal degeneration and neuromuscular synapse disconnection. However, studies on patients or animal models of these pathologies suggest that abnormal synaptic transmission occurs at connected neuromuscular junctions prior to axonal degeneration and could be the initial cause of motor unit dysfunction. To gain further insight into the long-term impact of neuromuscular transmission impairment, we generated a new mouse line in which about half of somatic motor neurons in spinal cord and brainstem are unable to produce acetylcholine. These mice were obtained by knocking out conditionally the gene encoding choline acetyltransferase (ChAT), the biosynthetic enzyme of acetylcholine. Mutant mice are viable and display spontaneously an abnormal phenotype worsening with age that attests progressive neuromuscular dysfunction. Unexpectedly, these changes appeared with a delayed onset relative to the start of ChAT breakdown, reminiscent of some pathological situations in the human. These mice could be an important tool to determine the sequence of pathological events that follow impairment of neuromuscular synaptic transmission.

P1.109

A substrate sheds light on a new role of the multifaceted E3 ubiquitin-protein ligase Parkin in mitochondrial physiology

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Mutations in the *Park2* and *Park6* genes - encoding respectively the E3 ubiquitin-protein ligase Parkin and the mitochondrial serine/threonine kinase PINK1 - are major causes for autosomal-recessive Parkinson's disease with early onset. In Drosophila, genetic studies demonstrated that *parkin* and *PINK1* belong to a common pathway, which upon alteration leads to severe mitochondrial phenotypes. Recent reports demonstrated that Parkin and PINK1 act in concert with key-components of the mitochondrial fusion/fission machinery, and highlighted a role for the PINK1/Parkin pathway in mitochondrial clearance (mitophagy). In a differential proteomic analysis of *parkin*-deficient and wild-type mice, we identified a novel Parkin substrate, the mitochondrial matrix enzyme Short-chain-3-hydroxyacylCoA dehydrogenase (SCHAD). By biochemical approaches, we demonstrated that Parkin interacts with and promotes the ubiquitylation of SCHAD. Confocal microscopy analyses revealed that overproduction of both proteins leads to massive recruitment of Parkin to the Outer Mitochondrial Membrane (OMM), in close proximity to SCHAD. Furthermore, FRET (Förster's Resonance Energy Transfer) and FLIM (Fluorescence Lifetime Imaging Microscopy) analyses revealed physical proximity between the two proteins and the TOM (Translocase of Outer Membrane) machinery. Comparable interactions were detected when mitochondrial protein import was blocked with the protonophore CCCP, and were abolished by PD-related *Park2* mutations. Parkin deficiency in mice induced SCHAD depletion from mitochondria, whereas its overproduction in cells increased mitochondrial SCHAD abundance. Moreover, siRNA-mediated gene silencing approaches excluded the involvement of SCHAD and the TOM machinery in Parkin-dependent mitophagy. Finally, FRET analysis revealed interactions between PINK1 and TOM subunits in the presence of Parkin. Taken together, our results suggest a novel role for Parkin in mitochondrial protein biogenesis through functional interaction with the TOM machinery. This function could contribute to the physiopathology of *parkin*-related Parkinson's disease.

P1.110

ERK-induced phosphorylation of the transcription factor Elk-1 is a key event in neuronal and behavioral responses to cocaine

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Elk-1 is a transcription factor activated by the Extracellular-signal Regulated Kinase (ERK) signaling pathway. Upon its phosphorylation by ERK, Elk-1 translocates to the nucleus where it controls SRE-driven gene regulation. Despite accumulating evidence that Elk-1 is a common target for drugs of abuse, no data yet describes its involvement in the neuro-adaptative and behavioural responses to these drugs. In the present work, we used a synthetic penetrating peptide (TAT-DEF-Elk-1, TDE), that mimicks the docking domain of Elk-1 towards ERK, to selectively impair Elk-1 phosphorylation mediated by ERK (Lavaur et al. J. Neurosci, 2007). Dose response curves and kinetics analyses revealed that systemic TDE administration efficiently altered striatal Elk-1 phosphorylation induced by cocaine (-50%), sparing ERK and Mitogen and Stressed activated Kinase-1 (MSK-1), a nuclear kinase downstream ERK. Nevertheless, TDE showed an unexpected role on striatal chromatin remodelling via inhibition of H3 (ser10) phosphorylation. This inhibition was confirmed *in vitro*, using a mutant version of the DEF domain of Elk-1. Consequently, TDE altered cocaine-induced regulation of genes bearing SRE in their promoters including c-fos, zif268 and arc/arg3.1. Of interest, in a chronic cocaine administration paradigm, the TDE totally reversed increased dendritic spine numbers. The TDE delayed the establishment of cocaine-induced psychomotor sensitization and conditioned-place preference. Thus, the TDE represents a unique tool to specifically address the role of ERK-induced Elk-1 phosphorylation in long term neuronal and behavioural adaptations to cocaine.

P1.111

BDNF-dependent hippocampal synaptic enhancement in a Tau transgenic mouse model

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Alzheimer's disease is characterized by extracellular amyloid-beta deposits and intracellular neurofibrillary tangles composed of hyperphosphorylated Tau proteins. Spatiotemporal spreading of Tau pathology has been shown correlated with cognitive deficits. Both brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor TrkB play a critical role in hippocampal-dependent synaptic plasticity and memory, particularly through mediation of synaptic enhancement. However, whether Tau pathology impairs the synaptic effects of BDNF remains completely unknown. Using the THY-Tau22 transgenic model, exhibiting a progressive development of both hippocampal AD-like Tau pathology paralleled by memory impairments, we found that Tau pathology was associated with the loss of BDNF-induced hippocampal synaptic enhancement from early pathological stages. This dysfunction was related to a defect in the coupling between TrkB and NMDA receptors. Indeed, whereas TrkB expression and activability remained unaltered, we observed a significant reduction of NMDA-induced fEPSP depression in the hippocampus from THY-Tau22 mice, supporting a decreased NMDA receptor activability. In accordance, we found increased NR2B levels in an insoluble protein fraction containing pathological Tau species. In addition, we found a decrease of its tyrosine phosphorylation at Y1472. Altogether, our data support that Tau pathology impairs hippocampal

BDNF-induced synaptic enhancement through defect of the TrkB-NMDA receptor coupling. These alterations could contribute to cognitive dysfunctions observed in Alzheimer's disease and other Tauopathies.

P1.112

Noradrenergic and serotonergic inhibitory feed-backs intervene in amphetamine-induced behavioural sensitisation

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One of the most important but difficult task in pharmacology of addiction is to specify the long-term neuronal changes induced by drugs of abuse. In rodents, amphetamine triggers a locomotor activity which increases with repeated injections and is maintained several months after the last amphetamine intake, a process called behavioural sensitisation. Previous work obtained by micro-dialysis in awake animals showed that, in parallel to the locomotor sensitisation, a neurochemical sensitisation of noradrenergic and serotonergic neurons occurred (1). This finding suggested a long-term uncoupling between these two noradrenergic and serotonergic systems, whom firing activity is controlled in part by α2a-adrenergic and 5-HT_{1A} inhibitory autoreceptors, respectively. We have therefore looked for possible modifications of these autoreceptors following repeated amphetamine injections. First of all, we measured the sensitisation state of 5-HT_{1A} autoreceptors in dorsal raphe nucleus using GTPγS binding in amphetamine sensitized C57BL/6J mice after a 4-day or 3-week withdrawal. We found a **20 % decrease in 5-HT_{1A} receptors stimulated G-protein coupling in sensitised mice after short or long withdrawals.** However, we also found that neither 8-OH-DPAT, a 5-HT_{1A} receptor specific agonist, nor WAY 100635, a 5-HT_{1A} receptor antagonist, could prevent amphetamine-induced behavioural sensitisation. Since amphetamine releases noradrenaline and not serotonin in mice, we assumed that behavioural sensitisation induction was controlled by noradrenergic autoreceptors. Accordingly, we found that **stimulation of α2a-adrenergic receptors by clonidine,** an α2-adrenergic receptor agonist, **completely prevents amphetamine-induced behavioural sensitisation** measured after a 3-week withdrawal.
These results highlight the critical role of autoreceptors in the induction and maintenance of amphetamine-induced behavioural sensitisation and suggest that inhibitory noradrenergic and serotonergic feed-backs are indirect targets of amphetamine, thus explaining long-term effects in addiction.
(1) Salomon L, Lanteri C, Glowinski J, Tassin JP. Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. (2006) *P.N.A.S. (USA) (Track II)* 103: 3476-3481.

P1.113

Sensory-motor deficits and Neurofilament disorganization in Gigaxonin-null mice

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Giant Axonal Neuropathy (GAN) is a fatale neurodegenerative disorder with early onset characterized by a severe deterioration of the peripheral and central nervous system, involving both the motor and sensory tracts and leading to ataxia, speech defect and intellectual disabilities. The broad deterioration of the nervous system is accompanied with a generalized disorganization of the Intermediate Filaments, including Neurofilaments in neurons, but implication of this defect in disease onset or progression remains unknown. The identification of gigaxonin, the substrate adaptor of an E3 ubiquitin ligase as the defective protein in GAN now allows to investigate the crucial role of the gigaxonin-E3 ligase in sustaining neuronal and Intermediate Filament integrity. To study the mechanisms controlled by gigaxonin in these processes and to provide a relevant model to test the therapeutic approaches under development for GAN, we generated a Gigaxonin-null mouse by gene targeting. We investigated for the first time the deterioration of the motor and sensory functions over time as well as the spatial disorganization of Neurofilaments in our Gigaxonin-null mice. We showed that gigaxonin depletion in mouse induces mild but persistent motor deficits starting at 60 weeks of age in the 129/SvJ-genetic background, while sensory deficits were evidenced in C57BL/6 animals. No apparent neurodegeneration was evidenced in our knock-out mice, but dysregulation of Neurofilaments in proximal and distal axons was massive. Indeed, Neurofilaments were not only more abundant they also showed the abnormal increased diameter and misorientation that are characteristics of the human pathology. Together, our results show that gigaxonin depletion in mouse induces mild motor and sensory deficits but recapitulates the severe Neurofilament dysregulation seen in patients. Our model will allow investigation of the role of the gigaxonin-E3 ligase in organizing Neurofilaments and may prove useful in understanding the pathological processes engaged in other neurodegenerative disorders characterized by accumulation of Neurofilaments and dysfunction of the Ubiquitin Proteasome System, such as Amyotrophic Lateral Sclerosis, Huntington's, Alzheimer's and Parkinson's diseases.

P1.114

Olfactory stem cells, a new cellular model for studying molecular mechanisms underlying familial dysautonomia

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Familial dysautonomia (FD) is a hereditary neuropathy caused by mutations in the *IKBKAP* gene, the most common of which results in variable tissue-specific mRNA splicing with skipping of exon 20. Defective splicing is especially severe in nervous tissue, leading to incomplete development and progressive degeneration of sensory and autonomic neurons. The specificity of neuron loss in FD is poorly understood due to the lack of an appropriate model system. To better understand and modelize the molecular mechanisms of *IKBKAP* mRNA splicing, we collected human olfactory ecto-mesenchymal stem cells (hOE-MSC) from FD patients. hOE-MSCs have a pluripotent ability to differentiate into various cell lineages, including neurons and glial cells.
We confirmed *IKBKAP* mRNA alternative splicing in FD hOE-MSCs and observed a significant lower expression of both *IKBKAP* transcript and IKAP/hELP1 protein in FD cells resulting from the degradation of the transcript isoform skipping exon 20. We localized IKAP/hELP1 in different cell compartments, including the nucleus, which supports multiple roles for that protein. Moreover, we showed that kinetin improved exon 20 inclusion and restores a normal level of IKAP/hELP1 in FD hOE-MSCs. Furthermore, we were able to modify the *IKBKAP* splicing ratio in FD hOE-MSCs, increasing or reducing the WT (exon 20 inclusion):MU (exon 20 skipping) ratio respectively, either by producing free-floating spheres, or by inducing cells into neural differentiation. To further identify processes altered during the physiopathology of FD, the transcriptomes of spheres and hOE-MCS-treated for neural differentiation were investigated at the genome-wide level. We confirmed that nervous system development was the most altered process in FD. Finally, we highlight kinetin role as a putative regulator of splicing factors which contribute to restore a correct splicing of *IKBKAP* mRNA. hOE-MSCs isolated from FD patients represent a new approach for modeling FD to better understand genetic expression and possible therapeutic approaches. This model could also be applied to other neurological genetic diseases.

P1.115

Targeting of *AIF1* mRNA to the mitochondrial surface counteracts optic neuropathy in the *Harlequin* mouse

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Depletion of the mitochondrial AIF protein causes respiratory chain dysfunction and triggers progressive ataxia and optic atrophy in the *Harlequin* mouse strain with a phenotype that recapitulates major hallmarks of human respiratory chain complex I deficiency. We characterized retinal morphology and function of *Harlequin* mice during the course of neuron cell death leading to blindness with the goal of preventing optic atrophy *via* AAV2-mediated gene therapy. Retinal ganglion cell loss and optic atrophy correlated with an isolated respiratory chain complex I defect in *Harlequin* mouse retinas and optic nerves. In control retinas, *AIF1* mRNA is 2.3-fold more abundant than *AIF2*, both mRNAs being sorted to the mitochondrial surface. In *Harlequin* mouse retinas, there is a 96% decrease of *AIF1* and *AIF2* mRNA steady-state levels. We attained substantial and long-lasting protection of retinal ganglion cell and optic nerve integrities as well as the stabilization of complex I function after intravitreal administration to 4-8 week-old *Harlequin* mice of an AAV2 vector containing the open reading frame and 3'UTR of the *AIF1* gene. Our findings indicate that ocular AAV2-mediated gene therapy is a realistic goal for preventing blindness from mitochondrial origin.

P1.116

Selenoprotein T expression is induced during astrogliosis in the MPTP model of Parkinson´s disease

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During neurodegenerative diseases, oxidative stress is a major cause of neuronal death. As a defense mechanism, nerve cells like all cells activate specialized protective enzymes including superoxide dismutase and various selenoproteins. Indeed, it has been shown that selenoproteins protect cells against reactive oxygen species and repair proteins damaged by the oxidative burst, owing to their strong reducing capacity conferred by the nucleophilicity of the selenium atom. We have recently identified a new selenoprotein, named selenoprotein T (SelT), whose expression is induced during neuronal differentiation. Using a model of cerebral ischemia in mice, we recently showed that the expression of SelT is strongly induced in astrocytes in these conditions, suggesting that SelT could be activated during oxidative stress associated with neuronal injury and neurodegenerative diseases. In order to determine the role of SelT during neurodegenerative processes, we sought to evaluate in this study the expression of SelT in the MPTP model of Parkinson's disease. To induce dopaminergic neuron degenerescence, adult mice were intraperitoneally injected with 4 doses of MPTP (15mg/kg each in 0.15ml 0.9%NaCl) at 1h intervals, and compared to control animals treated with the vehicle only. Mice were euthanized 2, 3, 4 and 8-days post-treatment, and brain sections were processed for immunohistochemical studies. Using anti-tyrosine hydroxylase and confocal microscopy analysis, we observed a marked decrease in dopaminergic neurons of the *substantia nigra* at 3 and 4 days post-

intoxication by MPTP. Concurrently, a strong astrogliosis was found in the striatum based on GFAP labeling, as previously reported. Interestingly, SelT expression was appreciably induced in the striatum of treated animals compared to controls, and this labeling colocalized with GFAP immunostaining. The intensity of SelT labeling paralleled the amplitude of astrogliosis reaction in the striatum and dopaminergic neuron degeneration in the substantia nigra. The present results show that SelT is induced during astrogliosis following dopaminergic neuron lesion, suggesting that this novel selenoprotein participates to neuroprotective mechanisms activated during neurodegenerative diseases, such as Parkinson's disease.

P1.117

Down-regulation of the potassium-chloride cotransporter KCC2 and up-regulation of the sodium persistent current both contribute to spasticity after spinal cord injury in adult rats

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Spasticity is a motor disorder that frequently appears after spinal cord injury (SCI). It is characterized by hyperreflexia and spasms, which seem to result from both reduced synaptic inhibition and hyperexcitability of motoneurones.
The reduction of inhibitory processes under the lesion results, at least partly, from an alteration of chloride (Cl^-) homeostasis. $GABA_A$and glycine receptors are associated with Cl^- channels and their activation has inhibitory effects only when intraneuronal Cl^- concentration is lower than in the extracellular compartment. We have shown that the expression of the potassium-chloride cotransporter KCC2, which extrudes Cl^- from neurones, is reduced in the plasma membrane of motoneurones in adult rats after SCI. This can account for the altered inhibition and reversal of reciprocal inhibition towards excitation observed in spastic patients. Reduction of the rate dependent depression (RDD) of the H-reflex is a good correlate of spasticity. Our results showed that RDD was reduced 1) after SCI in the rat, 2) in intact rats after intrathecal (i.t.) injection of the KCC2 blocker DIOA in the lumbar spinal cord and 3) in KCC2-deficient mice. Release of the neurotrophic factor BDNF after SCI plays a central role since sequestering endogenous BDNF at the time of the lesion prevents KCC2 downregulation, and conversely, i.t. injection of BDNF in the lumbar spinal cord of intact rats both downregulates KCC2 and reduces RDD.
Altered intrinsic motoneuron properties have also been involved in spasticity after SCI. An upregulation of voltage-dependent persistent inward currents is associated with spasms. Pharmacological experiments suggested the involvement of the persistent sodium current (I_{NaP}). We show that Nav 1.6 channels, strongly expressed in motoneurons and proposed to be the major molecular determinants of I_{NaP}, were significantly upregulated in the initial segments of lumbar motoneurons after SCI, whereas Nav 1.1 were not affected. Moreover, a pharmacological upregulation of I_{NaP} in the lumbar spinal cord of intact rats with i.t. injection of veratridine reduced the RDD of the H reflex.
These results are supporting the hypothesis of a synergic role of a downregulation of KCC2 and an upregulation of I_{NaP} in the physiopathology of spasticity.

P1.118

Effects of chronic stress on sleep structure in the model of prenatal restraint stress in rats

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The rat model of prenatal restraint stress (PRS) is particularly valuable for the study of the mechanisms involved in the pathophysiology of anxiety and depression since adult PRS rats show endocrine and behavioral abnormalities that are corrected by antidepressant medication. PRS induce alterations of circadian rhythms as a phase advance of both circadian corticosterone secretion and locomotor activity. Moreover, PRS rats present a prolongation of corticosterone response to stress

which is correlated to an increase REM sleep duration and fragmentation. Here we have considered the influence of two weeks manipulation on the sleep structure of control and PRS animal. For that, animal were surgically implanted with chronic electrode for tow EEG and one nuchal EMG. After two weeks of recovery and habituation to the sleep recording procedure, PRS and control rats were submitted for two weeks to a daily manipulation including intragastric saline gavage (chronic stress, CS) or were left undisturbed. PRS by itself reduced non-REM sleep duration and increased REM sleep duration and fragmentation. Two weeks of CS amplified the effect of PRS on non-REM and REM sleep. Moreover, the two weeks of CS induced an increase of wake duration in PRS animal and a decrease of REM sleep duration in control animal. Finally, the CS increased the number of wake, non-REM and REM sleep episodes in PRS animal indicating an increased sleep fragmentation. In conclusion, PRS animal show sleep alterations that are amplified by CS. These observations reinforce the concept of a strong linkage between sleep quality and stressing events and enforce to consider the circadian homeostasis in the mediation of the consequences of chronic stress on neuroplasticity in PRS animal.

P1.119

NP03, a novel low dose lithium microemulsion: a potential therapeutic approach for bipolar disorder and neurodegenerative diseases

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Objective: Lithium is used as the gold standard in the treatment of bipolar disorder for more than 40 years. Although its mechanisms of action are still unknown, there is currently a renewed interest in its use in neurodegenerative diseases. However, because of a narrow therapeutic window, lithium induces serious side effects which are exacerbated in patients suffering from neurodegenerative diseases, rendering the use of classical forms of lithium in these pathologies impossible.
Here we examined whether NP03, a novel low-dose lithium microemulsion, would be efficient in depressive behavioral mice models of bipolar disorder (BD) and would improve the disease phenotypes in two mouse models (the R6/2 and YAC128 transgenic mice) of a neurodegenerative disease, the Huntington's disease (HD).
Methods: C57Bl6J wild type mice were treated with vehicle, NP03 (160µg Li/kg/ day) or conventional lithium solution (16mg Li/ kg) for 4 weeks before depressive behavioral testing. R6/2 mice were treated with NP03 (40µg Li/kg) or conventional lithium solution (16mg Li/kg) for 13 weeks starting at 8 weeks of age (after onset of the disease). YAC128 and wild-type mice were treated with NP03 (40µg Li/kg) for 10 months starting at 2 months of age (before onset of the disease).
Results: In the BD experiment, NP03-treated mice had behavioral results equivalent to lithium solution-treated mice, decreasing depressive behavior. Furthermore, we show that postsymptomatic treatment with NP03 or lithium solution stabilized motor functions in HD R6/2 mice, without toxic effect using NP03 instead of lithium solution which provoked a decrease in survival. Presymptomatic treatment with NP03 in the HD YAC128 mice showed no difference in motor performance and striatal volume in comparison to wild type animals.
These positive effects are coupled with modifications of various pathways (GSK3 beta, caspase 6, etc.) usually modified by lithium.
Interpretation: Our findings demonstrate that NP03, a novel low-dose lithium microemulsion, has an equivalent pharmacological effect in BD and HD as a high dosed lithium solution, allowing a significant improvements in the therapeutic window. NP03 therefore represents a potential therapeutic approach for both BD and a range of neurodegenerative diseases.

P1.120

Sugar overconsumption during adolescence induces depressive-like behavior in adult male rats

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Major depressive disorder (MDD) is expected to reach the second cause of disability in the world by 2020 (Murray et al. Science 2005). In recent years, there has been growing interest in the association between diet quality and the possibility of developing depressed mood in adolescents (Jacka et al., Am J Psychiatry, 2010). Recently we showed that overconsumption of a palatable sweet solution during adolescence had a specific long-lasting effect on motivation and reward function at adulthood in male rats (Vendruscolo, Gueye et al., PLoS One 2010). Because anhedonia and loss of motivation are core symptoms of depression in humans, we tested the long lasting changes in behaviour, reported in sucrose pre-exposed animals during their adolescence (from Post Natal Day 30 to 46), on depressive-like behaviors and studied the effects of a chronic treatment with a tricyclic antidepressant (Imipramine, 10 mg/kg/ day i.p from Post Natal Day 47 onwards) in this model. We showed that, besides having a decreased sensitivity to sweet and non sweet reward, adult rats pre-exposed to sucrose during their adolescence also demonstrated an increased time spent immobile in the forced-swim test and an increased latency to feed in the novelty-suppressed feeding test. A chronic antidepressant treatment attenuated the reduced motivation for saccharin in adult rats pre-exposed to sucrose and reversed the depressive-like behaviors mentioned above. Antidepressant-induced hippocampal neurogenesis is believed to underlie their therapeutic behavioral effects (Santarelli et al. Science 2003). In accordance, we showed that sucrose pre-exposure generated decreased neurogenesis in the dentate gyrus which was restored by the antidepressant treatment. Altogether, these findings suggest that sugar overconsumption during adolescence induces enduring changes in the brain reward system and leads to a depressive-like state. Considering the day-to-day availability and excessive consumption of sugar (especially by adolescents) in our modern societies, these findings may indicate that sugar overconsumption might have unsuspected implications in the growing incidence of depression in humans.

P1.121

The mood stabilizers lithium and valproic acid prevent olfatory and memory alterations induced by intranasal administration of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rats

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Many studies have shown that alterations in olfactory, cognitive and emotional functions precede the classical motor symptoms seen in Parkinson´s disease (PD). Mood stabilizers such as lithium (Li) and valproic acid (VPA) are used to treat bipolar mood disorder and more recently they have been suggested as potential neuroprotective agents. The aim of the present study was to evaluate the potential of Li and VPA to prevent the olfactory and cognitive deficits in rats infused with a single intranasal (i.n.) administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an animal model of PD developed by our group. Methods. Adult male Wistar rats were pre-treated with Li (47.5 mg/kg) or VPA (200 mg/kg) by intraperitonial (i.p.) route for 7 days before a single bilateral i.n. infusion of MPTP (1 mg/nostril) and them the animals were evaluated during different intervals (2-14 days) in a

battery of behavioral tests that included the olfactory discrimination, social recognition, open-field and forced swimming tasks. Most of the impairments presented by rats infused with MPTP were similar to those observed during the early phase of PD, when a moderate loss of nigral dopamine neurons results in olfactory and cognitive deficits with no major motor impairments. Of high interest, the present results indicated that the pre-treatment with Li and VPA were able to prevent the olfactory deficits [%time in familiar compartment: Cont:59.7+4.3; MPTP:48.7+3.4; Li+MPTP:60.7+3.7; VPA+MPTP:57.5+4.4] and short-term social recognition memory [ratio of investigation duration: Cont:0.5±0.1; MPTP:0.8±0.1; Li+MPTP:0.6±0.1; VPA+MPTP:0.6±0.1). In the forced swimming test, although MPTP administration did not induce a depressive-like symptom, the treatment with Li and VPA reduced the immobility time [immobility time(s): Cont:9.2±6.2; MPTP:10.8±4.3; Li+MPTP:2.6±0.5; VPA+MPTP:2.8±1.3]. At this time, no significant alterations on the locomotor activity were observed in the open-field.
Conclusion: These results provide new insights in experimental models of PD, indicating that the mood stabilizers agents Li and VPA may represent new therapeutic tools for the prevention of olfactory and emotional symptoms associated to early preclinical phases of PD.
Financial support: CNPq, CAPES and IBRO.

P1.122

Modification of postural orientation after the retropulsion test and influence of a visual biofeedback in Pakinson's Disease patients

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Postural control is often affected in Parkinson's Disease (PD), both in its balance and in its orientation components. Recent studies have shown that these deficits could be in relation with sensory disorders across proprioceptive impairment partly compensated by the visual dependance of PD patients. In this research, we examined the role of the postural orientation control in the retropulsion test (a common clinical tool usually used to evaluate postural instability in PD). Moreover, the influence of a visual biofeedback device (VBf, restoring head and trunk orientation in a head-mounted display) was tested in this task.
PD patients (II-IV Hoehn and Yahr stages) participated in this study in the ON sate of medication. The experimental task consisted in sequences of 3 successive sudden but expected shoulder pulls delivered by the same examiner in 3 condition of vision (eyes open, eyes closed and with VBf) crossed with two different instructions (focus on balance maintain vs. focus on vertical orientation). Kinematics data were collected with the SMART (BTS Bioengineering) automatic motion analyzer and the postural orientation component was analyzed at head and trunk levels. The displacements of the centre of pressure were recorded with AMTI force platform. The percent of corrective steps to counteract the perturbation, the amplitude of the stabilization reaction and the final orientation adopted were quantified.
Preliminary results showed that PD patients had frequently balance loss and difficulties to recover a vertical orientation after sequences of retropulsion. This latter deficit is partly improved by the visual indexing provided by the VBf device.
This research suggests strong links between balance control impairment and vertical orientation control. The development of biofeedback technologies brings to consider ambulatory devices which could lead patients to more independence in the functional rehabilitation of their postural disorders.
The authors wish to thank France Parkinson Association for its financial support.

P1.123

Efficient episodic memory recovery induced by a copper chelator in non-transgenic Aβ-impaired mice

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Alzheimer's disease (AD) is a devastating neurodegenerative disease affecting almost 50% of people older than 85 years. AD is related to the progressive loss of neurons due to an oxidative stress inducing cognitive loss. Redox active metal ions, in particular copper, are mediating the oxidative stress and the toxicity of Ab amyloids. The activation by endogenous reductants of the excess of copper ions in amyloid plaques is able to produce the catalytic generation of reactive oxygen species. Bush et al. developed clioquinol, a copper chelating agent, up to a phase-II clinical trial for the treatment of AD but the intrinsic toxicity of clioquinol promoted the development of a new hydroxyquinoline derivative (PBT2) by the same group. This chelating agent is able to restore cognition in transgenic mice model of AD.
We decided to tackle the modulation of copper trafficking and homeostasis with cyclic and open bis-chelating agents able to generate stable tetradentate copper complexes. One of these, PA1637 is highly specific for copper chelation and is unable to complex zinc ions. This absence of chelation for zinc is a positive factor to avoid the myelo-optic neuropathy that has been evidenced for clioquinol treatment.
The pre-clinical evaluation of AD drug-candidates is highly challenging since there are no evident animal models in terms of efficiency and validity. Transgenic mice are “slow models” for the evaluation of new molecules and they are not highly predictive because none of those do fully recapitulate AD pathology. Therefore we decided to develop a « fast murine screening model » by using of non-transgenic mice suffering from cognitive deficits induced by the intracerebroventricular injection of Ab_{1-42} oligomers that are considered as a causative agent in AD associated with oxidative stress and aging.
Episodic memory deficits are a prominent feature of AD. In this study, these deficits were assessed in control and treated mice, using a well-validated learning procedure, Contextual Fear Conditioning, that requires intact declarative memory which is early affected in AD patients.
Here we report the capacity of PA1637 to fully reverse the cognitive deficits of $A\beta_{42}$ injected mice after a three-week treatment by oral administration or i.p. injection.

P1.124

Vitamin D_3 triggers axogenesis, myelination and induces a dramatic recovery in a rat model of nerve injury

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In a previous study, we demonstrated that vitamin D2, an FDA-approved compound, potentiates axon regeneration and improves functional recovery in a rat model of a transected peripheral nerve. However, before bringing this molecule to patient's bed, we performed a pharmacological study, in which we compared the efficiency of two doses of the two routinely used forms of vitamin D: vitamin D2 (ergocalciferol, the plant-derived form of vitamin D) and

vitamin D3 (cholecalciferol, the animal-derived form of vitamin D).
The rat left peroneal nerve was cut out on a length of 10 mm and immediately autografted in an inverted position. After surgery, animals were treated with either ergocalciferol (100 or 500 IU/kg/day) or cholecalciferol (100 or 500 IU/kg/day) or excipient (Vehicle) and compared to unlesioned rats (Control) (n =6 per group). Functional recovery of hindlimb was measured weekly, during 12 weeks post-surgery, using the peroneal functional index. At the end of this period, ventilatory, motor and sensitive responses of the regenerated axons were calculated and histological analysis was performed. We observed that vitamin D3 is more efficient than vitamin D2 and, when delivered at high dose (500 IU/kg/day), cholecalciferol induces a dramatic locomotor and electrophysiological recovery. In order to increase statistical significance, the experiment was repeated with a new lot of 6 animals in the D3-500, Vehicle and Control groups. We confirmed the above mentioned results and demonstrated that vitamin D3 increases
i) the number of preserved or newly formed axons in the proximal end;
ii) the mean axon diameter in the distal end and
iii) neurite myelination in both the distal and proximal ends.
Then, in order to get an insight on the molecular mechanisms underlying the role of vitamin D3, we performed an *in vitro* transcriptome study. Using pangenomic cDNA microarrays and qPCR techniques, we identified the genes regulated by calcitriol (1,25 (OH)D3, 10 nM) in dorsal root ganglia or Schwann cells. After 24 hours of calcitriol supplementation, we found a modified expression of many genes involved in axogenesis and myelination. Our data indicate that vitamin D3 is a neurotrophic and myelinating agent that can be tested in phase I clinical trials for nerve repair.

P1.125

Long term consequences of traumatic exposure in elderly general population

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Objective: Several recent studies have evaluated the impact of traumatic events on the development of post-traumatic disorder (PTSD) but very few studies have focused on their long term consequences on general health. Our study aimed to evaluate the association between the experience of a lifetime traumatic event and current mental as well as physical health in a community-based study.
Method: 1662 non-institutionalised persons aged 65 years and over, randomly recruited from the Montpellier district electoral rolls completed the Watson´s PTSD Inventory to evaluate PTSD and the Mini International Neuropsychiatric Interview to assess current and lifetime symptoms of psychiatric disorders, respectively. Medical history (e.g. vascular events, diabetes…), general health-related information and blood sample were collected.
Results: 55.9% of the sample had experienced a lifetime traumatic event, according to DSMIV criteria and 15.5% had developed re-experiencing symptoms, the most common PTSD symptom associated with trauma and one of the most clinically relevant. The lifetime and current PTSD prevalence was 2.4% and 1.2%, respectively. We observed an increase in the number and severity of health-related outcomes between groups, non-traumatized subjects having the lowest risk, and those with trauma leading to recurrent re-experiencing of events (non-resilient subjects) having the highest risk. Traumatized persons who did not report re-experiencing symptoms (resilient subjects) showed better current mental health than traumatized subjects who did and non-traumatized subjects. Both groups of traumatized subjects showed a higher rate of cardio-ischemic diseases notably current angina pectoris (multi-adjusted OR=2.27, 95%CI [1.31; 3.91] and 2.34, 95%CI [1.22; 4.49] for resilient and non-resilient groups, respectively). Traumatized persons, specifically those non-resilient, showed a higher waist/hip ratio, higher triglyceride levels and a greater frequency of hypertension.
Conclusion: Our findings suggest that lifetime experience of traumatic events is frequent in the elderly and could be associated with cardio-ischemic diseases independently of PTSD symptoms expression. However the presence of these symptoms appears associated with additional metabolic risk factors.

P1.126

Gene expression changes induced by canine adenovirus type-2 (CAV-2) in the brain of the nonhuman primate *Microcebus murinus*

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Canine adenovirus type-2 (CAV-2) vectors preferentially transduce neurons and undergo efficient axonal transport. Moreover, episomal expression leads to safe, long-term and efficient gene transfer in the CNS. We previously showed that CAV-2 survived at least 6 months in the brain of *M. murinus* without inducing neuronal notable death, suggesting that they could be used to prevent and/or treat numerous neurodegenerative diseases. A hallmark of Parkinson´s disease is the loss of dopaminergic neuron which induces a dysregulation in the striatum and in the frontal cortex producing motor disorders. How brain cells deal with the virion-induce signal transduction during attachment and internalisation is unknown. We therefore assayed changes in gene expression induced by helper-dependent (HD) CAV-2 in the striatum and in the frontal cortex of 4 females *M. murinus* following injection of CAV-2 (10^9 pp) into in the right caudate nucleus. We compared the gene expression in the ipsilateral side with the contralateral side, and with non infected brains using transcriptomic approach with human Affymetrix microarrays. We followed changes in gene expression in acute conditions (24h p.i.) and delayed time (28 days p.i.). Our data shows that about 20% of the probesets were detected. The 11,200 transcripts were analyzed by different statistical tools: Significance Analysis of Microarrays, ANOVA and Principal Component Analysis. About 20 genes were highly discriminating for differentiating the striatum and cortex containing infected by CAV-2 from those of uninfected tissue. Among them, the most important belonged to transcriptional regulation function and to synaptic transmission. Interestingly, clustering analysis showed that the HD CAV-2 injections induced different profiles at 24h and at 28 days. In the striatum, several genes involved in immune response were detected (e.g. an overexpression for TNFRSF18 at 24h and for HLAG, IGHM, IGLC2 at 28 days). Our results showed that the transduction of CAV-2 in neurons induced specific changes in gene expression and that the expression was different during the acute and the long-term stages. Our study will help better understand the changes induced by CAV-2 and assess its impact in the cells in relation to its safety as gene transfer.

P1.128

Sushi-repeat protein SRPX2 implicated in disorders of the speech cortex: *In utero* RNA silencing causes altered development of the rat brain cortex

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How the speech cortex develops and functions is a crucial challenge but remains poorly known given its human-specific nature. Mutations in secreted Sushi-Repeat Protein, X-linked 2 (SRPX2) cause

epileptic/developmental disorders of the speech cortex, *i.e.* rolandic epilepsy either with verbal dyspraxia (RESDX syndrome) or with bilateral perisylvian polymicrogyria (Roll et al. 2006). Together with its cell surface receptor uPAR (plasminogen activator receptor) and with its transcriptional regulator FOXP2 (forkhead-box transcription factor), SRPX2 forms a molecular network variably implicated in the aforementioned disorders (Royer-Zemmour et al. 2008; Roll et al. 2010). In the present study, we have questioned the possible role of SRPX2 in the development of the brain cortex. RNA silencing of rat *Srpx2 in utero* with shRNA constructs targeting the 5' or 3' UTR led to abnormal pattern of radial neuronal migration, whereas the corresponding mutant shRNAs were ineffective. Rescue experiments were successfully performed with rat and human SRPX2 proteins. In contrast, the mutant (pathogenic) SRPX2 proteins failed to rescue the phenotype and evidence for loss-of-function and for dominant-negative mechanisms, respectively, was obtained. No alteration in the proliferation of progenitors, in apoptosis, and in vascular and radial glia structures was detected. Consistent with previous reports on the role of SRPX2 in the migration of cancer and endothelial cells, ongoing analyzes suggest a defect in neuronal migration. Post-natal analyzes are currently being done to study the long-term morphological and behavioral consequences. Altogether, our data indicate a role for Srpx2 in the development of the rat brain cortex and support a developmental basis for the various SRPX2-related disorders of the speech cortex in human.

P1.129

Bilateral lesions of the subthalamic nucleus prevent escalation of cocaine intake and reduce cocaine intake after escalation

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The subthalamic nucleus (STN) is critically involved in reward-motivated behaviour. Lesion or high frequency stimulation (HFS) of the STN decreases the effort spent to obtain cocaine, without decreasing the effort spent to obtain more natural rewards (Baunez et al., 2005; Rouaud, Lardeux et al., 2010). This selective reduction in the motivation for cocaine leads to suggest that the STN could represent a promising brain target for therapy against cocaine addiction. The present study sought to further validate this hypothesis by first testing the effects of bilateral STN lesions on escalation of cocaine self-administration. Escalation of cocaine use is a hallmark of drug addiction that can be readily and validly reproduced in laboratory rats by increasing drug availability (Ahmed and Koob, 1998). First we tested the effects of STN lesions on the establishment of escalation. The animals were first trained to self-administer cocaine during short-access sessions (i.e. lasting 2 hours, 250µg/injection) and were then tested for 20 sessions of long-access (6 hours). We have shown that STN lesions prevent escalation in cocaine intake. Second, in order to test whether or not STN inactivation could have a 'curative' effect, we tested the effects of STN lesions in rats after they had exhibited escalation of their drug intake. For this, intact rats were subjected to the schedule of the first experiment. After 20 sessions of long-access, they were subjected to surgery and then re-tested for an extra 20 sessions in long-access conditions. STN lesion significantly decreased the drug intake after established escalation of cocaine self-administration. These outcomes suggest a role for the STN in the development of cocaine addiction and confirm recent research that highlights the STN as a possible brain target for the treatment of cocaine addiction.

P1.130

Exonskipping mediated restoration of dystrophin reverses physiological alterations in the *mdx* mouse hippocampus

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Duchenne muscular dystrophy (DMD) is caused by the lack dystrophin, protein which hold important functions in both muscle and brain. The *mdx* mouse model of DMD, in which a non-sense mutation in exon 23 results in the absence of dystrophin, shows a deficit in γ-aminobutyric acid type A ($GABA_A$)-receptor clustering in central inhibitory synapses and enhanced long-term potentiation (LTP) at CA3-CA1 synapses of the hippocampus. Our recent investigations demonstrate that U7 small nuclear RNAs modified to encode antisense sequences and expressed from recombinant adeno-associated viral (rAAV) vectors are able to induce skipping of the mutated exon, thereby rescuing expression of a functional dystrophin-like product both in the muscle and nervous tissue *in vivo*. In the brain, this rescue was accompanied by restoration of both the size and number of hippocampal $GABA_A$-receptor clustering. In the current sudy, we report that 25.2 ± 8% of re-expression two months after intrahippocampal injection of rAAV reverses the enhanced LTP phenotype at CA3-CA1 synapses of *mdx* mice. These results suggests that dystrophin expression indirectly influences synaptic plasticity through modulation of $GABA_A$-receptor clustering and that re-expression of the otherwise deficient protein in the adult can alleviate deficits in neural functions associated with DMD.

P1.131

Human umbilical cord blood mononuclear cell transplantation against perinatal brain injury: a pre-clinical study

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Cerebral palsy (CP) is a neurological disorder that affects the developing brain causing motor and cognitive deficits. Major brain lesions associated with CP are white matter damage in preterm infants and cortico-subcortical lesions in term newborns. There is no specific treatment for these perinatal brain lesions.

Cell therapy seems promising to repair brain damages. Human umbilical cord blood mononuclear cells (hUCBMNCs) are a rich source of various stem cells that could be of interest for neurological diseases. They have many advantages over other stem cell sources, including easy access and isolation.

Our goal is to investigate the potential of hUCBMNCs to prevent or repair white and grey matter lesions in an animal model of excitotoxic brain injury.

The animal model of perinatal brain lesions consisted of an intracranial injection of ibotenate, a glutamate agonist, in Sprague-Dawley rats and Swiss mice at P5. hUCBMNCs (from 10^6 to 10^7) were injected either intraperitoneally or intracranially immediately after the ibotenate injection. Effects of cell transplantation on brain lesions were studied with histological methods and immunohistochemistry.

In mice, single or repeated i.p. injections of 10^6 or 5.10^6 hUCBMNCs, immediately, 1 day or 3 days after the ibotenate injection did not have any effect on the lesion size neither in the cortex nor in the white matter.

In rat pups, the injection of 10^6 cells had no significative effect on lesion size. However, the injection of 10^7 hUCBMNCs immediately after ibotenate increased exclusively the white matter lesion size (40%, $p< 0,002$). This deleterious effect was associated with perilesional microgliosis.

Preliminary experiments with an intracranial injection of 10^6 cells showed a tendency towards a decrease in the cortical lesion size (24%, $p=0.06$) with no impact on white matter.

So, rats and mice respond in a species-dependent manner to xenotransplantation. Our results show a deleterious effect of the i.p. transplantation of 10^7 hUCBMNCs immediately after the excitotoxic insult in rat pups. In contrast, an i.c. transplantation of 10^6 cells was not associated with a deleterious action. All together, our results highlight the importance of the administration route in preclinical cell therapy experiments.

P1.132

High frequency stimulation of the subthalamic nucleus impacts adult neurogenesis in a rat model of parkinson's disease

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Chronic high-frequency stimulation of the subthalamic nucleus (STN-HFS) efficiently alleviates motor symptoms of advanced Parkinson's disease (PD). Here, we looked for possible STN-HFS-induced changes on adult brain neurogenesis in the hippocampus and olfactory bulb that may be related to non-motor deficits associated to PD, such as mood disorders and olfaction deficits. Cell proliferation (Ki-67 immuno-positive-cells) and survival (bromodeoxyuridine (BrdU)-immuno-positive cells) were assessed in the subventricular zone-olfactory bulb continuum and the dentate gyrus of the hippocampus of hemiparkinsonian rats with or without continuous STN-HFS for 8 days. Dopamine lesion impaired cell proliferation and survival through different mechanisms, the effect on proliferation being correlated to the level of dopamine depletion whereas the effect on survival was not. Prolonged STN-HFS did not affect cell proliferation, but increased cell survival bilaterally. In these regions of constitutive neurogenesis, the percentage of new neuroblasts (BrdU-doublecortin-positive cells) was unchanged, suggesting that STN-HFS can lead to a net increase in newly formed neurons later on. STN-HFS also increased new cell survival in the striatum and promoted dopamine system recovery detected by tyrosine hydroxylase immunostaining. These data provide the first evidence that prolonged STN HFS has a neurorestorative action and support the view that the action of this neurosurgical treatment can bypass the cortico-basal ganglia-thalamocortical loop circuits and largely impinge neuroplasticity and brain function.

P1.133

Loss of *PARK9* expression enhances the toxicity of α-synuclein and produce abnormal lysosomal accumulation in mammalian cells

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Recent evidence suggests lysosomal function impairment in Parkinson's disease (PD). Mutations in *ATP13A2*, a lysosomal type 5 P-type ATPase (*PARK9*) have been associated with hereditary parkinsonism as well as in some juvenile and young-onset PD. We have associated different cellular models and in vivo models to understand where the putative lysosomal dysfunction by loss of ATP13A2 function may lead to insufficient lysosomal protein degradation and membrane permeabilization. In fibroblasts from one patient harboring pathogenic *PARK9* mutations, we observed an abnormal lysosome accumulation under basal conditions compared to control fibroblasts by electron microscopy and with different lysosomal markers. Our data suggest that PARK9 fibroblasts may replicate some of the lysosomal dysfunction observed in PD patients. We propose that PARK9

fibroblasts may represent a unique cellular model to study the role of abnormal lysosomal function in the pathogenesis of PD without artifacts derived from over-expression or lack of endogenous translational regulatory elements. To confirm this hypothesis, we seek to analyze the lysosomal membrane permeabilization upon treatment with different stressors. To strengthen the results, we have knocked down ATP13A2 in M17 neuroblastoma cells overexpressing or not wild-type α-synuclein.. An enhancement in toxicity was observed when ATP13A2 was knocked down in neurons overexpressing wild-type α-synuclein confirming previous reports on the toxic link between α-synuclein and ATP13A2. To evaluate *in vivo* the role of endogenous ATP13A2, we will test whether knockdown of ATP13A2 with lentivirus-delivered shRNA would cause dopaminergic neurodegeneration and aggregates formation after SNpc-stereotactically injection. Overall, these results indicate that ATP13A2 mutations may participate in lysosomal-mediated dysfunction and PD-related neurodegeneration.

P1.134

Striatal medium spiny neurons of mouse models of Parkinson's disease generate gigantic GABAergic currents that are abolished by subthalamic nucleus lesion

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We have shown that in the striatum from 6-hydroxydopamine-treated mice, the projection neurons of the striatum (medium spiny neurons, MSNs) spontaneously generate gigantic GABAergic currents, single or in bursts due to excessive bursting of LTS interneurons (Dehorter, 2009). We investigated whether the same electrophysiological signature is present in the striatum of mouse models of familial forms of Parkinson's disease and whether it is sensitive to the lesion of the subthalamic nucleus (STN). We took the example of PINK1 KO mice where the loss of function mutations in the mitochondrial protein PINK1 (PTEN induced putative kinase) cause the recessive PARK6 variant of Parkinson's disease (PD) in humans. Evoked DA release and DA content become significantly decreased in the striatum of such mice when deficits in spontaneous locomotion appear in the absence of neuronal loss (Kitada 2007, Gispert 2009). We patch clamp recorded the spontaneous GABAergic currents in MSNs from 6-8 months-old control mice, PINK1 KO mice and PINK1 KO mice bearing a chronic lesion of the subthalamic nucleus (STN). We lesioned the homolateral STN with stereotaxic injection of kainic acid (0.3 µl, 1mg/ml), 2-4 weeks before recordings.
In 70% of the MSNs from control mice we recorded spontaneous, small amplitude (73 ± 11 pA), regular (4.5 ± 1.6 Hz), $GABA_A$-mediated currents. In the other 30% of MSNs, these currents were giant $GABA_A$ sPSCs (270 ± 6 pA, 0.4 ± 0.1 Hz). In contrast, in 71% of MSNs (10/14) from PINK1 KO mice, we recorded the gigantic $GABA_A$ sPSCs, single (340 ± 37 pA, 0.3 ± 0.1 Hz) or in bursts (77 ± 4 Hz). STN lesion entirely reversed the situation since 79% of MSNs (27/34) displayed the tonic pattern of small amplitude $GABA_A$ sPSCs (46 ± 2 pA, 2.9 ± 0.4 Hz) and only 20% still exhibited giant $GABA_A$ sPSCs, single (251 ± 16 pA, 0.2 ± 0.1 Hz) or in bursts (77 ± 13 Hz).
Our results show that dopamine depletion of the striatum in neurotoxic and genetic PD models lead to the same electrophysiological signature. These pathological striatal currents are strongly reduced by STN lesion which was used in patients to ameliorate PD motor signs. Thus, they constitute a functional correlate of early and possibly reversible stages of Parkinson's disease.

P1.135

EGCG improves learning and synaptic plasticity markers in Ts65Dn mouse model of Down syndrome

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Trisomy of chromosome 21 results in Down syndrome (DS), a disorder marked by mental retardation. Trisomy is associated with the overexpression of a large part of the HSA21 genes. It is generally thought that defects in plasticity may account for the cognitive disability. We have demonstrated that a YAC transgenic model carrying three copies of Dyrk1a, a kinase with multiple targets among which genes involved in synaptic plasticity and memory processes, present deficits in paradigms assessing long term memory. This deficit was shown to be rescued after a treatment, given from gestational age to adult stage, with an inhibitor of Dyrk1a, a polyphenol from green tea (PGT), the epigallocatechin gallate (EGCG). Ts65Dn, a more complex model with trisomy of 123 genes, is closer to the situation of Down syndrome: it exhibits reduced LTP in the dentate gyrus and poor learning in many memory paradigms. The effect of a chronic treatment with EGCG was assessed on adult male Ts65Dn mice after a one month treatment: using Morris water maze tasks we have shown that, after the treatment, Ts65Dn mice were reaching performance levels similar to the control mice. At the molecular level the effect of the treatment was assessed by investigating the levels of components of markers previously shown as Dyrk1a dosage sensitive: plasma homocysteine level and pathways involved in control of LTP and synaptic plasticity (CamKII, Akt, Mapk pathways): for most of these biomarkers the treatment rescues significantly these alterations. Another molecular feature of Ts65Dn is the excess of inhibition, consequences of which have been rescued by picrotoxine treatment. As GAD65 and GAD67 are controlling the level of GABA we have investigated the mRNA and protein levels and found that a PGT treatment decreases also the level of these proteins. These results open new perspectives for the treatment of Down syndrome patients with green tea extracts.

P1.136

Prenatal ischemia induces white matter damage, mild cognitive deficits and hyperactivity in adult rats

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Prenatal ischemia (PI) has been shown to provide a promising rat model of white matter damage (WMD) that induces hypomyelination, axonal degeneration, neuronal damage and astrogliosis in various brain areas, as observed in preterm infants with a history of perinatal hypoxia-ischemia (Olivier et coll., 2005; Delcour et coll., 2011). In addition to sensory and motor disabilities, many premature children exhibit deficits in visual and spatial memory, and spatial navigation, as well as attention deficits with hyperactivity disorders (ADHD). The aim of the present study was to assess cognitive impairments and the neuro-anatomical substratum in the involved brain areas of adult rats with WMD induced by PI. The PI was induced by unilateral ligation of the uterine artery in pregnant rats at E17. PI rats exhibited hyperactivity in open-field test. They also showed short-term memory deficits in the object-recognition and object-location tasks, suggesting visuospatial memory deficits as observed in premature children. These deficits were related to neuronal loss and astrogliosis found in the parahippocampal areas of PI rats. Short-term impairments may indicate attention deficits, possibly underlain by increased GABAergic interneurons and concomitant decreased glutamatergic neurons in the prefrontal cortex. In addition to possible attention deficits, hyperactivity in open-field likely recapitulates ADHD often observed in preterm children. PI rats also exhibited long-term memory deficits in object-recognition task that may involve learning disorders. However, PI rats did not exhibit any impairment in various Morris watermaze tasks, indicating an absence of spatial memory and working memory deficits after PI. This absence seems consistent with a lack of neuronal loss in the hippocampus of PI rats, highly involved in the spatial cognition. Our data shows a differential time-dependent vulnerability of hippocampal structures to ischemic insult at E17. Thus, this model of WMD and gray matter injury reproduces some of the cognitive deficits observed in premature children. It could allow us to better understand the neuro-anatomical substrates of some cognitive disabilities found in preterm children with a history of perinatal hypoxia-ischemia.

P1.137

Modulation of potassium KCa2 channels as an antiparkinsonian strategy

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Parkinson's disease (PD) originates from the progressive loss of dopaminergic neurons substantia nigra. L-DOPA therapy is effective on motor symptoms and motor fluctuations but leads over the years to dyskinesias. KCa2 channels (calcium-activated potassium channels, SK channels) have emerged recently as a potential drug target because they regulate neuronal firing of midbrain dopamine neurons. The involvement of these channels in the expression of motor symptoms at different stages of PD has thus been tested in different rodent models. Using systemic injection of apamin, a KCa2 channel blocker, we show that this compound can reverse haloperidol-induced catalepsy a pharmacological model of akinesia. This effect of apamin is blocked by intracerebroventricular injection of CYPPA, a KCa2 channel opener, showing the specificity of apamin action on KCa2 channels. Apamin reverses the effect of a partial 6-OHDA lesion in a reaction time task only after a 6 days subchronic treatment. Apamin potentiates apomorphine-induced contralateral rotations in the rotameter test following unilateral total 6-OHDA lesion. When administered alone acute apamin treatment induces ipsilateral rotations while subchronic treatment had no effect. These results suggest that apamin acts on the spared dopaminergic system in a partial lesion model of the disease. The fact that KCa2 channels are heavily expressed on dopaminergic neurons strengthens this hypothesis. Blocking these channels could increase the release of dopamine since it enhances the firing in bursts. However KCa2 channels are not only expressed on dopaminergic neurons but are widely distributed in the basal ganglia. Consequently anti-parkinsonian effects of apamin could also be mediated by the modulation of the dysregulated gabaergic and glutamatergic activity in these structures as well. Overall these results underlie the critical role of KCa2 channels in the physiopathology of PD and as a possible drug target. Funded by Fondation de France.

P1.138

Autosomic recessive hereditary spastic paraplegia-type SPG11: a *Drosophila Melanogaster* model

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SPG11 spastic paraplegia represents the majority (~21%) of autosomal recessive complicated forms. It is characterized by the degeneration of the pyramidal tract, the presence of a thin corpus callosum (TCC) and white matter abnormalities at brain MRI. The mechanisms underlying the degeneration of the corticospinal tract and other structures remain unknown. The disease is predominantly associated with loss of function mutations in the *SPG11* gene, encoding spatacsin.
SPG11 is conserved in *Drosophila*; (*dspg11*) exhibits 22% of similarity with its human ortolog.
We generated a fly model of SPG11 disease by RNA interference (RNAi) to investigate the function of SPG11 protein *in vivo*. Transgenic flies containing an inducible UAS-RNAi construct against *dspg11*

are available. Expression of this construct was possible exploiting the GAL4-UAS system. We used various tissue/stage-specific GAL4 drivers, to discriminate between developmental and adult-specific effects. When *dspg11* was inactivated during development, at ubiquitous as well as CNS-specific level, we observed up to 90% of pupal lethality; the rare escapers had a clear locomotion phenotype and a drastically decreased life span. *Dspg11* transcript levels, measured under ubiquitous inactivation conditions, were decreased of 60% in adult males and larvae, and of 40% in adult females. Inactivation (ubiquitous and CNS-specific) induced in adult flies conferred a normal phenotype and no difference in life span between induced/non induced flies.
In the hypothesis that dSPG11 plays a role in the control of axonal transport, like other human SPG proteins, we are also planning to analyze if there is an impairment of axonal transport of mitochondria and synaptic vesicles in dissected larvae in which the inactivation of *dspg11* is specifically driven in motoneurons.
Moreover, in order to identify possible dSPG11 interactors, we are planning a genetic screening with fly strains inactivated for other SPG-genes and for genes involved in potential common pathways: intracellular trafficking, transport, microtubules and actin metabolism.
From our study it emerges that dSPG11 seems to play an important role during development; further data are still necessary to delineate its exact function.

P1.139

Activators of the cyclic GMP pathway modulate cocaine self-administration by rats: involvement of the epigenetic parameters MeCP2 and HDAC2

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Previous results from our laboratory showed that elevating the intracellular levels of cyclic 3´,5´-guanosine monophosphate (cGMP) in several brain areas results in the inhibition of cocaine-induced events, such as dopamine release in the striatum, immediate early gene expression, or hyperlocomotion. In this study, we investigated whether activation of the cGMP pathway was able to modulate intravenous cocaine self-administration, an operant conditioning test recognized as the most adequate animal model for studying the reinforcing properties of a given substance. The natriuretic peptide CNP, an endogenous ligand of the membrane-bound guanylyl cyclase GC-B, and 8-bromo-cGMP, a membrane-permeant activator of the cGMP-dependent protein kinase, were directly injected into the medial prefrontal cortex (mPFC) of rats self-administering cocaine. The treatments decreased cocaine self-administration behavior dose-dependently under both a fixed and a progressive ratio schedule of reinforcement, which measure reinforcing properties of cocaine and motivation to obtain the drug, respectively. As the effect was noticeable only after a few days, the involvement of middle- to long-term mechanisms was explored. Since epigenetic processes were previously shown to be induced by cocaine self-administration, we checked whether these mechanisms were under the control of an elevation of brain cGMP. Immunohistochemical studies unveiled a decreased expression of the methylated CpG-binding protein MeCP2 and the histone deacetylase HDAC2 in the brain of CNP- and 8-bromo-cGMP-treated rats. The data therefore show that the cGMP pathway in the anterior cingulate cortex participates in the analysis of the reinforcing properties of drugs of abuse. Furthermore, they suggest that CNP and the cGMP pathway counteract some long-term effects of cocaine by inhibiting plastic modifications triggered by the drug.

P1.140

Transcriptome evolution and distinct gene expression profiles of brain aging and Alzheimer´s disease-like pathology in the primate *Microcebus murinus*

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Aging is the primary risk factor of neurodegenerative disorders such as Alzheimer´s disease (AD). However, the molecular events occurring during brain aging are extremely complex and still largely unknown. New approaches such as transcriptomic approaches are required to unravel the complex biology of brain aging and neurodegenerative disease. For a better understanding of these age-associated modifications, animal models as close as possible to humans are needed. In this study, we analyzed the transcriptome of the temporal cortex of *Microcebus murinus*, a relevant primate model for aging and AD studies because some of them present, as they age, ß-amyloid plaques and cortical atrophy, which are pathognomonic signs of AD in humans**.** We compared the transcriptomes of 6 young adults, 10 healthy old animals and 2 "AD-like" animals using human Affymetrix microarrays (HG U113plus2). Over the 14,911 transcripts detected present, 152 genes were identified with significant changes in their expression in relation with age or with AD pathology by ANOVA (p-value1%) whose 10 were used to confirm their expression by RT-PCR. Among these genes, 47 were revealed very discriminative by Significance Analysis of Microarrays. Hierarchical clustering indicated that each group showed a specific expression profile. In addition, Principal Component Analysis showed that each group could be distinguished from others. Biological function of these genes was investigated by Gene Ontology, EASE and pathways by IPA-Ingenuity softwares. Interestingly, the network including about twenty genes with significant expression changes in AD belong to neurological diseases, and some of them were common with others neurodegenerative diseases. In conclusion, transcriptomic analysis represents a relevant approach to investigate molecular changes occurring during complex process like aging and AD. Because we identified distinct and specific profiles for each group, these patterns represent a specific signature for each group. These results open the way to explore physiological cerebral and "AD-like" aging in primates.

P1.141

Resistance to epilepsy: a new genetic model in the mouse to understand the physiopathology of absence epilepsy

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Absence-epilepsy is a prototypic form of generalized non convulsive epilepsy characterized by short impairments of consciousness concomitant with synchronous and bilateral spike-and-wave discharges (SWD). Two main genetic models for absence-epilepsy have been described in the rat (i.e. GAERS and WAG/Rij) for which control or « neutral » strains with no SWD were also selected. However, an animal model with a *resistance* to epilepsy, instead of a neutrality, could be very informative, as it is likely that resistance involves different mechanisms than susceptibility.
Two mouse lines were derived for either their hyper- (BS/Orl) or hypo-susceptibility (BR/Orl) to convulsive seizures induced by methyl-ß-carboline-3-carboxylate (ß-CCM). These lines also differ in terms of electroencephalographic activity: BS/Orl displays spontaneous, bilateral and synchronous SWD whereas BR/Orl do not. This is in line with the greater susceptibility of GAERS to the convulsive effects of ß-CCM and suggest a genetic link between the mechanisms which control ß-carboline-induced seizures and those controlling absence-epilepsy. The hypothesis that BR/Orl are not prone to SWD, rather than a neutral line was tested by injecting pentylenetetrazol (PTZ) or gamma-butyrolactone (GBL), two SWD-inducers. Significantly higher doses of PTZ or GBL were necessary to induce SWD in BR/Orl, as compared to BALB/c, CBA/H and C57BL/6J. Indeed, the doses of PTZ which were able to induce 50% of the time spent in SWD were 23, 27 and 60 mg/kg respectively for BALB/c, CBA/H and C57BL/6J. In BR/Orl mice, the dose of 60 mg/kg of PTZ was only able to induce 20% of the time spent in SWD. This mouse model, and more especially when combined with BS/Orl, offers therefore a unique tool to carry out genes and physiological pathways involved in resistance to absence-epilepsy.

P1.142

Role of serotonergic and noradrenergic autoreceptors in amphetamine behavioural and neurochemical sensitisations

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Addicts have difficulty to stop their consumption of drug of abuse and avoid relapse, even after long periods of withdrawal. Long term neuronal modifications may explain this feature of addiction. Behavioural sensitisation induced in rodents by repeated injections of drugs of abuse seems therefore an accurate model to study these long term neuronal changes, this phenomenon lasting several months and being specific to drugs of misuse. Previous results obtained by microdialysis have shown that the development of behavioural sensitisation is correlated with a long term hypereactivity of noradrenergic and serotonergic systems (1). However, mechanisms explaining maintenance of this hypereactivity are still unknown. We postulate now that a maintained disinhibition of these systems can explain these enhanced responses. Actually, noradrenergic and serotonergic neuronal firing activities are in part controlled by α2a-adrenergic autoreceptors located in the locus coeruleus and 5-$HT1_A$ serotonergic autoreceptors located in the raphe nuclei, respectively. We have studied the sensitisation state of these autoreceptors in amphetamine sensitized C57BL/6J mice by using two functional methods. First, GTPγS binding to 5-$HT1_A$ receptors was performed in the dorsal raphe nucleus after 4-day or 3-week withdrawals. Second we have measured by microdialysis the efficiency of α2a-adrenergic and 5-HT_{1A} receptor agonists, clonidine and 8-OH-DPAT respectively, to decrease basal extracellular levels of noradrenaline and serotonin in the prefrontal cortex of sensitised animals. Both **GTPγS and microdialysis experiments indicate that a desensitisation of these autoreceptors occurs after 4-day and 3-week withdrawals**. Moreover, dexefaroxan, an α2a-adrenergic receptor antagonist, can reproduce, in naïve animals, noradrenergic hypereactivity found in sensitised mice (1). Our results suggest that noradrenergic and serotonergic inhibitory feed-backs could be indirect targets of amphetamine and that maintained desensitisations of autoreceptors explain long-term effects in addiction.

(1) Salomon L, Lanteri C, Glowinski J, Tassin JP. Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. (2006) P.N.A.S. (USA) (Track II) 103: 3476-3481.

P1.143

Metyrapone modifies systemic and cerebral energy metabolism

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Metyrapone is a cytochrome P450 inhibitor that protects against brain damages elicited by ischemia and excitotoxicity. The aim of this study was to examine whether its neuroprotective effect would be related to changes in energy metabolism. In a first experimentation,33 rats were instrumented with telemetric devices to measure abdominal temperature and locomotion. They received either 150 mg.kg-1 of metyrapone (n=17) or saline (n=16). One hour after injection, their blood and brain were

sampled. Metyrapone acutely decreased locomotion and provoked a slight hypothermia. Metyrapone did not modify blood corticosterone level but changed systemic metabolism with increased blood glucose and lactate concentrations. Moreover, hyperglycemia was negatively correlated to abdominal temperature and locomotion. Hippocampal metabolites concentrations were measured using 1H high-resolution magic angle spinning magnetic resonance spectroscopy (1H HRMAS MRS). Compared to saline rats, metyrapone rats exhibited decreased GABA and glutamate levels but increased glutamine and N-acetyl-aspartate contents in the hippocampus. Western blot analysis showed that mTOR and AMPK hippocampal phosphorylated levels were similar between treatment groups. No difference in brain c-fos and HSP70-2 mRNA transcription was observed between groups.
In a second investigation, 20 rats received either metyrapone (150 mg.kg-1, n=10) or saline (n=10). One hour after injection, their brain was sampled and the mitochondria functioning was studied. No difference among groups was observed in oxygen consumption whatever the substrates used. In conclusion, it appears that metyrapone would modify systemic and brain metabolism in line with its neuroprotective properties without affecting blood glucocorticoid levels.

P1.144

Characterization of mice with GFAP knock-in mutations, as models of Alexander disease

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Introduction: Alexander disease (AXD) is a rare leukodystrophy due to dominant mutations in glial fibrillary acidic protein (GFAP), the main intermediate filament protein (IF) in astrocyte. AXD is characterized by cytoplasmic protein aggregates containing ubiquitinated GFAP and other proteins, such as small heat shock proteins.
Astrocytes exert many essential complex functions at the interface of other neural cell functions in the central nervous system. Astrocytes are organized in networks and communicate through specialized channels gap junctions.
They participate to synapse maturation during development and in the mature brain by controlling neurotransmitter metabolism; they are pivotal in water homeostasis, influencing blood-brain barrier integrity, and controlling extracellular homeostasis. They also participate in processes involved following different types of lesions (reactive gliosis).
To investigate the pathophysiological mechanisms of GFAP mutation, two knock-in mouse models bearing different mutations have been generated (Clinical Mouse Institute, Illkirch) that are currently studied.
Result: We notice an increase of GFAP, vimentin and nestin (other IF partners) expression in hypertrophic astrocytes in several brain regions in KI mice indicating a reactive gliosis. We also observe an accumulation of alpha B-crystallin, a chaperone involved in the formation of the intermediate filament network. Iba1 overexpression indicates a microglial reactivity. The presence of vimentin and nestin (FI partners) and especially the MTOC (microtubule organizing center) in huge peri- or juxtanuclear GFAP aggregates, suggests that these structures could be ´aggresome´ structures. We also observe an increase of Ki67 labeling in different regions
Conclusion: The presence of aggresomes and their role, protective or harmful, in the pathophysiology of AxD has to be confirmed and clarified.
The increase of Ki67 labeling could be due to reactive gliosis, and/or result from the proliferation of other neural cell types.
This work is supported by ELA Research Foundation, Jerome Lejeune Foundation, and INSERM.

P1.145

Loss of tyrosine hydroxylase expression in the Substantia nigra and ventral tegmental area and reduced locomotor activity in rat with minimal hepatic encephalopathy

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Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of chronic liver disease. The underlying mechanisms for HE are still not fully understood. Liver failure is generally associated with changes of many neurotransmitter systems including dopamine which is an important neurotransmitter implicated in various brain functions including its role in voluntary movement. The present study aims to describe the effect of liver failure due to double bile duct ligation (BDL for 4 weeks) on voluntary locomotor activity using the open-field test, and the dopaminergic system in the brain using the immunohistochemistry of the tyrosine hydroxylase (TH) within the ventral tegmental area (VTA) and the substantia Nigra pars compacta (SNpc). Using the open-field test, our data show a significant loss of voluntary locomotor activity in cirrhotic rats as compared with sham-operated animals. Immunohistochemistry using TH antibodies showed evidence for a decreased TH-immunoreactivity in both SNpc and VTA of cirrhotic rats which may reflect a compensatory mechanism to increased dopamine turnover known to be increased in several animal models of HE. Our data also show a significant reduction of TH-immunoreactivity within the nigro-striatal projections. Alteration of the nigro-striatal and thalamo-cortical circuitries may be involved in locomotor activity changes of cirrhotic rats.

P1.146

Glial modulation of medial prefrontal cortex deep brain stimulation

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Purpose of the study: High frequency deep brain stimulation (DBS) of the subgenual cingulate gyrus 25 produces a surprising fast antidepressant response in treatment-resistant depressed patients (Mayberg et al., 2005). Although the mechanism of action of DBS is not yet fully defined, it has been shown that rat infralimbic prefrontal cortex (IL-PFC) DBS induces a rapid antidepressant-like behaviors that is prevented by a lesion of the serotoninergic (5-HT) system and curiously unchanged after an IL-PFC neuronal ibotenic acid lesion (Hamani et al. 2010). In the present study, the effects of DBS on 5-HT neuronal activity and hippocampal metaplasticity were investigated in sham operated and IL-PFC glia-lesioned rats to explore a putative cortical non-synaptic modulation of DBS.
Methods used: Unitary extracellular recordings of dorsal raphe nucleus 5-HT neurons were carried out in anesthetized Sprague-Dawley rats before and after 1 hour of bilateral stimulation (130Hz) of IL-PFC. Moreover, we have evaluated the effects of unilateral IL-PFC DBS on field excitatory postsynaptic potential recorded in the CA1 area of dorsal hippocampus prior and after a low and a high frequency stimulation of the Schafer's collaterals (LFS and HFS). Both recordings were assessed in sham operated and lesioned rats with the astrocyte specific toxin L-α-aminoadipic acid (100 µg/µL/hemisphere).
Summary of the results: IL-PFC DBS increased the spontaneous firing rate of 5-HT neurons by ≈30%. Interestingly, this enhancement was attenuated by the glial lesion. As expected in the dorsal hippocampus of sham operated rats, LFS failed to induce a long term depression (LTD) whereas HFS provoked a long term potentiation (LTP) of ≈20%. Unexpectedly, concomitant IL-PFC DBS induced a LTP of ≈20% after LFS and a doubled LTP after HFS. Importantly, the glial lesion significantly reduced the enhancing action of the concomitant IL-PFC DBS. The effects of IL-PCF DBS on hippocampal neurogenesis are actually assessed.

Conclusions: Taken together, these in vivo electrophysiological results unveil for the first time a key role of the glial system in the mechanism of action of medial prefrontal cortex deep brain stimulation.

P1.147

Automated subcellular imaging for drug screening in *C. elegans*

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Screening for drugs at the subcellular level may enhance drug discovery for degenerative disease. To develop drug screening at the subcellular level in nematodes, we took advantage of the Plate Runner HD® (Trophos, France), a 96/384-well device that collects fluorescence at resolutions ranging from 1024x1024 to 8192x8192. This device has high depth-of-field, thus allowing fluorescent signals to be quantified from whole animals after paralysis. This device also allows a single image of the whole well to be acquired at once.
We developed and normalized a drug screening assay at the subcellular level to search for compounds that protect from mutant PABPN1, the oculopharyngeal muscular dystrophy (OPMD) protein. Transgenic nematodes co-expressing GFP and mutant PAPBN1 in body wall muscles show defective motility, a phenotype that is accompanied by muscle cell degeneration. These phenotypes are also accompanied by the loss of GFP nuclei, a subcellular phenotype that we used for drug screening. The results obtained after screening greater than 2000 compounds indicate the screen is robust and selective.
As part of a drug discovery program on neuromuscular diseases, we also developed a drug screening assay at the subcellular level to search for compounds that may protect from defective axonal transport. Here, we generated nematodes that carry a weak, temperature-sensitive allele of dynactin-1, an axon motor involved in retrograde transport, and that express a pre-synaptic fluorescent reporter in motor neurons. Preliminary data suggest that the dynactin-1 mutation induces a strong change in the intensity and size of fluorescent signals at permissive temperature. This effect is unrelated to a change in transgene expression and can be quantified by the Plate Runner after paralysis of the animals in the 96-well plate. An assay is being normalized to screen chemical libraries.
Data will be presented to illustrate the use of our method for sub-cellular imaging and drug screening in *C. elegans*.
Work supported by Inserm and AFM.

P1.149

Reaction time of neurons submitted to operant conditioning in a brain-machine interface task

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Among the different strategies used to implement a brain-machine interface, operant conditioning of neurons is a simple method that has proven robust enough to restore goal-directed movements using a prosthetic device [Moritz 2008, Schmidt 1980]. Although multiple conditioning on several neurons has been investigated for a long time, the precise temporal relations of spike discharge in a small network during training have not yet received full attention.
Here, head-fixed awake Wistar rats were trained to increase the firing rate of motor cortex neurons, such as to control in a graded way the position of a prosthetic device (on which was placed a liquid

reward) along a 1D-axis. Each time the smoothed neuronal activity crossed a threshold controlled by the experimenter, a water bottle moved on a linear axis perpendicular towards the rat mouth until it was within tongue reach, allowing the animal to drink. Several neurons were recorded simultaneously throughout training using microwire arrays (MWA), and the operant conditioning task could be carried out successively in the same animal by choosing a different single unit, for each training session, as the operant response. Peri-event time histograms around trial start were computed off line and showed that successful learning was associated with a significant increase in the firing rate of the conditioned unit after 100-200 ms. Unconditioned neurons sometimes showed a significant change in their firing rate as well, but the conditioned neuron tended to have a shorter latency. This finding suggests that neurons not directly involved in the conditioning phase could be recruited in a newly formed assembly of neurons that collectively increase their activity in relation with the task, sequentially to the early signaling by the conditioned neuron. This serial regulation process could be of interest for designing fast and efficient real-time prosthetic devices.
Funded by CNRS, ANR (NATACS), EU FP6 FET (FACETS), EU FP7 FET Open (Brain i Nets) and EU FP7 FET (BrainScales). PJ A. thesis was supported by ENP and FRM.

P1.150

What is mapped in the barrel cortex? A two-photon study of the topography of sensory inputs in layer 2/3 of the barrel cortex

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Layer 2/3 neurons in the barrel cortex may represent the substrate for multiwhisker integration. Indeed, receptive fields of these cortical neurons are large and cover several whiskers. In addition, this layer displays dense input connections coming from several adjacent barrels.
The primary somatosensory barrel cortex is endowed with several complementary maps of neuronal functional responses: one is the mapping between the whiskers and layer 4 barrels; another is the map of the tuning to the direction of whisker deflection: a tuning that develops during the rat adolescence (Kremer et al., 2011 under revision). Finally, a recent study using reverse correlation methods has revealed an additional dimension of whisker stimulation encoding: the phase. This study also brought initial evidence of the spatial coherence of adjacent neurons for phase encoding.
We hypothesized that both single whisker deflections and inter whisker interactions are encoded in a topographical manner in layer 2/3, from the scale of whiskers spatial arrangement, down to the direction and the phase of whisker deflections.
To explore this potential multi-scale topographical mapping of barrel cortex processing, we developed a two-photon imaging system incorporating an adapted version of a 24 whisker stimulation device (Jacob et al., 2010) that had been previously used to demonstrate phase encoding in the barrel cortex (see the abstract by Estebanez et al., 2011). The two-photon microscopy setting was specifically designed to allow an efficient exploration of neuronal activity. It allows the real-time display and recording of structures (sulforhodamine staining) and functional activity (calcium imaging) over large fields of view (300 x 300 µm) and with a high imaging rate (40Hz).
We designed two protocols, addressing
a) the mapping of first order properties (whiskers, direction, phase), and
b) the mapping of second order interactions (effects of multi-whisker deflections with different phase and direction relations).
We will present our first observations on the mapping of sensory stimuli in the barrel cortex, obtained using this experiment setup and this protocol.
Funded by ANR (NATACS and TRANSTACT), EU FP6 FET (FACETS), EU FP7 FET Open (Brain i Nets) and EU FP7 FET (BrainScales).

P1.151

Texture-whisker transduction in anaesthetized rats: influence of sampling conditions

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Rats gather information on objects and textures with their whiskers. In this study, we characterized how texture micropatterns are transduced into a temporal series of force on the whisker and how this depends on the whisker geometry and exploratory conditions. For this, we mimicked a situation where a rat explores a texture and sweeps its whiskers on it.
We recorded movements of β, C1 and C2 whiskers in anaesthetized animals (urethane/n=6). Using custom-made textures with well-controlled 1D roughness (random pattern with a mean period of 0.6mm), we excited vibrissae with white noise tactile stimulus and studied the resulting dynamics of whisker movements. A motorized rail moved the textures at different speeds (100/150/200mm/sec) and radial distances ($d_{skin\text{-}texture}$: 14.5-32.7mm). Whiskers were filmed with a high speed video camera (Photron, Fastcam) at 2kHz.
Considering whiskers as mechanical oscillators, resonant mode decomposition associated with random noise stimulation allow us to extract dynamic parameters. Our model reliably predicts the frequency of each mode. We found that the shorter the whisker, the higher the amplification performed by the vibrissa ($p< 10^{-15}$)[a] while this amplification also increases with the texture speed in a less substantial manner ($p< 3.10^{-11}$)[a] (n=768).
From the whisker dynamics we extracted the instantaneous friction force exerted by the texture on the whisker tip. This force was cross-correlated with the substrate topography, yielding a linear transfer function. We found that transfer functions can be classified into two families of similar shape but opposed polarity, corresponding to a preference for bumps or troughs (n_{trough}=40/n_{bump}=59). The width of those transfer functions can depend on whisker identity (p_{trough}=0.0001/p_{bump}=0.07)[b] but not on radial distance (p_{trough}=0.22/p_{bump}=0.82)[b]. Texture speed also influenced the width of this integration window (p_{trough}=0.02/p_{bump}=0.0003)[b].
We are now achieving a prediction of the vibrissa motion from the topography of a substrate exciting the whisker by using the transfer functions computed by our method.
[a]: ANOVAN / [b]: MANOVA
Funded by CNRS, ANR (NATACS and TRANSTACT), EU FP6 FET (FACETS), EU FP7 FET Open (Brain i
Nets) and EU FP7 FET (BrainScales).

P1.152

Nonlinear integration of multiwhisker tactile stimuli in the rat somatosensory system

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While exploring objects, rats make multiple contacts with their whiskers. Our goal is to understand how the different stages of the somatosensory system process this information. We have shown previously that responses in the barrel cortex, the primary somatosensory area representing the whiskers, are modulated by the spatiotemporal pattern in which whisker deflections occur (Jacob et al. 2008, Neuron).

Here, we explored whether emergent properties of multiwhisker stimulations are already encoded by sensory neurons in the ventro-posterior medial nucleus (VPM) of the thalamus, projecting to the barrel cortex. Using a 24-whisker stimulator centered on whisker C2 , we first characterized the receptive fields of VPM neurons in urethane-anesthetized rats using forward correlation analysis. We selected cells responding preferentially to C2 and tested them with sequences of caudal deflections that generated an apparent motion in 8 different directions across the mystacial pad.
45% of VPM neurons (n=33/73) exhibited an increase in firing rate significantly selective to the apparent direction of motion of this global stimulus. Periods of suppression of firing rate were often observed, but were rarely selective to the direction of apparent motion (5/73). Direction selectivity was unrelated to the extent or structure of receptive fields, or to the selectivity for the local direction of motion of whisker C2.
Recordings of trigeminal ganglion cells, the first-order neurons of the vibrissal pathway, showed no selectivity in these monovibrissal neurons. Thus, the global selectivity of VPM neurons could be generated a) in the thalamus or before by nonlinear integration of multiwhisker stimuli, or b) in the barrel cortex, modulating VPM responses through cortico-thalamic feedback connections. On a subset of neurons, we also recorded while inactivating the barrel cortex by applying Mg^{2+} on its surface. For most VPM neurons, the direction selectivity decreased (10/13) but was not abolished. These results suggest that nonlinear integration of stimuli from different whiskers emerges in subcortical nuclei and is amplified by the trigemino-thalamo-cortical loop.
Funded by ANR (NATACS and TRANSTACT), EU FP6 FET (FACETS), EU FP7 FET Open (Brain i Nets), EU FP7 FET (BrainScales) and HFSP.

P1.153

Coexistence of neuronal coding specific to different levels of spatial correlation in a sensory cortex

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Rodents explore their tactile environment by touching objects with several whiskers simultaneously. The level of correlation between whiskers may vary greatly: correlated when large scale objects are contacted; uncorrelated during sweeping on textured surfaces.
The encoding of inter-whisker correlation, however, is unknown. We investigated this question with a new multi-whisker stimulator that delivers any stimulus to the 24 caudal whiskers of rats (Jacob et al., 2010): we applied Gaussian noise with different levels of inter-whisker correlation on the right 24 caudal whiskers, while extracellularly recording single units (silicon probes) in the primary somatosensory barrel cortex. Using spike triggered covariance analysis, LN models of the neuron functional response were built for each correlation level. This analysis provided an estimate of the functional selectivity of neurons for these different conditions, revealing that sensory inputs are encoded in the barrel cortex by two coexisting coding schemes, which corresponded respectively to low and high levels of correlation.
At low levels of inter-whisker correlation, we distinguished two categories of barrel cortex neurons, analogous in the temporal domain to the well-known 'simple' and 'complex' cells.
When increasing the level of correlation, many of these neurons displayed dramatic shifts in their receptive field properties and were found to be specifically tuned to local centre/surround antagonist stimulations. Remarkably, an otherwise unresponsive sub-population of neurons was specifically sensitive to correlated whisker deflections across the whisker pad, thus responding only to coherent stimuli. Such responses are reminiscent of those reported in the higher order visual area MT/V5, an area that analyses visual motion.
In conclusion, by studying the cortical representation of spatial correlation, we found that several coding strategies can coexist among the same population of sensory neurons. Similar dependence to correlation could exist in other sensory areas, providing a general principle by which sensory systems detect coherent structure in the incoming stimuli.
Funding: ANR NATACS, HR-CORTEX, TRANSTACT ; EU FP6 FET (FACETS), FP7 FET Open (Brain i Nets), FP7 FET (BrainScales).

P1.154

Descending hypothalamic A11 dopaminergic projections to the medullary dorsal horn

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Descending pain-modulatory systems, either inhibitory or facilitatory, play a critical role in both acute and chronic pain. While descending noradrenergic and serotonergic controls have been intensively investigated, the actions of descending dopaminergic controls, originating from the hypothalamic A11 nucleus, on spinal and medullary dorsal horn (SDH and MDH, respectively) are less known. The aim of our study was to characterize the A11 dopaminergic projections to the MDH.
Combining behavior (orofacial formalin test: 50µl, 1.5% in the upper lip) and phospho-ERK immunocytochemistry (anatomical marker of neuronal activation), we demonstrate that neurons in the A11 nucleus are activated following persistent noxious trigeminal stimulation. Our immunohistochemical analysis shows that the A11 nucleus contains different types of neurons: dopaminergic (tyrosine hydroxylase positive; TH; 201 ± 12 neurons/side), calcitonin gene-related peptide (CGRP; 62 ± 11 neurons/side) and GABAergic (GABA decarboxylase positive; GAD; 200 ± 64 neurons/side); 17% of TH-positive neurons are also CGRP-positive. Microinjections of the retrograde tracer, fluorogold (FG), into MDH reveal that A11 projections to MDH are bilaterally organized with about 20% of dopaminergic neurons (TH-positive/FG-positive co-labelling) throughout the A11 nucleus projecting to the MDH. Interestingly, only 60% of the A11 neurons projecting to the MDH are dopaminergic neurons. We are currently assessing the neurochemical phenotype of the other 40% A11 neurons projecting to MDH.
Therefore, descending hypothalamic A11 projections to the MDH control tonic trigeminal nociception. While descending A11 projections to the SDH are ipsilateral, those to the MDH are bilateral. Finally, descending A11 projections to the MDH are not exclusively dopaminergic raising the question as to what is the phenotype of the other A11 neurons projecting to the MDH.

P1.155

Adult neurogenesis improves olfactory discrimination learning and memory

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Thousand of local interneurons are continuously recruited every day in the olfactory bulb (OB) of adult rodents. The impact of incorporating the newcomers into the preexisting neuronal circuits is still debated for olfaction. Here we assessed the role of adult neurogenesis on odor discrimination learning and memory using channelrhodopsin selectively expressed by adult-born bulbar neurons. We find that exciting adult-born neurons during an odor discrimination task, on a go/no-go operant conditioning, dramatically accelerates the rate of learning. This boosting effect was observed when light stimuli were paired with odorant exposure, but not when lagging 500 ms after the odorant exposure. Remarkably, 40 Hz light stimulation was efficient in facilitating the discrimination learning, but not when delivered at 10 Hz. When mice were examined again 50 days later, those who received paired light stimuli delivered at 40 Hz, exhibit stronger olfactory memory than control mice. Finally, patch-clamp recordings of mitral cells revealed that 40 Hz-stimuli provide the bulbar output neurons with a tonic GABAergic action while 10 Hz-stimuli elicit phasic GABA responses

Overall, this study reveals, for the first time, that inhibition mediated by adult-born neurons improves odorant discrimination learning and olfactory memory. We propose that adult-born neurons achieve this boosting effect by improving the function of pattern segregation ensured by the OB circuitry.

P1.156

Slow oscillation of membrane potential participates to the determination of respiratory spiking patterns of mitral/tufted cells, in freely breathing anesthetized rat

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An important characteristic of olfactory bulb activity is its modulation by respiration. This modulation is distinguishable by the existence of several respiratory spiking patterns and the presence of slow oscillations of the membrane potential. Relationships between these two activities have never been studied. In this study, we recorded intracellular activity of mitral/tufted (M/T) cells in order to examine relationships between the two forms (positive and negative forms) of intracellular slow oscillations (ISOs) and the different discharge patterns among which: excitatory-simple-synchronized patterns (S+: single increase in firing activity synchronized with the respiratory cycle), inhibitory-simple-synchronized patterns (S-: single decrease in firing activity synchronized with the respiratory cycle), and non synchronized patterns (noS: uniform distribution of action potentials along the respiratory cycle).
We showed that, both discharge patterns and ISOs were modulated by odor stimulation. The proportion of cells presenting S+ or S- respiratory-synchronized pattern together with an ISO is increased during odor stimulation. Two strong relationships were observed, during control period as well as during odor stimulation: i) noS pattern was only combined with none oscillating membrane potential and ii) S- patterns only with ISO- form. Furthermore, cells exhibiting S+ pattern never presented an ISO- form. Surprisingly, some cells with respiration-synchronized discharge pattern did not exhibit any observable ISO. We hypothesized these cells received a rhythmic input but ISO were not observable. This was supported by our M/T cell model showing that M/T cells receiving rhythmic input exhibited synchronized discharge activity at resting MP even if no ISO was distinguishable. Taken together, these results suggest that ISO participates to the synchronization of discharge activity on the respiratory cycle.
This work was supported by a grant from ANR-07-neuro-030.

P1.157

Contribution of the voltage-gated sodium channel Nav1.9 to inflammatory pain hypersensitivity

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Inflammatory pain perception is the result of an increased excitability of sensory neurons. This process involves the modulation of many ion channels resulting in a complex but poorly understood mechanism.
Among all these channels, the Nav1.9 sodium channel is particularly interesting because
1) it is selectively expressed in nociceptors,

2) it generates persistent voltage-gated current that is thought to contribute to subthreshold electrogenesis and
3) it is upregulated by inflammatory mediators. Collectively these data suggest a key role of Nav1.9 in inflammatory pain.
In this study, we examined the role of Nav1.9 in both persistent and chronic inflammatory pains. We combined behavioral, molecular and electrophysiological approaches . Persistent inflammatory pain was induced by intraplantar injection of Carrageenan, which produces an inflammation lasting for at least 48h. The chronic model of monoarthritic pain was induced by a peri-articular injection of Complete Freund Adjuvant (CFA), which produces an inflammation over 21 days. In the persistent inflammatory pain model, Carrageenan caused thermal hyperalgesia and mechanical allodynia, which were significantly reduced in Nav1.9 knock-out (KO) mice compared to wild-type animals. These behavioral data were correlated with an increased expression of Nav1.9 in the cell body and nerve terminals of sensory neurons. In the chronic inflammatory pain model, Nav1.9 KO mice showed diminished thermal hyperalgesia and mechanical allodynia.
Overall this work demonstrates the fundamental role of the sodium channel Nav1.9 in early stage of inflammationory pain. Further studies should clarify and characterize the underlying mechanisms.

P1.158

Morphological and molecular changes in mouse olfactory epithelium following postnatal odorant exposures

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Within the olfactory epithelium of Vertebrates, specialised neurones (olfactory sensory neurones or OSNs) represent the first step of the olfactory system and are responsible for the detection of odorant molecules. At their apical pole, ciliary structures bear the sites for odorant binding and olfactory transduction events. At the basal part of OSNs, axons project to the olfactory bulb. Therefore, olfactory neurones form an interface between the outside world and the brain. Another peculiar property of these neurones, is that they are constantly renewed which suggests a level of plasticity. While many reports addressed the consequences of long term odorant exposures on behaviour and CNS properties, little data exists on the olfactory epithelium itself. We address this question through anatomical and molecular approaches. MOR23-GFP and M71-GFP transgenic mice were exposed to lyral or acetophenone daily for 21 days starting at birth.
First, animals were killed, dorsal olfactory epithelia dissected, fixed and flatmounted. Fluorescence images were taken and the whole septal olfactory epithelium was reconstructed. GFP-containing neurones were counted and plotted against the total septal olfactory epithelium surface. In control mice, MOR23-GFP neurones' density was 82.8 +/- 5.6 neurones/mm^2 (n=12). Postnatal exposition to lyral dramatically reduced the density of MOR23 containing neurones to 29.4 +/- 4.2 neurones/mm^2 (n=10, $p< 0.001$). In order to verify whether lyral only affects MOR23-GFP or has unspecific effects we exposed M71-GFP mice to this odorant. In these conditions, no reduction of M71-GFP containing neurones could be seen. Meanwhile, the postnatal exposition to acetophenone does not reduce the density of M71 neurones in M71-GFP mice.
Second, we aimed to investigate molecular changes within individual OSNs following odorant exposures. Transcriptomes of isolated GFP-containing neurones were quantitatively analyzed using real-time qPCR. CNGA2, ACIII and olfactory receptors (M71 and MOR23) mRNA levels were drastically down-regulated in exposed mice (to acetophenone and lyral respectively).
Taken together, these observations suggest that odorant exposures lead to profound cellular and molecular changes within the olfactory epithelium during development.

P1.159

Proprioceptive integration in adolescent idiopathic scoliosis: postural control deficits could be explained by position and / or movement sense impairments ?

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Idiopathic scoliosis is a developmental pathology which expresses spinal deformity mainly during adolescence. Adolescent idiopathic scoliosis (AIS) patients exhibit deficits in balance control, which could be linked with a multisensory integration disorder. In the present research, we investigated the hypothesis of a differential integration of the proprioceptive inputs, by focusing both on static proprioceptive cues (position sense), assessing by slow oscillation paradigm and dynamic proprioceptive cues (movement sense), assessing by tendon-vibration stimulations.
In Experiment 1, 10 AIS patients (mean Cobb angle: 18.3° +/- 5°, 10°-35°) and 10 age-matched healthy adolescents (CS) were asked to maintain their vertical body orientation with or without vision, despite the imposed slow lateral oscillations of the support, at chosen amplitude (+/- 5°) and frequency (0.01 Hz) below the semicircular canal threshold. In Experiment 2, two groups of AIS and CS participants were submitted to 12-s Achilles tendon or tibialis tendon vibration stimulation (100 Hz) in a standing and in a sited position. In such situations, we analysed respectively the postural responses and the illusory perceived foot movements induced by the vibratory stimulation. In both Experiment 1 and 2, orientation and segmental stabilization were analyzed at head, shoulder, trunk and pelvis levels with kinematics recordings (SMART automatic motion analyzer).
Firstly, Experiment 1 showed no difference between CS and AIS in both orientation and stabilization components of postural control: without vision, the two groups tended to slightly follow the movement of the platform, especially at trunk level. Secondly, vision improved postural performances in CS as well as in AIS participants particularly in postural orientation. Data of Experiment 2 are currently acquired.
To conclude, when static proprioceptive sub-system was challenged, the postural control of both control adolescents and AIS patients was affected, suggesting that this neglect of the static proprioceptive cues relies more on a developmental effect than a pathological effect. Therefore, proprioceptive disorders in AIS could be rather reflected by an impairment of the sense of movement, as speculated with tendon vibration investigations.

P1.160

Stabilization of mutant rhodopsin in treatment of retinal degeneration in Retinitis pigmentosa

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Retinitis Pigmentosa (RP) is an inherited disease that progressively leads to blindness. Mutations in the rod photoreceptor and prototypical G protein coupled receptor rhodopsin associated with RP have been shown to cause misfolding of rhodopsin, but this knowledge has not yet been leveraged in developing treatments for RP. We showed recently that the chlorophyll-derivative, chlorin e6 (Ce6), alters rhodopsin structure and stability *in vitro* [1]. Computational docking and NMR studies demonstrate direct binding of Ce6 to rhodopsin in its cytoplasmic domain. Thermal denaturation studies at 55^{o}C followed by circular dichroism and fluorescence spectroscopy show that the secondary and tertiary structure of rhodopsin is stabilized in the presence of Ce6, both in terms of shifting the denaturation mid-point temperature values to higher temperatures, as well as a significantly increased retention of helix content at 100^{o}C. To test of stabilization of wild-type rhodopsin by Ce6 may impact

and possibly prevent misfolding of rhodopsin carrying RP mutations, we created stable cell lines of the RP mutants P23H and N15S. In the presence of added 9-cis retinal during expression, both mutants are stabilized and form wild-type like chromophore. The purified mutants were analyzed with regard to their structure and stability in vitro in the presence and absence of Ce6. To test if the stabilizing effects of Ce6 on rhodopsin in vitro may alter RP progression in vivo, we studied the RP rat models, P23H and S334ter. Electroretinographic and retinal tissue analysis indicate that Ce6 exerts a positive functional effect on P23H in vivo, slowing the rate of photoreceptor degeneration. This is supported by an observed increase in the ERG waves and an overall preservation of the retina during the treatment period. In contrast, Ce6 enhanced photoreceptor degeneration of the S334ter rat in vivo. We are currently generating a stable cell line of this mutant to investigate the molecular basis for this in vivo effect. In summary, our studies indicate that Ce6 affects RP progression in vivo and the effects are likely linked to the binding of this molecule to rhodopsin demonstrated in vitro. We hope that this observation may ultimately lead to new approaches to treat or prevent RP in patients.

P1.161

Influence of hyperdirect pathway on the cortex-basal ganglia dynamic properties: A combined electrophysiological and optogenetic study of subthalamic neuronal activity in the anesthetized rat

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This study investigates the functional role of the cortico-subthalamic (hyperdirect) pathway on dynamic states of the cortex-basal ganglia loop in physiological and afterdopaminergic depletion (such as in Parkinson´s disease). We combine electrophysiology and optogenetic tools to solve the technical issue related to the specific stimulation of the hyperdirect pathway. We analysed the modulation of the electrophysiological activity of the subthalamic nucleus (STN) neurons in response to the hyperexcitation of hyperdirect neuronal activity induced by optogenetic stimulation in physiological conditions. This study is performed in anaesthetized transgenic rats in which cortico-subthalamic neurons were transduced by a anterograde viral vector injected in the orofacial motor cortex. Viral construction includes channelrhodopsine ChR2 transgene (LV-CaMKIIa-ChR2-EYFP-WPRE) when expressed leads 570 nm light-activated Na^+ channels and enhanced neuronal excitation. Targeting transfected cortico-subthalamic neurons is performed by implanted the light stimulation directly in the subthalamic nucleus closed to the recording electrode. An electrical stimulation electrode is placed in orofacial motor cortex to induce evoked-response in subthalamic neurons. Electrical recordings are performed before and during optical stimulation. The subthalamic neuronal activity is analysed using two electrophysiological parameters: the spontaneous activity and the evoked-activity in response to cortical stimulation.
Our results show that spontaneous activity is enhanced by optical stimulation of cortico-subthalamic neurons. Orofacial motor cortex stimulation allows to induce different evoked-responses of subthalamic neurons (mono-, bi- or triphasic responses). 60% of cortical stimulation-responsive subthalamic neurons present a characteristic early excitation (post-stimulation latence : 7.27± 2.04 ms) of the recruitment of cortico-subthalamic neurons. During the light activation, amplitude and latency of early excitation are respectively increased and decreased. In conclusion, these results show that cortico-subthalamic neurons can be efficiently transducted and their excitability is light-sensitive. Subthalamic neuron activity can be modulated by cortico-subthalamic neuron activity.

P1.162

Acquisition of anticipation during a new coordination between posture and movement in adolescents: a kinematic and EMG analysis

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The postural body scheme is used to control posture according to a feedforward process based on internal representations. In the current study, we adopted the hypothesis that body scheme

disturbances occurring during adolescence might affect the internal representations and might consequently affect the feedforward mode of postural control. The aim of this experiment was to test the capacity of adolescents to develop anticipation during a new co-ordination in a bimanual load-lifting task. We studied the learning process of an artificial co-ordination by means of a procedure of double unloading in two groups of adolescents (11-13 versus 14-15 years of age) and a group of adults. Elbow-joint angle measurement and EMG recordings were used to assess the improvement of the forearm stabilization through six learning sessions. Kinematics and electromyographic data were recorded simultaneously.
Preliminary results report a difference between adolescents and young adults concerning the learning dynamics, but also the final level of performance following learning. Electromyographic analysis showed that the onset of the muscular inhibition of the postural arm appeared after the onset of unloading in the first trials, and before unloading in the last trials suggesting a transformation of feed-back postural correction into a feed-forward control associated with voluntary movement. In adolescents, a similar timing adjustment of the muscular pattern was observed. However, more learning sessions were needed for this timing adjustment to become efficient. Our results suggest that the mechanisms underlying the acquisition of new abilities are still maturing during adolescence, which might constitute a transient period in the general maturational process of the postural control.

P1.163

A simple read out of population activity in the mouse auditory cortex predicts behavioral generalization across sounds

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In order to better understand how sounds are represented in patterns of neuronal activity in the mouse auditory cortex, we compared perceptual decisions of mice involved in an auditory go-nogo task with predictions obtained from single trial responses of large ensembles of cortical neurons recorded optically.
During the task, mice were trained to discriminate between two target sounds predicting a water reward or an air puff, respectively. We interleaved various non-reinforced sounds at a low probability and mice reacted with a choice that was independent of the valence of the target sounds. This allowed us to behaviorally map generalization across stimuli with high precision.
Neuronal activity was derived from temporally deconvolved 2-photon calcium imaging performed *in vivo* in cortical layers II and III during isoflurane anesthesia. A large number recordings from local populations of 50 to 100 neurons within a ~200x200 mm area yielded a pooled dataset of few thousand neurons from widespread locations of the auditory cortex, as previously identified with intrinsic imaging.
We show that a linear classifier that is optimized to discriminate between single trial cortical ensemble responses to the two target sounds even predicts the generalization curves of choices for a variety of non-target sounds. Such a classifier represents a simple and robust decoding mechanism equivalent to a binary neuron or population summing up inputs from selected cortical neurons. Furthermore, detailed analysis of the coding properties of local cortical populations revealed highly stochastic dynamics with eventually few attractors that allowed only representation of few groups of sounds. Coding efficiency largely increased by combining several local populations.
We therefore propose a quantitative model of auditory cortex function in which sounds are encoded by large scale activity patterns, emerging from local stochastic dynamics. Moreover, the procedure we use to emulate perceptual decisions of the animal based on these patterns is known to have biologically feasible implementations. We believe that this model can help formulating hypotheses on how the discriminative behavior is actually learnt and performed by brain circuits.

P1.164

CCL2 increases Nav1.8 current and nociceptor excitability through a $G_{i/0}$ dependent-mechanism

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Altered function of voltage-gated sodium channels is responsible for the hyperexcitability produced by inflammatory agents in primary nociceptive sensory neurons. The tetrodotoxin-resistant (TTX-R) sodium channel Nav1.8 is known to be expressed predominantly in nociceptive sensory neurons and to play a major role in inflammatory pain. Monocyte chemoattractant protein-1 (CCL2), a pro-inflammatory cytokine, which binds to the CCR2 receptor, is involved in the development and maintenance of chronic pain states. However, the mechanisms by which CCL2 elicits pain behaviors are still poorly understood. Nevertheless, compelling evidence suggests that CCL2 sensitizes primary afferent neurons. In the present study, we thus examined the effects of acute application of CCL2 on TTX-R sodium Nav1.8 channels as well as its influence on membrane conductance in acutely small/medium dissociated lumbar dorsal root ganglion (DRG) neurons from adult rats. Using a whole-cell patch-clamp approach, we found that CCL2 (100 nM) increases TTX-R currents by 79 % ($p< 0.0001$) and 36 % ($p< 0.01$) in small and medium DRG neurons, respectively. CCL2 also shifted both the conductance-voltage relationship curve and the steady-state inactivation curve in a hyperpolarizing direction in small DRG neurons. No difference in the activation and inactivation kinetics was observed in medium DRG neurons. Moreover, we demonstrated that these steady-state Nav1.8 sodium channel activity was reversed by a CCR2-selective antagonist. These data thus reveal that CCL2-induced pain facilitation is mediated by direct spinal activation of CCR2. Pertussis toxin (250 ng/ml) blockade of CCL2-induced increase in Nav1.8 currents in small/medium neurons also supports the involvement of CCR2 via $G_{i/o}$ signaling in pain processing. Altogether, our data strongly demonstrate that Nav1.8 currents can be increased during painful conditions via a G-protein dependent mechanism associated with CCL2/CCR2 signaling pathway.

P1.165

Behavioral and metabolic adaptation to high-fat diet involves PSA-NCAM-mediated rewiring of the hypothalamic melanocortin system

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The hypothalamus is of major importance in the control of food intake and energy homeostasis. Interestingly, this brain area remains “plastic” in the adulthood, meaning that neuronal networks can undergo functional or morphological remodeling. Although hormone-triggered hypothalamic plasticity has been mainly studied in the field of reproductive biology, recent studies showed that this process would be involved in energy homeostasis as well. Indeed, in adult laboratory animals rapid rewiring of hypothalamus can be achieved by various experimental procedures including treatments with metabolic hormones such as leptin or ghrelin. Nevertheless, whether adult hypothalamic plasticity could play a role in the regulation of eating behavior and energy homeostasis in physiological condition is still a largely unexplored question, which we addressed in the present study. For this

purpose, we explored hypothalamic plasticity in adult mice fed with high-fat diet (HFD) for one week. Here, we report that HFD rapidly induces overeating together with dyslipidemia, and glucose intolerance. Despite the persistent unbalanced diet, mice adapt to the new nutritional condition and the HFD-induced altered phenotype is progressively reversed within a week. In this model, synaptogenesis occurs specifically in the arcuate nucleus of the hypothalamus increasing the anorexigenic tone due to activated proopiomelanocortin (POMC) neurons. Diet-induced rewiring of arcuate POMC neurons is mediated by the PSA-NCAM glyco-protein and is required to adjust energy intake. Finally, inhibition of this mechanism rapidly induces overweight and could thus be determinant in development of metabolic diseases including obesity.

P1.166

The nutritional state modulates the spatiotemporal coding of nutritive and novel odors in the olfactory bulb

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In the last ten years, many studies have demonstrated that the nutritional state can modulate odor detection in awake animals but the supporting mechanisms remain widely unknown. In this context, we focused our study on the Olfactory Bulb (OB) which is the first central relay of the olfactive information. In fasted versus fed rats, we compared the spatiotemporal bulbar responses to different odorants.
We used Intrinsic Optical Signals Imaging (IOSI) to study the spatial coding and Local Field Potential (LFP) recordings to study the temporal coding. We used 2 experimental groups with 2 odorants linked to distinct valence: i. bitter almond odor was used as a nutritive stimulation after the rats were habituated to consume a bitter almond odorized cake. ii. hexanal odor was used as a novel odorant since rats have never been confronted to this odor before.
Results: Nutritive odorant: in IOSI 4/4 fasted animals responded to 2% and 5% vapor pressure (vp) of bitter almond while only 1/4 of the fed animals responded with a clear spatial map. LFP recordings showed that the fasted animals (n=6) responded with a decreased gamma activity to both concentrations while the fed animals (n=6) showed this pattern only for 5%.
Novel odorant: in IOSI, 4/4 fasted animals responded to 0.1% vp hexanal by activating 2 zones while only 2/4 of the fed animals activated a single zone. All fasted animals responded to the 0.5% vp hexanal by activating three glomeruli and 4/4 fed animals activated only one glomerulus. LFP recordings have shown that fasted animals (n=6) responded to 0.1% vp hexanal by a decrease of two bands in gamma activity (70-100Hz and 100-130Hz) while fed animals (n=6) decreased only the higher band (100-130Hz). The response induced by 0.5% vp hexanal was a decrease in the two bands of gamma activity and an increase in the beta band (15-40Hz) in fasted rats while we observed only the decrease of the two gamma bands in fed animals.
This study demonstrates that the fasting affects deeply the spatiotemporal coding of odorants in the OB, at least by shifting the threshold of odorant detection of both nutritive and novel odor.

P1.167

Static and dynamic postural control in patients with cochlear implants

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Cochlear implantation (CI) in patients with severe hearing loss can induce vestibular symptoms that resolve usually with time. Postural stability was reported to be impaired, unchanged or even improved (Buchman et al., 2004) depending on the study. This study was aimed at examining the long term impact of CI on posture control, if any. Its originality was to evaluate the postural performance in ecologic conditions with a concomitant cognitive task (dual-tasking) and in different sensory conditions.
Postural performance was measured in 16 unilateral CI patients tested one-six years after CI surgery, using a static/anteroposterior (AP) translational platform. Different levels of postural task difficulty were obtained by:
(i) suppression of auditory information (device “on” vs “off”),
(ii) suppression of vision (eyes closed vs eyes open),
(iii) addition of a concomitant cognitive task (single task vs dual-tasking),
(iv) increasing the sinusoidal translation frequency (0.25 Hz vs 0.5 Hz). Analyses were performed on the center-of-pressure displacements with both statistical and non-linear methods (wavelet transform). Head, hip and knee position and stabilization were recorded with a 3D motion analysis system.
The ANOVA showed that CI patients differed significantly from the controls, and that suppression of visual input (eyes closed) at 0.5 Hz sinusoidal translation is the main source of variation between the two groups. Suppression of the auditory input had no influence on the postural performance and adding a concomitant cognitive task induced similar effects in both groups. Results from the 3D motion analysis corroborated these data. The strategy used to control balance remained unchanged in the CI patients compared to the controls, except during 0.5 Hz (AP) translation without vision. In this latter condition, head stabilization was strongly impaired in the CI patients who exhibited an inverted pendulum strategy, while controls showed a rigidification of all body segments.
In conclusion: CI patients behave like controls in easy postural conditions when examined a long time after CI surgery. But they are strongly dependent of vision, particularly in the most challenging postural tasks. This can reflect a rehabilitation process based on lip-reading.

P1.168

Cholinergic partition cells and lamina X neurons induce a muscarinic-dependent-short-term potentiation of commissural glutamatergic inputs in lumbar motoneurons

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Acetylcholine and activation of muscarinic receptors influence the activity of neural networks generating locomotor behaviour in the mammalian spinal cord. Using electrical stimulations of the ventral commissure, we show that commissural muscarinic (CM) depolarizations could be induced in lumbar motoneurons. We provide a detailed electrophysiological characterization of the muscarinic receptors and membrane conductance involved in these responses. Activation of the CM terminals, originating from lamina X neurons and partition cells, induced a pathway specific short-term potentiation (STP) of commissural glutamatergic inputs in motoneurons. This STP is occluded in the presence of the muscarinic agonist, atropine. At a more integrated level, during fictive locomotion, the activation of the commissural pathways transiently enhanced the motor output in a muscarinic dependent manner. This study describes for the first time a novel regulatory mechanism of synaptic strength in spinal motor networks that can potentially account for motor task-related modulatory influences in these circuits.

P1.169

Does visual, tactile and muscle proprioceptive information contribute complementarily or redundantly to kinesthesia?

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In addition to the signal derived from the motor command, muscle proprioception, vision and touch are the 3 main sources of information involved in body limb position and movement perception. This study deals with the controversial issue as to whether these 3 sensory inputs convey complementary or

redundant information, and whether they are integrated to encode the kinematic properties of hand movements.
Illusory sensations of clockwise right hand rotation (ulnar deviation) were induced by stimulating these three sensory channels either separately or simultaneously at low, medium and high intensities. ***Muscle Proprioception*** was activated by applying mechanical vibration to the *pollicis longus* muscle tendons, ***Touch*** was activated by scrolling a textured disk under the subject's hand, and ***Vision*** was stimulated by projecting a background scene rotating under the subject's hand. The kinesthetic illusions induced were copied by the subjects on-line with their left hand using a potentiometer and the EMG activity of their right wrist muscles was also recorded.
Results show that the velocity of the perceived movements and the amplitude of the corresponding EMG activities depended on the stimulation intensity applied in each modality and on the combinations tested. Combining muscle proprioceptive messages with visual or tactile ones resulted in stronger and faster illusory movements. This did not occur when the visual and tactile modalities were co-stimulated. When a third sensory input was added to the previous combinations, the subject's perceptual responses increased only with the muscle proprioceptive + visuo-tactile combination.
These findings confirm that ***Vision***, ***Touch*** and ***Muscle Proprioception*** alone are able to encode the kinematic parameters of hand movements. When all 3 sensory modalities are available, as occurs in everyday life, they probably contribute unequally to kinesthesia. In addition to muscle proprioception, the CNS might use complementary kinesthetic components of visual and/or tactile inputs to assess the velocity of ongoing movements more accurately; By contrast, movement velocity perception might not benefit from the kinesthetic redundancy of the visual and tactile inputs in absence of additional muscle proprioceptive information.

P1.170
The role of prediction in motion integration

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Due to the aperture problem, the initial direction of tracking responses to a translating tilted bar is biased towards the direction orthogonal to the orientation of the bar. This directional error is largely reduced over the first 200ms of tracking, consistent with the neural solution of the aperture problem (Pack & Born, 2001) and is fully corrected during the steady-state. We have proposed that pursuit dynamics reflects that of visual motion processing and can be modeled as a dynamical Bayesian inference of 2D target motion (Bogadhi et al., 2010). Such simple paradigm also offers a powerful way to explore interactions between sensory and predictive signals in controlling action (Montagnini et al., 2006). We conducted two experiments to investigate these interactions by transiently blanking the target at different moments of pursuit. First, a 45^0 or 135^0 tilted bar translating horizontally was blanked for four different durations (0,200,400 ms) during steady-state tracking. Bar orientation after reappearance changed on half of the trials. We found a marginal directional bias (compared to initial bias) when the target reappeared with no change of orientation and when target changed orientation with no transient blanking. However, there was a significant directional bias when the target reappeared with a change in orientation. Second, the target (45^0 or 135^0 tilted line) was blanked (duration: 200ms) on half of the trials during the initiation phase of pursuit, starting at either 100 , 120, 140, 160 or 180ms after pursuit onset. Line orientation was constant. We found no directional bias when the tilted line (45^0 or 135^0) reappeared after a 200ms blank starting at 100ms. These results suggest for a conditionally weighted mixing of retinal and extra retina signals in driving smooth pursuit.

P1.171
Optogenetic control of various hypothalamic neuropeptide systems differentially affects the initiation of physiological stress responses

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Hypocretins (Hcrt) are known to play an important role in wakefulness and arousal. Although the activity of Hcrt neurons correlates with increased levels of arousal, discharge activity patterns can be irregular and particularly elevated during specific behaviors requiring increased vigilance.
In the present study, we show that optogenetic control of Hcrt tone is sufficient and necessary to initiate some physiological stress responses with different kinetics. Acute Hcrt stimulation immediately affects sympathetic pathway with a significant increase in heart rate. Chronic stimulation of Hcrt neurons leads to HPA axis activation underlied by elevated plasmatic corticosterone levels and hypothalamic paraventricular (PVN) neuronal activation. Also the Hcrt-induced elevated plasmatic corticosterone might be under both circadian and homeostatic controls.
Our data suggest that a specific amount of Hcrt neural activity is coupled to an increased arousal related to stress. Thus, we propose to dissect the neuroendocrine response dynamics using both optogenetic manipulation of Hcrt and PVN corticotropin-releasing factor (CRF) neurons.

P1.172

Mental representation of space is altered after vestibular loss

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Introduction: The vestibular system contributes to a wide range of functions, from postural and oculomotor reflexes to spatial representation and cognition. After vestibular loss, disorganization of spatial memory has been shown in participants moving in physical as well as in visual (virtual) environments, that is, in the absence of any solicitation of vestibular signals. These results suggest that spatial deficits originate from an erroneous elaboration of the mental representation of space. In order to test this hypothesis, we evaluated the implication of vestibular signals in mental imagery processes, in the absence of any real or imagined whole-body displacement.
Methods: The present study assessed how vestibular loss can affect the metric properties of mental images involved in mental rotation of 3D objects and mental scanning in an environment recently learned or already consolidated in memory. Menière's disease patients were tested before unilateral vestibular neurotomy and during the recovery period (1 week and 1 month). They were compared to healthy participants tested at similar time intervals, and to bilateral vestibular-defective patients tested during the compensated stage.
Results: Patients were impaired in all imagery tasks; bilateral patients were frequently the most impaired. Mental scanning was affected in both environments but more severe deficits were observed in the environment recently learned, suggesting that vestibular loss disrupts the ability to elaborate and use mental images.
Discussion: The present data emphasize the consequences of the status of the vestibular system, assessed both through the level of loss (bilateral *versus* unilateral loss) and through the compensation level (acute stage *versus* chronic stage), on the ability to elaborate and maintain metric properties of mental representations, and to rotate mental images. We believe vestibular loss can disorganize brain structures commonly involved in mental imagery, and more generally in mental representation.

P1.173

Retinas of the diurnal rodent *Arvicanthis ansorgei* are highly resistant to experimentally-induced stress and degeneration

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Environmentally-induced stress plays a significant role in retinal degeneration and blindness, both in animals and humans. Among such sources of stress, phototoxicity is well studied and has been shown to lead to photoreceptor-specific loss in a number of species. However, the vast majority of studies have been conducted in nocturnal, albino rod-dominant rat and mouse strains, and the pertinence of such findings to human pathology and cone loss is debatable. We decided to examine the retinal vulnerability to induce damage in a diurnal murid rodent, *Arvicanthis ansorgei*, a species possessing a large number of cones. We used established protocols for either:
1) exposing animals to a wide range of lighting conditions (variable intensity, duration, spectrum, previous light history and time of exposure); and
2) injection of N-methyl-nitrosourea (MNU), both reported to produce rapid and complete photoreceptor-specific damage.
Animals then underwent electroretinography to record rod and cone function, and were subsequently euthanized and used for immunohistochemical analysis of retinal structure and quantification of free fatty acids. Using these standard regimes, there were no detectable detrimental effects on *A. ansorgei* retinal phenotype, function or structure. Partial retinal damage in *A. ansorgei* was induced by very intense blue light or elevated doses of MNU. This resistance was not due to differences in lipid composition (specifically docosahexaenoic acid) between *Arvicanthis* and susceptible strains of mice and rats. Hence the retina of this species exhibits generally high resistance to retinal damage from light and toxins such as MNU.

P1.174

Persistent sodium current-dependent plateau potentials in neonatal rat lumbar motoneurons

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Rats achieve a raised quadruped posture during the second postnatal week. Changes in the excitability of motoneurons are likely an important factor underlying the development of a postural tone. In adult motoneurons, tonic firing appears to arise from plateau potentials. The plateau-generating current has been identified as a persistent calcium-dependent current mediated by L-type calcium channels. However, studies investigating the ionic nature of plateau potentials have been done with extracellular concentrations of calcium ($[Ca^{2+}]_O$) higher than the 1.2 mM measured from the cerebrospinal fluid. It follows that calcium currents are upregulated to the detriment of the persistent sodium current (I_{NaP}), which is downregulated when $[Ca^{2+}]_O$ is increased (Tazerart et al. 2008). With a developmental perspective, this study was designed to reevaluate the contribution of I_{NaP} in generating plateau potentials in motoneurons of lumbar spinal cord slices isolated from neonatal rats (P0-P6). When $[Ca^{2+}]_O$ was set within a physiological range, about half of motoneurons elicited a sustained action-potential discharge in response to a brief depolarizing pulse. These plateau potentials were prevented when the holding potential was hyperpolarized. The L-type calcium channel blockers nifedipine and nimodipine did not affect plateau potentials. In contrast, low concentrations of Na^+ channel blockers such as TTX and riluzole completely abolished plateau potentials, before affecting the action potential itself, suggesting a critical involvement of I_{NaP}. Furthermore, enhancement of I_{NaP} by veratridine consistently revealed a latent plateau potential in motoneurons. Bistable properties could also be revealed when temperature was raised above 28°C with a concomitant upregulation of I_{NaP}. This study provides evidences for an early acquisition of I_{NaP}-dependent plateau potentials by lumbar motoneurons in neonatal rats. Together with the thermoregulation that becomes effective within the first two postnatal weeks, the emergence of I_{NaP}-dependent plateau properties in motoneurons may represent one of the mechanisms contributing to the development of posture.

P1.175

Activity-dependent modulation of pacemaker properties in the central pattern generator for locomotion of rodents

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Rhythm is an important feature of central pattern generators (CPGs) that coordinate repetitive movements. Our recent investigation (Tazerart et al., 2008) provided the demonstration of a new pacemaker property in the locomotor CPG, dependent on the persistent sodium current (I_{NaP}), which is

critical for the expression of locomotion (Tazerart et al., 2007). Intriguingly, I_{NaP} is upregulated after subtle changes in the extracellular concentration of some ions such as calcium ($[Ca^{2+}]_O$) and potassium ($[K^+]_O$). That the concentration of both ions depends on the recent experience of the network, raises the possibility that the relative contribution of pacemaker properties to rhythm generation may change depending on the functional state of the network. To bring a new perspective into the operation of the locomotor CPG, this study was aimed at identifying the fluctuations of $[K^+]_O$ and $[Ca^{2+}]_O$ during locomotion and their significance in the firing patterns of CPG interneurons. In spinal cord preparations isolated from neonatal rats, variations of $[K^+]_O$ and $[Ca^{2+}]_O$ were recorded during NMA-induced fictive locomotion by means of ion-sensitive microelectrodes inserted into the CPG region (L1-L2). We observed a significant increase of $[K^+]_O$ from 4 to 6 mM and a decrease of $[Ca^{2+}]_O$ from 1.2mM to 0.9mM. Whether pacemakers are modulated by these locomotor-related changes in $[Ca^{2+}]_O$ and $[K^+]_O$ was then tested. Neonatal rat spinal cord slices from upper lumbar segments were equilibrated with $[K^+]_O$ and $[Ca^{2+}]_O$ reported to occur during locomotion. Simultaneous changes of both ions induced I_{NaP}-dependent pacemaker activities in 25% of interneurons located in the CPG region. The synergistic action of both ions in eliciting pacemaker activities was also observed in half of genetically identified Hb9 interneurons which have been proposed to be intimately involved in rhythm generation. In sum, considering the dynamic fluctuations in the ionic composition of the extracellular space as relevant signals triggering I_{NaP}-dependent pacemaker properties, the locomotor network may be viewed as a hybrid pacemaker-network model in which pacemaker neurons might be the kernel for locomotor rhythm generation.

P1.176

Neural correlates of an equivalent of "mach bands" in auditory modality

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It is well-known that tinnitus is accompanied by hearing loss in the majority, if not all, subjects, and that tinnitus pitch corresponds to the frequency band of hearing loss. Hearing loss has been shown to reduce sensory inputs sent towards the auditory centers, resulting in dramatic central changes which may ultimately cause tinnitus. An auditory illusion, called Zwicker tone (ZT), shares some properties with tinnitus and as such has been considered as a transient tinnitus. The ZT can be induced after the presentation of a notched noise, and interestingly, the ZT presents a pitch corresponding to the notch of the noise (as tinnitus corresponds to the frequency band of hearing loss). The notched noise, which induces a contrast of neural activity over frequencies (similar to what cochlear insults do), may induce comparable central changes than those causing tinnitus. In this context, studying the central changes induced by a notched stimulus can give some insights into the mechanisms of tinnitus. In the present study, we recorded neural activity (single units, multi-unit activity and local field potentials) obtained in the auditory cortex of anesthetized guinea pigs evoked by control and notched stimuli. All stimuli used in the study served two purposes simultaneously; they provided a given sensory environment to the animal (simulating a hearing loss for instance), and at the same time, they allowed the characterization of the Spectro-Temporal Receptive Fields (STRFs) of cortical neurons. MUAs and LFPs were collected in 10 guinea pigs from 180 cortical sites. Neural activity during stimulation with the notched stimulus was increased at both edge frequencies of the notch and decreased within the notch frequencies. This result is reminiscent of the perceptual phenomenon called the "Mach bands" where the sensation of brightness does not correspond to a gradient of luminance at the edges of the gradient.

P1.177

Task2 K+ channel gene knockout causes deafness in mice

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In the mammalian inner ear, K+ channels are important for various cellular processes including basolateral conductance in sensory hair cells and the regulation of the endolymph homeostasis. The presence of some K+ channels with two pore domains (K2P family) has been reported in vestibular end organs and cochlea, but no specific function for any of them have been described. Task1, -2, and -3 channels (gene nomenclature: KCNK3, KCNK5, and KCNK9) belong the K2P channels. They produce background conductance, are involved in resting potentials and are sensitive to external pH. In addition, Task2 channels participate in $HCO3^-$ reabsorption in kidney as well as central chemosensitivity to O2 and CO2 levels. The other two members of the TASK family (Task1 and Task3) are expressed in many brain areas, including the central auditory pathway.
Electrophysiological assessment of auditory function using auditory brainstem responses revealed deafness in $Task2^{-/-}$ mice and normal hearing in Task2 heterozygotes. In opposition, $Task1^{-/-}$, $Task3^{-/-}$ and Task1-3 double knockout mice showed normal auditory-brainstem responses. Cochlear structure examined from resin embedded specimens revealed an absence of Corti's organ along the cochlea associated with considerable spiral ganglion degeneration in $Task2^{-/-}$ mice whereas a normal structure was observed in Task2 heterozygotes. The use of the β-galactosidase as gene reporter allowed localizing Task2 expression in frozen sections of the cochlea from both heterozygote and homozygote mice. Specific patterns of Task2 expression within the epithelial gap junction network and root cells were revealed. Both networks are known to be involved in K+ recirculation from the organ of Corti to the stria vascularis. Thus, this study is the first demonstration of the presence and the essential role of Task2 channels in the cochlea.

P1.178

Perceiving human movement (HM) performed under microgravity: an fMRI investigation

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Psychophysical studies showed that the shift of the HM (180° returned) disturbed the recognition of motion (Shipley, 2003). Moreover, HM presented backward would be perceived as strange, so the human features of the movement would be more difficult to extract (Jacobs et al., 2004).
Furthermore, Indovina et al. (2005), through an fMRI study, showed that the component of terrestrial gravity of non-HM is encoded in the vestibular cortex of human brain. On Earth, HM also involves this terrestrial gravity component.
The question raised here is which brain areas are recruited during the perception of HM performed with (1G) or without (0G) the Earth gravity? We propose to compare brain activity during the presentation of HM performed under 1G or 0G.
Stimuli were collected during parabolic flights both 1G and 0G periods using an optoelectronic system. 4 cameras recorded the movements of 22 retroreflective markers taped onto actors' bodies. We used this minimalistic point-light displays to depict the characters, as they provide the most straightforward way to isolate mechanisms used to extract information from motion without any interference with other visual information.
A preliminary behavioral study was performed to test the relevance of the stimuli. Any ambiguous stimuli were eliminated on the basis of the error rate (72 stimuli were used). Subjects were asked to classify the stimuli as 1G or 0G on a 3T whole-body imager (Bruker).
Our preliminary results tend to highlight the role of the superior temporal sulcus, identified in several fMRI studies as key brain area involved in the perception of the HM (Pelphrey et al., 2003).
We expect to show brain activations in: parietal cortex (Vaina et al., 2001), premotor and inferior frontal cortices (Sayginer et al., 2004), prefrontal cortex (Stevens et al., 2000), and particularly in the vestibular system (insular cortex, temporo-parietal junction), selectively engaged when acceleration of object-movement is in conformity with the laws of gravity.
The authors wish to thank the Centre National d'Etudes Spatiales (CNES) for its financial support.

P1.179

Crossmodal compensation during face-voice integration in cochlear implanted deaf patients

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Any dysfunction in the capacity of voice or face recognition can negatively impact on the social communication of a person, this is particularly the case in profoundly deaf patients. A cochlear implant (CI) allows deaf patients to understand speech but because of the limitations of the processor, patients present strong difficulties in voice recognition. Here we investigated the possibility that the visual system can exert in CI users a strong influence on the multimodal perception of voice attributes. We used a speaker discrimination task between sounds taken from a voice continuum obtained by morphing between a male and a female voice. Proficient CI patients (n=15) were tested under auditory-only or audiovisual conditions in which a female or male face was simultaneously presented. Their performance was compared to those of normal hearing subjects (NHS n=50) tested with a vocoded voice stimuli that simulate the processing of an implant. A visual impact index computed from the A and AV psychometric functions revealed that CI users are significantly influenced by visual cues. This is expressed by a shift in categorization of the voice toward the gender carried by the face in incongruent AV conditions. No such visual effect was observed in NHS tested with the vocoder in spite of a deficit in the A-only categorization. Thus, in case of ambiguity in the stimuli and uncertainty in the auditory signal, CI users perceptual decisions are based mainly on vision their most reliable sensory channel. These results, coupled to our brain imaging study showing in CI patient a functional colonization of the voice sensitive areas by visual speechreading, suggest a crossmodal reorganization of the mechanisms of face-voice integration after a prolonged period of deafness.

P1.180

Cortico-muscular coherence over the SMA region in a bimanual precision grip task: a human EEG study

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Introduction: We assessed communication between motor cortical areas and motoneurons by computing corticomuscular coherence (CMC) between electroencephalography (EEG) and electromyography (EMG) in human subjects. We analysed the scalp topography of CMC, with special interest for the SMA region.
Methods: Ten right-handed subjects were instructed to keep a cursor on a curve that moved from right to left on a screen, indicating the force level to produce. The cursor´s vertical position varied with the pression they applied with their right hand on a small device containing force sensors. With their left hand, the subjects had to hold the device in order to correctly perform the task. High resolution EEG (64 channels, ANT, The Netherlands) was recorded simultaneously with surface EMG from the first dorsal interosseous and abductor pollicis brevis of each hand. CMC was computed from rectified EMG signals and mean referenced EEG signals using the multitaper method implemented in the open-source FieldTrip Matlab toolbox.
Results: A clear difference in the topography of the CMC can be seen between both hands: Although for the left hand, CMC is found over the right sensorimotor area only, for the right hand, not only CMC is found over the left sensorimotor area, but also over the SMA region. In order to perform statistical

testing, three cortical regions of interest were defined: left and right sensorimotor cortex and SMA. For each subject, cortical region and condition, the maximum CMC value in the 15-44 Hz frequency band was determined, revealing significant differences ($p < 0,05$) in CMC (1) between left and right M1 for the right hand, (2) between both hands for left M1, with a higher CMC for the right hand, and (3) between SMA and right M1 for the right hand, with a higher CMC for SMA.
Conclusion: Corticomuscular coherence was found in a bimanual precision force control task, not only between the sensorimotor areas and the opposite hand, but also between SMA and right hand muscles. Our results suggest a role of direct corticospinal projections from SMA in precise bimanual force control.

P1.181

Influence of the choice of the anesthetic on the spontaneous and odor induced activity of the mouse olfactory bulb

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Electrophysiological recordings under anesthesia have shown that following odorant stimulation, the functioning of neural networks of the olfactory bulb (OB), first relay of the olfactory information's processing, give rise to large oscillations of local field potentials. While this approach has been used extensively to decipher the odorant neural representation at the level of the OB in anesthetized rodents, little is known about the influence of the drugs used to maintain sedation on the olfactory network activity. Anesthesia is commonly obtained by injecting a cocktail of ketamine associated with one of the two available agonists at the alpha 2 class of adrenergic receptors, xylazine or medetomidine. The objective of this work is to compare the recorded signals under these two different conditions of anesthesia. We use chronically implanted macroelectrode (100µ diameter) in order to record in the same mouse spontaneous and odor-induced OB activity under the two types of anesthesia. We observed that basal amplitude of the signal in the gamma band (65-120Hz) was higher under medetomidine when compared with xylazine. Stimulation with hexanal (5 and 10%) or carvone (25 and 50%) elicited a diminution of the amplitude of the signal in the gamma band frequency and an augmentation in the beta band (15-45Hz). We are currently further analyzing the characteristics of these odor responses in order to see if the modifications observed are different between the two conditions and compare them with recordings from freely moving animals.

P1.182

Role of xenobiotic metabolizing enzymes in the perception of caffeine in *Drosophila melanogaster*

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Detoxification and elimination of potentially harmful chemicals are crucial processes for most organisms. Xenobiotics Metabolizing Enzymes (XME: CYP, UGT…) are able to take in charge exogenous molecules, biotransform and eliminate them out of the organism. These enzymes are highly expressed in tissues involved in detoxification processes such as fat body, malpighian tubules, and in the chemosensory organs, but there is little experimental evidence showing their functional role in chemoperception.
The aim of this project is to investigate the potential role of cytochromes P450 (CYP) in the perception of caffeine in *Drosophila melanogaster*. We highlight that the expression of several CYP, expressed in

chemosensory organs, is up- regulated after an exposure to caffeine in adult flies. This result suggests that these CYP genes could be involved in the perception of this component.
We hypothesized that a modulation of CYP expression should provoke a modification of caffeine detection and alter animal feeding behaviour. To confirm this hypothesis, we targeted the inhibition of candidate genes expression in sensory organs using interference RNA (RNAi). Then, we evaluated the ability of these transgenic flies to detect caffeine using a behavioral test (MultiCAFE), which allows to estimate the consumption of caffeine solution. We noted that a reduction of the expression of these CYP genes disturbs feeding behaviour. RNAi-CYP animals fed significantly more than controls on food containing caffeine, which is normally repellent.
This suggests, for the first time, that some CYP genes, expressed in sensory organs, may be involved in chemoperception in Insects.

P1.185

Transcriptomic analysis of blood-derived macrophages identifies 5-lipoxygenase activation protein as a key tumor-induced immune molecule in glioma patients

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In many types of tumors, infiltrating macrophages so-called tumor-associated macrophages engage a peculiar activation program that favors tumor outgrowth and supports neovascularization. Major efforts are currently developed to identify the full range of molecules characterizing such macrophage activation program. In this context, our study was aimed to identify a molecular signature of glioma-induced immune alterations in the monocyte/macrophage lineage. For that purpose, we performed a transcriptomic analysis of blood-derived macrophage cultures obtained from
i) 4 glioma patients untreated at the time of blood sampling and
ii) 4 sex- and age-matched healthy subjects.
Surprisingly, from more than 25 000 genes analyzed, only one immune-related gene, the 5-lipoxygenase activating protein gene, was identified as being up-regulated in blood-derived macrophages from glioma patients as compared to controls. Such an up-regulation was confirmed and found to be statistically significant by q-PCR analysis of macrophage cultures obtained from a larger cohorte of patients. Analysis of CNS tumor samples showed that expression of 5-lipoxygenase activating protein was up-regulated in glioma (n =15) as well as in meningioma samples (n = 10). However, only in glioma samples the expression of 5-lipoxygenase activating protein correlated with the expression 5-lipoxygenase genes suggesting a functional activation of the leukotrien synthesis pathway in glioma but not meningioma. Finally, in blood-derived macrophages obtained from healthy subjects, we demonstrated that supernatant from glioma cell lines induced a significant up-regulated expression of 5-lipoxygenase activating protein. The role of 5-lipoxygenase activating protein in glioma cells/macrophages crosstalk is currently being assessed using an in vitro co-culture model.

P1.186

Hypothalamus-specific deletion of *socs3* in adult mice enhances hindbrain sensitivity to endogenous satiety signals via oxytocin signaling

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Leptin is a major contributor to long-term energy homeostasis, through an intracellular transduction pathway involving activation of Stat3 and its feedback inhibitor Socs3, which limits Stat3 activation. Previous studies have shown that Socs3 haploinsufficiency or *socs3* deletion in the whole brain or in selective neuronal populations triggers an increased sensitivity to exogenous leptin, through increased Stat3 activation, and protects against diet-induced obesity in mice fed a high fat diet. Intriguingly however, no phenotype was detected when Socs3 mutant mice were maintained under standard diet, suggesting that Socs3 may only contribute to energy homeostasis in pathological conditions and not in physiological conditions. In this study, we show that deletion of *socs3* in the medio-basal hypothalamus, when performed in adult mice by stereotaxic injection of an adenovirus encoding Cre recombinase, produces an attenuation of body weight gain, a decreased adiposity, and a decreased food intake in animals maintained under standard diet. The decreased food intake was associated with an increased hindbrain sensitivity to endogenous satiety signals, which was blocked by fourth ventricular injection of a selective oxytocin receptor antagonist. Thus, our study indicates that Socs3 controls energy homeostasis in physiological conditions, and that oxytocin signaling functions as a downstream effector of hypothalamic leptin to enhance hindbrain sensitivity to endogenous satiety signals. This result suggests that oxytocin could be of therapeutic interest to circumvent leptin resistance associated with common forms of obesity.
Support: Société Française de Nutrition (to SB)
Keywords: CCK, devazepide, SSR126768A, nucleus tractus solitarius

P1.187
Hypoglycemic stimuli activate central nesfatinergic neurons

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The regulation of feeding behavior implicates various compounds originating from both peripheral organs and central structures. Nesfatin-1, a recently isolated peptide (Oh-I et al, 2006) is expressed by neurons of different brain areas including hypothalamic and brainstem nuclei and by peripheral organs such as the stomach and pancreas. Both peripheral and central injections of nesfatin-1 result in a food intake reduction and this peptide has been proposed to contribute to satiety (Noetzel et al, 2009). Glucose is an important regulatory signal that activates glucose-sensing systems located in the CNS which in turn control glucose homeostasis, feeding behavior and energy storage. The purpose of the present work was to determine whether nesfatin-1 expressing neurons partake in the central signalling involved in glucose homeostasis. The expression of nesfatin-1 in pancreatic beta cells and the altered blood and pancreatic nesfatin-1 levels in diabetic subjects support our hypothesis.
We first show that after a peripheral insulin injection (10 U/kg) in the rat, resulting in a robust hypoglycemia and food intake increase, a part of the activated neurons (c-Fos+) exhibited a nesfatinergic phenotype. Nesfatin-1 neurons activated in response to hypoglycemia were located in the hypothalamus (PVN, ARC) and the brainstem (NTS, DMNX). Similar results were obtained after either peripheral (300 mg/kg) or central (4 mg/rat; 12 µl) 2-deoxyglucose (a non-metabolisable glucose analogue) injection. Hypoglycemia-associated autonomic failure (HAAF) is a complication of recurrent hypoglycemia in diabetic patients characterized by the loss of responsiveness of brain structures to hypoglycaemia. Accordingly, we next determined whether nesfatin-1 neurons sensitivity was altered after repeated glucoprivic challenge reproducing HAAF syndrome. Preliminary results show a lack of nesfatin-1 neurons activation in numerous brain areas in response to glucoprivation in HAAF animals. Altogether, these results suggest a multifaceted role for nesfatin-1 neurons in energy homeostasis. In addition to its satiety effect, this peptide could belong to the central signalization which contributes to glucose homeostasis in response to hypoglycemia.

P1.188

Annual variation of daily expression of the gene *Clock* in the suprachiasmatic nuclei of Syrian hamster is not affected by pinealectomy

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In mammals, the suprachiasmatic nuclei (SCN) contain the master circadian clock. Endogenous rhythmicity is derived from transcriptional-translational feedback loops of “clock genes” including the gene named *Clock*. The SCN drive their rhythmicity via neuronal and endocrine outputs, for example by regulating the nocturnal secretion of melatonin from the pineal gland. Mammals also anticipate upcoming seasonal changes in their environment. In hamsters, the transition from a long photoperiod (LP, long day length / 24h) to a short photoperiod (SP) induces a lengthening of nocturnal secretion of melatonin which initiates winter physiology such as gonadal regression within 8 weeks. After 6-7 months in SP, while nocturnal peak of melatonin secretion is still long, a seasonal “interval timer” governs the gonadal recrudescence. This part of the annual cycle, named the photorefractory phase, although a key for spring reproduction in the hibernator species like the hamsters is poorly understood.
We have previously shown that the SCN are sensitive to the photoperiod. In Syrian hamster, daily mRNA profiles of most clock genes are correlated to the photoperiod. Moreover, among the studied genes, the changes in expression profile of *Clock* are unique: mRNA levels which are high and constant throughout the day/night cycle in LP, become rhythmic with low values during the day in SP, and surprisingly again change to constant low values in RP. Our current hypothesis is that melatonin, the main actor hormone of seasonal physiology, is not essential for this seasonal adaptation of the SCN. Hamsters from LP were pinealectomised or sham-operated and kept for 8-10 weeks more in LP or transferred in SP and kept in this photoperiod for either 8-10 weeks (until testis regression in sham animals) or 28 weeks (until testis recovery in sham animals). We demonstrate that, under these 3 photoperiodic conditions, the profiles of daily expressions of *Clock* are similar between intact and pinealectomised groups. Whatever the photoperiodic conditions, the pineal is not essential for the daily functioning of the SCN. Thus, the circadian clock can build seasonal messages throughout the year, even in the absence of melatonin.

P1.189

Effect of water restriction on the serotoninergic system and glycoprotein secretion in the subcommissural organ Comparative study between Wistar rat and a semi-desert rodent: *Meriones shawi*

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Water is an important element for life in each living organisms, its deficiency can trigger a several neurological disorders involving some changes in many neurotransmitters. These disorders depend to the specie studied and the duration of dehydration. We aimed by this work to describe the effect of a graded durations (7 and 14 days) of water restriction, in rat, on the serotoninergic system and the secretory activity of the SCO; a part of the circumventricular organs implicated in the regulation of water balance by the double release of a glycoprotein: Reissner's Fiber (RF) in the cerebrospinal fluid and the blood stream. Using the technique of immunohistochemistry (IHC) of serotonin (5-HT) and RF,

in rat brain, we showed an alteration of both of them (5-HT and RF), following 7 and 14 days of total water deprivation. These data show an implication of 5-HT as a neurotransmitter and RF in the response to the water deprivation in the rat.
Keywords: Rat; Water restriction; Serotonin; Reissner's Fiber; SCO; Immunohistochemistry.

P1.190

The anorexigenic cytokine Ciliary Neurotrophic Factor (CNTF) activates its receptor in the nucleus of hypothalamic neurons that control energy balance

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CNTF (ciliary neurotrophic factor) is a neural cytokine that belongs to the same family as leptin with similar central anorexic action. Furthermore, CNTF anorexic action persists in obese states characterized by a leptin-resistance (Lambert et al., 2001). We have recently demonstrated that CNTF and its receptor are expressed in the anorexigenic neurons of the arcuate nucleus (ARC), a key hypothalamic region controlling food intake, and that CNTF distribution shares similarities with that of its receptor subunits. Furthermore, CNTF levels are inversely correlated to body weight in rats fed a high-sucrose diet, thus endogenous CNTF is positively correlated with protection against diet-induced obesity in some individuals (Vacher et al., 2008).
However, because CNTF lacks a signal peptide that tags secreted proteins, the comprehension of the physiological significance of neural CNTF action was still incomplete.
In this study, combining different cellular, biochemical and molecular approaches, we examined the potential direct intracrine effect of CNTF in ARC cells.
We demonstrated that CNTF translocates with its receptor subunits to the nucleus of the rat ARC cells and that the stimulation of hypothalamic nuclear fractions with CNTF induced the phosphorylation of several signaling proteins such as Akt, as well as the transcription of the POMC (pro-opiomelanocortin) gene in anorexigenic neurons.
These data strongly suggest that intracellular CNTF activates signaling pathways in the nucleus of ARC cells, regulating gene expression, and thus providing a novel plausible mechanism of CNTF action in the control of energy homeostasis.

P1.191

Glial cells of the dorsal vagal complex express the anorexigenic octadecaneuropeptide ODN

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Autonomic functions are controlled by central structures among which the dorsal vagal complex (DVC), a caudal brainstem center, plays a pivotal role. Comprising the nucleus tractus solitarius (NTS), an integrative nucleus, the area postrema (AP), a circumventricular organ and the dorsal motor nucleus of the vagus nerve (DMNX), the DVC participates in the control of food intake and satiety. To date, the literature dealing with the involvement of astroglial cell in the signalization regulating feeding

behavior and energy homeostasis remains scarce. Some clues are now available indicating that DVC glial cells could play a dynamic role in the regulation of energy balance (Dallaporta et al, 2010). Octadecaneuropeptide (ODN), a peptide derived from diazepam-binding inhibitor and mainly expressed by glial cells, has been shown to reduce food intake in rodents. Here, we investigated by confocal imaging the ODN expression at the brainstem level. We observed a strong ODN immunoreactivity within the DVC while surrounding brainstem structures were devoid of labeling. ODN labeling was mainly located within the AP and at the interface between the AP and NTS, a region called *funiculis separans*. We first confirmed the absence of neuronal ODN expression as attested by the lack of ODN and the neuronal marker MAP2 co-localization. Within the *funiculis separans,* ODN was associated with a subpopulation of GFAP+/vimentin+/nestin+ radiating glial cells that we recently characterized (Pecchi et al, 2007). At the AP level, ODN was expressed by DARPP-32+/vimentin+/GFAP negative stellate glial cells whose phenotype was evocative of 3rd ventricle tanycytes. The localization of glial ODN expression, within the AP and the AP/NTS interface, corresponds to the main area where hormonal and circulating information are received and integrated. Our results suggest that a glial source of ODN within the DVC could partake in the feeding behavior signalization. The effectors able to stimulate ODN release and the downstream neuronal targets of this peptide at the brainstem level remain to be elucidated to confirm our hypothesis.

P1.192

Transcriptional mechanisms controlling RAE-1 expression within neural stem/progenitors cells

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RAE-1 is known for its immune functions as a ligand of the activating receptor NKG2D expressed by NK cells, NKT, Tγδ and some T CD8 lymphocytes. Rae-1 transcripts are expressed in the central nervous system (CNS) during development and until the embryonic day 11.
We described that RAE-1 is also expressed in the adult subventricular zone (SVZ).
In vitro, neural stem/progenitor cells (NSPCs) in culture expressed RAE1, the level of expression correlated with the rate of cell proliferation and the expression was rapidly down regulated when differentiation in neurons, astrocytes and oligodendrocytes was induced. This observation mimics the developmental situation. In the C57BL/6 genome two Rae-1 genes are encoded: *d* and *e*. We described that the SVZ and NSPCs mainly expressed Rae1*d* transcripts. The expression profile of RAE1*d* and RAE1*e* proteins correlated with the level of transcripts. This suggests that transcriptional regulation is one of the main mechanisms involved in the control of RAE-1*d* and *e* expression. We indeed identified two different promoter regions for each gene. Moreover, the 3' untranslated regions of Rae1*d* and Rae1*e* are barely identical. We thus hypothesize that micro-RNAs are involved in repressing Rea-1 expression. Using bioinformatics tools, we identified micro-RNAs candidates and analyzed their expression in NSPCs and during NSPCs differentiation.

P1.193

IGF-BP3 is a determinant of Alzheimer's disease

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Insulin-like growth factor-I (IGF-I) and insulin-like growth factor-binding protein-3 (IGFBP-3) are involved in protein synthesis, carbohydrate homeostasis, bone metabolism and longevity. Circulating IGF-I and IGFBP-3 concentrations predict anthropometric traits and risk of cancer and cardiovascular disease. Since IGF-1 levels have been associated with cognitive processes, the relationship between serum IGF-1, IGFBP-3 level, IGF-1/IGFBP-3 ratio and cognitive function were measured in 481 elderly subjects consecutively assessed in a memory center for memory complaint. A control group of 213 patients without memory complaint was also included. All subjects benefited from a comprehensive cognitive assessment, brain imaging and APOE genotyping. IGF-1 and IGFBP-3 levels were determined by ELISA. Mean age was 78.6 ± 6.7 years, 69% of female. 50% of the population suffered from hypertension, 11% from diabetes. Mean albuminemia was 40.3± 2.7 g/l and 29.7% of cases were APOE 4 +. Subjects were divided into 3 groups: cognitively normal (controls, n=213), Mild Cognitive Impairment (MCI, n=257) and Alzheimer Disease (AD, n= 224), according to international criteria. Age, female gender, ApoE 4 +, low educational level status were significantly higher in cognitively impaired patients ($p< 0.05$). IGFBP-3 serum level was significantly higher in controls (4413+/-1635 ng/ml) than MCI (4172+/-1575) than AD patients (3747+/-1412 ng/ml) ($p< 0.001$). The main determinants of AD were age (0R (95%CI)=1.13 (1.1-1.2)), female gender (0R (95%CI)= 0.49 (0.25-0.95), ApoE 4 + (0R (95%CI)=3.1 (1.7-5.7)), low educational level status ((OR (95%CI)=4.7 (1.8-12), and IGFBP-3 level (0R (95%CI)=0.78 (0.64-0.95). No significant differences considering IGF-1 serum level were found between the 3 groups (respectively for AD, MCI patients and controls (ng/ml):145±72; 159±.81; 157±79; p=0.15). Such results may explain discrepancies between previous studies on smallest cohorts (high or low IGF-1 serum level associated with AD), because IGFBP-3 can regulate IGF-1 bio-availability.
This work was supported by the PHRC SIGAL
J. Epelbaum and E. Duron: equal contribution.

P1.194

The food-contaminant Deoxynivalenol modifies by targeting anorexigenic neurocircuitry

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Deoxynivalenol (DON), one of the most abundant trichothecenes found on cereals, has been implicated in mycotoxicoses in both humans and farm animals. Low doses toxicity is characterized by anorexia, reduced weight gain, diminished nutritional efficiency and immunologic effects. The levels and patterns of human food commodities contamination justify that DON consumption constitutes a major public health issue. DON stability during processing and cooking explains its large presence in human food. Despite this widespread human exposure, the mechanisms by which this toxin exerts its anorexigenic action remain unknown. In the present work, we show that acute *per os* DON intoxication in mice, resulting in hypophagia, decreased body temperature and activity, stimulated specific anorexigenic neurocircuitry in main structures involved in food intake regulation. We also report the first evidences for a DON-induced central inflammation, attested by the strong up-regulation of IL-1β, IL6, TNF-α, COX-2 and mPGES-1 mRNA. However, mPGES-1 KO mice, which are resistant to inflammation-induced anorexia, exhibited a feeding behavior similar to their control littermates in response to the toxin. Finally, when centrally injected at doses ineffective after *per os* administration, DON resulted in a rapid modulation of feeding behavior and stimulation of anorexigenic neurocircuitry. In conclusion, we propose that DON having reached the brain, after consumption of contaminated food modifies central regulation of energy balance by targeting anorexigenic networks. These new results raise the question of the potential consequences of chronic DON consumption on the development of pathological alteration of food intake behavior.

P1.195

Early effects of high-fat diet feeding on hypothalamic cell proliferation

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The hypothalamus is one of the main brain structure involved in the control of energy homeostasis. Recent reports indicate that hypothalamus exhibits neuroproliferative potency in adult. Moreover, it has been found that hypothalamic cell proliferation could be modulated by numerous intrinsic factors such as CNTF, IGF-1, bFGF and EGF, and by external and internal conditions such as dehydration, variation in ambient temperature and during ovarian cycle. Interestingly, experimental manipulation of neurogenesis can affect body weight control. However, whether nutritional conditions could influence hypothalamic cell proliferation and thereby modify energy homeostasis is still unknown. To address this question, mice were subjected to a high fat diet (HFD) for one week and hypothalamic cell renewal was assessed through central chronic infusion of Bromodeoxy-Uridine (BrdU). Using this approach, we report that hypothalamus constitutively exhibits ≈2000 BrdU-positive neoformed cells per day. This proliferative rate was significantly higher in hypothalamus of mice fed with HFD for 3 days (+60%). This rate was then decreased by 50% when HFD persisted during 5 days. To assess whether these HFD-induced modifications of cell renewal were linked to change in cell proliferation, we counted Ki67 proliferative cells. We found 1,937 Ki67 positive cells in hypothalamus from mice fed with standard chow. However, number of Ki67 positive cells was higher in mice fed with HFD for 1 and 3 days (+30 and 50%, respectively), and then returned to basal value after 5 days. Further experiments are needed to assess phenotypes and roles of these HFD-induced newborn cells.

P1.196

Octreotide and pasireotide decrease cell proliferation of human meningiomas in vitro

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Introduction: Meningiomas are one of the most frequent adult brain tumors. Complete surgical removal is the reference treatment. But, despite radiotherapy and radiosurgery, meningiomas post-operative recurrence or incomplete removal management could be problematic. For many years, multiple therapeutic tries have been performed, but no medical treatment has ever been proven to be efficient. Many studies performed on meningiomas demonstrated an important somatostatin receptor (sst) expression, essentially sst2 receptor, targeted by somatostatinergic agonists: octreotide, lanreotide, already use in acromegaly for their anti-secretory and anti-proliferative effects.Pasireotide is a new molecule considered as a pansomatostatinergic agonist with a longer life time.
Objective of our study was to evaluate human meningiomas cell viability, in vitro, in presence of octreotide and pasireotide.
Material and methods: Primary cultures were performed from 25 operative fresh pieces of human meningiomas. Somatostatin receptors subtypes 1,2,3,5 were quantified by real-time PCR and the presence of sst2 protein was verified by immunohistochemistry analysis. Increasing doses of pasireotide and octreotide (10^{-10} to 10^{-8} M) were applied on the primary cultures from 20 meningiomas.

The cell viability was measured by Cell titer glo assay, cell count or brdU incorporation after 3 or 5 days. The cell cycle was analysed by FACS.
Results: Sst2 receptor mRNA was expressed in all tumors, however the expression levels were highly variable between them (from 0,2 to 10 copies). We observed a stronger expression of sst2 receptor in grade 1 meningiomas and in meningothelial subtype in comparison with grade 2 and 3. The mRNA expression of other sst subtypes was not significant in primary culture. Octreotide and pasireotide induced a dose inhibitory effect (varying from 0 to 50%) of cell viability. The pasireotide effect was significantly higher than the octreotide effect in 90% of cultured meningiomas.
Conclusion: This study demonstrated the inhibitory effect of octreotide and pasireotide on cell viability of meningiomas in vitro, with a stronger effect of pasireotide, allowing to set up a clinical study evaluating the use of octreotide and pasireotide in case of meningioma therapeutic failure or impasse.

P1.197
Cognitive performances of anemic preadolescents in Morocco

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Iron deficiency, the major cause of anemia worldwide, is associated with neuro-cognitive impairment in studies reported elsewhere.
The aim of this work is to determine the levels of anemia and iron deficiency in Moroccan school children and to look for associations with cognitive performances.
All pupils from an urban elementary school from grade one to four are recruited. After approval of the parents and administrative clearing, a medical team examined the children and venous blood samples were drawn. Ferritin level was determined by ELISA on the serum of the anemic children (HB < 11.5g/dl). A battery of objective tests were administered to the children (Extracts from WISC; Raven Progressive Matrix and the bells test).
Anemia was observed in 23.4% of the pupils. Iron deficiency was the cause in 57% of children. Children did not perform well in Raven test but did better in WISC (Numbers recall) and the bells. The psychometric tests used showed that the temporary memory performance and the visual attention are higher than those of global intelligence in this sample and are higher in non iron deficient than in iron deficient children.

P1.198
Brain dynamics of cognitive control during lexical selection in overt speech

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Lexical selection is the process by which we select words for speaking, writing or signing. This process is under cognitive control [Ferreira, V.S. &, Pashler, H. (2002, *J EXP PSYCHOL LEARN*). Previous research has shown two different frontal areas that may be involved: the left inferior frontal gyrus (LIFG) [Schnur T. et al. (2009, *P NATL ACAD SCI USA*)] and medial frontal regions [Alario et al. (2006, *BRAIN RES*)]. Our goal was to clarify the relative roles of these regions by studying the spatio-temporal dynamics of lexical selection in overt speech.
We used the blocked picture naming task [Damian et al. (2001, *COGNITION*)] to elicit a semantic interference effect believed to reflect the competitive nature of word selection. This was compared to a task involving arbitrary stimulus-response associations: the verbal Simon task [Wühr, P. (2006, *ACTA*

PSYCHOL)]. Brain activity was measured using high-resolution electroencephalography. A blind source separation algorithm based on the Canonical Correlation Analysis helped reducing articulation-related artifacts [De Vos, et al. (2010, *NEUROINFORMATICS*)] and estimating Current Source Density of the signal helped improving its spatial resolution [Nuñez, P. (1981). *Electric fields of the brain: the neurophysics of EEG*. New York, NY: OUP].
Behavioral performance was as expected: semantic interference in naming, and laterality congruency effect in the Simon task. Early effects of the task performed, but not of within-task difficulty, were present on frontal recording sites. Semantic context effects were visible starting 250/300 ms after stimulus presentation on a left frontal activity. This activity peaked around the onset of the electro-myographic (EMG) activity linked to the articulation of the utterance; it was virtually absent in the Simon task. By contrast, a medial frontal activity peaking about 250 ms pre-EMG onset was present in both tasks. It was affected by the semantic context in picture naming and by congruency in the Simon task.
These results suggest the left and medial frontal regions are differentially involved in word selection. Whereas left frontal activities seem associated specifically to lexical selection, medial frontal activities seem associated to response selection in general.

P1.199

Cognitive, behavioral and psychological effects of a computer-based memory and attention training program in older adults with Mild Cognitive Impairment

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Considering the high risk for individuals with amnestic Mild Cognitive Impairment (A-MCI) to progress towards Alzheimer disease, we focused on the potential efficacy of non pharmacological interventions such as cognitive training that could generate neuroprotective effects, enhance cognitive functioning and delay the cognitive decline. To this aim we evaluated the efficacy of a 12-week computer-based memory and attention training (MAT) program in 11 patients with A-MCI (MAT group) and compared their performance with those of 11 A-MCI controls that participated in a cognitively stimulating activities training (CSAT group). The effect of MAT was assessed by the comparison of outcome measures in pre-and post-test that carried out respectively 15 days before and after training. In order to evaluate the maintain of training benefits' over time, a follow-up test session was performed 6 months after training or cognitively stimulating activities.
The results showed that MAT group improved cognitive performance in target cognitive domains (i.e., memory and attention) as well as in non target cognitive domain as executive domain and global cognitive status. In addition, a benefit effect was found on measures of subjective memory, self-esteem, and quality of life, and on the frequency of depressive symptoms. Six months after training, the results show that memory is the cognitive domain the most resistant to the decline.
Thus, cognitive training offers promise as a preventive therapeutic method for “at risk' individuals to convert to Alzheimer disease and could be proposed as a non-pharmacological early intervention strategy. Future investigations need to focus on methodological constraints and delineating possible neuroplastic mechanisms of action.

P1.200

Impact of environmental enrichment on recent and remote spatial memory over aging in the rat

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Aged rodents, as aged humans, exhibit alterations in cognitive functions, particularly when memory is concerned. The identification of environmental factors that might contribute to the preservation of

these functions with the age is one of the challenges that research has dealt with for over more than half a century. In this respect, we recently demonstrated in rats that environmental enrichment during the whole post-weaning life limited the age-related alterations of the acquisition of a spatial task. In the present study, the impact of such rearing conditions on recent and remote spatial memory retrieval was evaluated. Long-Evans female rats (1 month) were housed in standard or enriched conditions during 3 (young), 12 (middle-aged) or 24 (aged) months. Spatial learning and memory were assessed in the Morris water maze using a standard reference memory protocol. After a first probe trial assessing recent memory (1-day post-acquisition delay), rats were further trained and remote memory was assessed at a 25-day post-acquisition delay. Middle-aged rats reared in standard conditions swam a longer distance to reach the platform than their young counterparts but performed similarly during the first probe trial, suggesting a weak acquisition deficit but no marked impact on recent memory. In contrast, aged standard rats were unable to learn the task. No deficit was observed in middle-aged rats reared in enriched conditions and despite the presence of a learning impairment in enriched aged rats, their recent memory was preserved. Remote memory retrieval, which was altered independently of age under standard rearing conditions, was rescued by enrichment, but only in young and middle-aged rats. These results show that environmental enrichment both delayed the onset of spatial learning/retention deficits during the aging process and limited their severity. They also indicate that, in young and middle-age rats, environmental enrichment allowed the preservation of the spatial memory at a time at which it had disappeared in rats housed in standard conditions. This may suggest that enriched environment facilitates spatial memory consolidation and/or allows the formation of a memory trace which is more precise and/or less sensitive to degradation.

P1.201

Long-term effects of negative vs positive emotions on learning strategies

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There are different types of memories processed in human and animal brain. “Declarative / cognitive” memory depends on hippocampal circuitry, whereas “habitual / procedural / response” memory would rely on a neural system involving the dorsal striatum. Although distinct, these memory systems may operate either cooperatively or competitively. Increasing evidences exhibit that these two systems have to cooperate during creation of new memories. For instance, procedural learning needs the involvement of cognitive functions to be acquired and, conversely, hippocampus-dependant tasks rely also on the integrity of the dorsal striatum. Much remains to be done to better understand the key factors which modulate memory systems interactions. There is increasing evidence that emotions have a crucial impact on these interactions. It is well known that stress has a deleterious effect on acquisition of both spatial and procedural learning. Chronic restraint stress strongly impairs spatial learning in rats. Interestingly, drug use induces learning disturbances that maybe compared to the effects of chronic stress. For example, morphine-treated rats exhibit spatial deficits in a water maze task. The common neural elements of learning disturbances induced by positive and negative emotions could involve dopamine (DA). The DA system provide wide array of inputs from the ventral tegmental area (VTA) to memory-relevant brain regions, in particular to the hippocampus, dorsal striatum and amygdala. DA underlies reward learning. Yet it is also involved in fear conditioning. In this context, we will present new experimental data showing how negatively (stress) and positively (drug or food rewards) -charged stimuli modify the use of a spatial learning strategy, thus revealing striking similarities in the long-term effects of emotional events, whether negative or positively valuated. The results presented will concern the cellular, system and behavioral levels. They provide evidence that effects of emotionally-charged events on learning and memory processes rely on modification of brain regional-specific expression of the phosphorylated form of the cAMP-related binding protein (pCREB).

P1.202

Neural correlates of observational learning in the prefrontal cortex of the macaque monkey

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Numerous animal species can learn either through their own experience (trial and error learning, TE) or through the observation of conspecifics. Here, we address the question of how the brain of an observer encodes the outcome of other's behavior, with particular focus on error and success signals. In this study, two monkeys were trained on a visuo-spatial task. The experimental design allowed the monkeys to face each other, and to have access to the touch screen displayed face up between them. Only one of the two monkeys had access to the touch screen at a time, the other could observe but not reach the screen. To control that the observer looked at the touch screen, its gaze direction was monitored. Neural activity was recorded from the dorso-lateral prefrontal cortex (DLPF) of the observer, an area known to play a key role in the processing of action and its outcome during learning. The actor initiated a trial by putting his hand on a lever. Then a visual cue was presented together with 2 or 4 targets. Pressing the correct target led to a success signal, followed after a delay by a reward; incorrect response led to an error signal and no reward was given. To investigate the neural activity, several conditions were used: the observation of familiar or new cue-target associations, the execution of new associations that were (observational learning) or were not (TE) observed before. Gaze data showed that the observer did monitor the actor´s behavior. Behavioral results showed that the observation led to performance improvement of up to 13% for associations learned after observation relative to those learned by TE. Neuronal data indicate that the DLPF contains two populations of neurons encoding error and/or success signals. One responds both to the outcomes of own and other´s actions. The other differentiates the source of the outcome, responding either to signals triggered by the monkey´s own actions or only to those triggered by the other monkey´s actions. Taken together, the neuronal properties provide evidence that the monkey DLPF cortex integrates signals about own and others' successful and erroneous behavior. We conclude that the DLPF cortex may play a key role in encoding pertinent social information which allows learning from others' experience.

P1.203

The addiction to thinner and glue in mice

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Paint thinner and glue constitute a large class of volatile compounds that are voluntarily inhaled for their euphoric effects. They have been shown to cause some behavioural alterations and may lead to irreversible brain damage.
The aim of the present study was to highlight a possible dependence on these drugs in mice animal model using a place preference apparatus. This consists of two compartments in which the drugs (thinner or glue) were associated to one of the two compartments. Three groups (n=15) were used ; a control group and two groups exposed by inhalation to 0.4 ml of the thinner or 5g of the glue in a static chamber for 45 days (1h/j). The conditioned place preference was started one day after the last exposition of the groups. In the post-conditioning test, the time spent in each compartment was noted. Animals were sacrificed one day after this test to investigate the glial reactivity in the hippocampus by immunohistrochemistry using glial fibrillary acidic protein (GFAP) antibody.
Our results show that control mice show no preference for any compartment. However mice receiving the solvent and the glue spent significantly more time in the compartment containing the drugs (+42, 94 % and +47.08%, respectively). Immunohistochemical GFAP study shows no significant difference

between the control group and that treated by the thinner, whereas, the difference was highly significant with the group having sniff glue ($p < 0.05$).
These data show that chronic exposure to the glue and thinner leads to addiction to these drugs in mice. They suggest that behavioural alterations induced by inhalation, especially by the glue, could be explained by the brain dysfunction including in part, glial changes in the hippocampus which is involved in mediating reward processes.

P1.205

Cocaine withdrawal-induced anxiety is associated with impaired reactivity of the rat medial prefrontal cortex

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Impairment in emotion regulation is often observed during abstinence in cocaine abusers and has been suggested to increase relapse susceptibility. The aim of this study was to provide further insights into the functional neurobiological alterations that might contribute to this pathologic state.
Male Sprague-Dawley rats were injected with cocaine (20 mg/kg, i.p.) or saline once daily for 14 days and anxiety-related behavior was assessed in different paradigms during withdrawal. Two days after the last cocaine injection, cocaine-treated rats exposed to one open arm of the elevated plus maze test (OA) exhibited longer periods of freezing (38.21 ± 9.16 vs 7.4 ± 2.93; $p < 0.01$) and less time head scanning (43.35 ± 6.3 vs 79.5 ± 15.56, $p < 0.01$) than saline-treated rats. In the shock-probe burying test, another well-validated test of anxiety, periods of immobility were longer in cocaine-treated rats compared to saline-treated rats for up to 28 days of withdrawal (119.21 ± 19.73 vs 62.87 ± 19.36, $p < 0.05$). Such marked difference in behavior indicates that cocaine-withdrawn rats have more difficulties to cope with anxiogenic environment than saline-treated rats.
Using immunohistochemistry of Fos, a marker of neuronal activation, we next demonstrated that after OA exposure cocaine-treated rats displayed lower densities of Fos-positive cells than saline-treated rats in the anterior cingulate (54% reduction), prelimbic (38% reduction) and infralimbic (31% reduction) cortices, pointing to a marked alteration in mPFC reactivity of cocaine-treated rats.
We then sought to determine whether transient inactivation of the cortical neurons recruited in response to OA exposure had an impact on anxiety-related behavior in cocaine- and saline-treated rats. We showed that bilateral microinjections of muscimol (a GABA-A receptor agonist) in the ventral mPFC prior to OA exposure completely abolished the expression of freezing postures in cocaine-treated rats. Altogether, our findings point to a strong reactivity of the ventral mPFC during the processing of emotionally relevant information that contributes to impaired coping with anxiogenic situations during cocaine withdrawal.

P1.206

Dorsomedial and dorsolateral striatal activity in a continuous T-maze alternation task

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Several authors have demonstrated a specific involvement of the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) in the acquisition of instrumental action-outcome associations and habit formation, respectively. However, the functional properties of neuronal activity in each striatal subregion have been poorly investigated in relation to goal-directed spatial learning.
The aim of this study is to compare DMS and DLS cells activity in a continuous T-maze alternation task, in which rats were required to run up the central stem of the maze and enter the left and right choice arm in alternance to obtain a reward. Rats were implanted with a movable bundle of 4 tetrodes and single unit activity from DMS or DLS was recorded from the first acquisition session.
Preliminary results show that a significant proportion of DMS neurons fire differentially at goal locations during left and right turns, and that this differential signal depends upon animals' performance. DLS neurons generally increase firing activity during reward delivery, irrespective of its position. These data suggest that DMS may participate to the acquisition of spatial alternation behaviour by generating the signal necessary to link a reward with a specific spatial sequence. In contrast, DLS activity is more sensitive to reward delivery, than to reward-response contingencies. Taken together these results confirm and expand previous data by showing a functional dissociation between DMS and DLS in the acquisition and performance of a spatial goal-directed behaviour.

P1.207

Early consumption of a high-fat diet is more detrimental than adult consumption for spatial and relational memories in rats and mice

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Increased consumption of energy-dense food, especially high-fat diets (HFD), is directly linked with the new obesity pandemic. In addition to metabolic and cardiovascular disorders, obesity is associated with adverse cognitive outcomes. This can be particularly problematic during childhood and adolescence as these periods shape the neurobehavioural processes required for life-long cognitive function. However, the existence of critical periods of development that differ in terms of sensitivity to the detrimental effects of HFD remains unexplored. In adult rodents, long lasting consumption of HFD induces obesity and cognitive impairments, particularly in hippocampal-dependent spatial memory. The aim of this study was thus to compare the consequences of a short duration HFD (8 weeks) starting when animals were either 3 weeks-old (early HFD exposure, i.e. covering late infancy and adolescence) or 12 weeks-old (adult HFD exposure, i.e. during adulthood only) on hippocampal-dependent memory.
We first assessed spatial memory in Wistar rats using the Morris water maze task. Early HFD exposure impaired long-term memory retention (assessed 4 days after acquisition) without affecting acquisition and short-term memory retention (tested 2 hours after the last day of acquisition). On the contrary, adult HFD exposure did not alter any memory step. In order to further establish that early HFD exposure is critical for cognitive impairment, we explored another hippocampal-dependent task in another species. Relational memory was evaluated in C57/BL6 mice using radial maze. During concurrent spatial discrimination of pairs of arms, early HFD exposure induced a greater impairment than adult exposure. Mice performing this discrimination were then exposed to a recombined pair (made of previously discriminated pairs) to evaluate their relational memory, i.e. their ability to use previously acquired spatial memory in a flexible manner. Again, early HFD exposed mice were specifically impaired: they responded at chance level whereas controls and adult HFD exposed mice responded well above chance (~70% accuracy).
Our results identify a critical period of development (late infancy and adolescence) with higher sensitivity to the detrimental effects of HFD on hippocampal-dependent memories.

P1.208

Dissociation of "place" and "cue" strategies in a spatial navigation learning task in primates

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Two separate sub-cortical brain systems have been shown, in rodents, to learn environmental navigation in parallel but at different rates and encoding the environment in different ways. In the rodent, the hippocampal system has been shown to support allocentric or place learning strategies and the basal ganglia egocentric or cue learning, with the place learning dominating in early stages of training and the cue learning later. This has not been reproduced in the primate and taxonomic differences between the groups mean that it is not possible to extrapolate from rodents to primates. The two groups rely on different basic perceptual systems, olfaction and vibrissae in rodents and vision in primates. As a consequence, in contrast to the place cells of spatial maps formed in the rodent hippocampus, primates form a hippocampal mapping more related to visual orientation. As this issue has consequences for day-to-day functioning in the most common human neurodegenerative diseases, we are in the process of quantifying the interactions between the two neural networks in the non-human primate. We have developed a task that is adapted from the classical X-maze of Packard & McGaugh (1996) that showed the dissociation of learning between the two systems. We have trained two female rhesus macaque monkeys to navigate the maze in a powered wheelchair. In short training sessions (three repetitions of the same path) we show that the monkeys use a place learning strategy to reach the goal and in long training sessions (10 repetitions) we show that they use a cue learning strategy. This is the same dissociation as shown in rodents. In order to investigate the neural basis of this dual system learning we will continue the study using a combination of electrophysiological recording and region-specific pharmacological inactivation.

P1.209

Behavioral characterization of the LOU/C/Jall rat, a model of healthy aging

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Because Lou/c/Jall rat does not show obesity or any severe pathologies when it gets old, and because its longevity is better than other common rat strains (median life span: 29 and 33-34 months for males and females respectively), it has been described as a potent model of healthy aging *(Alliot et al., 2002)*. Moreover, brain electrophysiological properties, that sustain memory function, are preserved in Lou/c/Jall across aging *(Kollen et al., 2010)* contrary to Sprague Dawleys rats. Unfortunately, very few behavioural data regarding this strain have been collected till now. Herein, we characterized Lou/c/Jall rats' behaviour over aging.
We compared performances of male and female animals aged 6, 12, 24, and 30 months,. Behaviours analyzed were: locomotor activity, anxiety-related behaviour (plus maze and open-field), working memory (spontaneous alternation), episodic memory (place and object recognition), spatial learning (Morris water maze), and despair-like behaviour (forced swimming test).
Our results showed that there is no modification during aging of spontaneous activity, anxiety-like behaviour (or even a decrease). Working memory, object and place recognition capacities, and spatial learning were preserved until the age of 12 months in males and 24 months in females. Despair-like behaviour decreased with age in females but not in males.

Globally, these data suggested that, compared to the control Wistar rat, LOU/c/jall rats displayed a delay in age-related decline in locomotor and cognitive performances *(Hunt et al, 2009; Kollen et al, 2008)*. Interestingly, the memory capacities were better preserved in females than in males. Our results are in accordance with previous electrophysiological observations, and argue that the LOU/c/Jall rat is a model of healthy aging. Nevertheless, further studies need to be performed to characterize the neurochemical substrates related to such a powerful behavioural preservation during aging.

P1.210

Simple model of control of verbal fluency in Arabic language studied using BOLD-fMRI

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Introduction: The adult's cognitive functions are based on activation of specialized neuronal networks. Studies showed that a set of well determined brain cortical tissue are involved in the control of language function. In addition, Arabic language is shown to be very complex and might involve a complicated network of brain cells during language performance. The study goal is to establish the functional map of the verbal fluency Arabic language using simple model of paradigm and making a use of BOLD-fMRI approach.
Materials and methods: 12 healthy-adult Arabic-speaking volunteers were recruited. They were non-smoking, right-handed, and without any neurological, psychiatric disorder. We used BOLD-fMRI to map the neuronal network involved in the control of verbal fluency of Arabic. The functional paradigm consisted of silent words generation alternating with counting during 30 seconds in each cycle.
For achieving a better activation of cortical brain involved in this language task; the Arabic characters were chosen according to the most important prevalence of use in the vocabulary of volunteers of this study.
Results and discussion and conclusion: The Arabic letters generating most words were used (أ, ج, ح, م, ق, س, ر) Obtained fMRI results in Arabic-speaking adults showed that the network of the Arabic verbal fluency is structured in an equivalent way compared to already studied languages, in other words the right hemisphere is less stimulated than the left one, and this has considered volume and intensity of activations. This could be explained by the fact that all volunteers are right-handed and have a left hemispherical lateralization. However, different aspects were essentially found in cerebral lateralization in women. Similarities indicate the continuity in the processes and the neuronal structures underwent the functional control of different languages. While differences suggest that Arabic language have appropriate functional control characteristics.
This study of Arabic language opens new perspectives of exploration which would contribute to better information about the involved neuronal network in functional control of Arabic language.

P1.211

A critical episodic buffer in long term declarative memory formation

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Recently, A. Baddeley revised his model of working memory by adding a new component: the episodic buffer (EB). It is a temporary storage system capable of binding and integrating multimodal informations from various sources and systems. The EB occupies a strategic position at the interface between short-term and long-term declarative memory (LTDM). A recent human study suggested that the EB would play a role in the aging-related decline of LTDM, but this hypothesis needs to be further explored.
In the present study, we used a behavioural model of LTDM developed in mice in a radial maze. We manipulated the demand on EB during the encoding phase of LTDM by separating individual arms presentation with a temporal gap (Inter-Trial Interval, ITI). We studied the consequences of this ITI manipulation in young and aged mice (i) on characteristic flexible expression of LTDM, assessed by a specific flexibility test, (ii) on memory systems' activity during encoding by Fos immunohistochemistry. We found that both memory flexibility (i.e. LTDM) and encoding-related Fos activities were sensitive to the ITI manipulation. Our behavioural results indicated that a short-term buffer of limited capacity was needed to encode temporally discontiguous events into a unitary, flexible LTDM representation. A reduction in the EB capacity could explain the LTDM deficit seen in aged mice. Only non-flexible procedural memory was formed when the task demand exceeded the buffer's capacity (ITI longer than 20 sec. in the young, 5 sec. in the aged mice). Our Fos data highlighted an EB's correlate in the activity of a subfield of the hippocampus, critical brain structure for LTDM formation. Namely, in young mice, increasing demand on the EB was associated with CA1 activation. This CA1 activation was not seen in aged mice and disappeared in young animals when the task demand exceeded the EB's capacity (ITI=60 sec.). In both these conditions, the dorso-medial striatum, critical structure for procedural memory was activated.
In conclusion, our data show that an EB is needed for the formation of flexible LTDM representation and an aging-related decline in this short-term memory system contributes to LTDM degradation. Our findings also suggest that hippocampal CA1 activity is critical to this EB.

P1.212

Differential roles of caudate nucleus and putamen during instrumental learning

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The dorsal striatum is crucial for the acquisition and consolidation of instrumentalbehaviour, but the underlying computations and internal dynamics remain elusive. Toaddress this issue, we combined a model of key computations supporting decision-makingduring instrumental learning with human behavioural and functional magnetic resonanceimaging (fMRI) data. The results showed that the associative and sensorimotor dorsalstriatum host complementary computations that, we suggest, may differentially supportgoal-directed and habitual learning. The anterior caudate nucleus integrates informationabout performance and cognitive control demands, whereas the putamen tracks how likelythe conditioning stimuli lead to correct response. Contrary to current models, the putamenis recruited during initial acquisition. As the exploratory phase proceeds, the relativecontribution of the caudate nucleus becomes dominant over the putamen. During earlyconsolidation, caudate nucleus and putamen settle to asymptotic values and share control.We then investigated how dorsal striatal computations may affect decision-making. Wefound that portion of reaction times´ variance parallels the combined cost associated withthe dorsal striatal computations. Overall, our findings provide a deeper insight into thefunctional heterogeneity within the dorsal striatum and suggest that dynamic interplaybetween caudate nucleus and putamen underlies the acquisition and early consolidation ofinstrumental behaviours.

P1.213

Individual trait for emotionality operates as a switch between memory systems

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Recent investigations pointed out a critical role of emotions in facilitating the switch between different memory systems. In the present study we tested whether individual trait for emotionality can modify the use and selection of different strategies for solving a spatial learning task. To that end, lines of Japanese quail divergently selected for a typical fear response in birds, the duration of tonic immobility (TI), were used. Previous investigations demonstrated that the selection program modified the general underlying emotionality of the birds rather than exerting only effect on tonic immobility.
In the first experiment, lines of quail were trained in a spatial reference task where birds had to learn the position of a cup that contained a food reward among 8 identical cups placed in the test arena. Data obtained by the end of training (distance moved to reach the target cup) and during the probe test (time spent in the zone surrounding the target cup) revealed that birds selected for a high emotionality acquired a more accurate representation of the location of the target cup.
In the second experiment, quail from both lines were similarly trained for locating a target cup among 8 cups placed in the same arena. In this experiment, the target cup was of white colour whereas the other cups were black. To find the food reward animals could thus either learn the position of the target cup ("spatial strategy") or simply learn that the white colour indicates the presence of the reward in the cup ("cue-based strategy"). The strategy used was assessed during a probe test by relocating the white target cup to another position in the arena. Results showed that animals selected for their low emotionality systematically chose the relocated white cup ("cue-based strategy"). In contrast, birds selected for their high emotionality preferred the position of the cup used during training ("spatial strategy").
Overall these findings indicate that high emotionality (1) facilitates spatial memory performances and (2) promotes the selection of a spatial learning strategy. The neurobiological mechanisms involved in the selection process between memory systems are currently under process.

P1.214

Age-related cognitive impairment occurs before overt plaque pathology in the macaque

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A central question in research on age-related cognitive decline is the relationship between the cognitive performance and the cerebral pathology. Due to its rich behavioural repertoire, the rhesus monkey provides an excellent animal model for assessing age-related cognitive impairments. Such impairments occur in animals over the age of 19 years. After that age, cognitive performance becomes impaired in a variety of domains. For example, in a delayed matching to sample (DMTS) task, aged rhesus monkeys perform long delay trials significantly worse than short delay trials and performed the task worse than young mature animals. Aged animals also exhibit deficits in performing the most difficult levels of a paired associative learning (PAL) task and perform worse than mature animals. Both deficits are reminiscent of preclinical Alzheimer's disease (AD) with the DMTS and PAL deficit being improved by the cholinesterase inhibitor tacrine (0.3 to 1 mg/kg, i.m., 45 minutes prior to testing). Cerebrospinal fluid from young, mature and aged rhesus monkeys were collected and the Aß ratio (Aß-42/ Aß-40 * 10) was determined by Elisa detection. Surprisingly, the ratios were identical regardless of age of the animal (i.e., ratio >3, no recognizable risk for AD). We then characterized the distribution and aggregation of βA4-amyloid and tau in post mortem brain tissue using immunohistochemistry. Analysis was performed by a trained pathologist. While subcortical gliosis was noted in aged animals, only scarce ßA4 positive senile plaques were observed in the neo-cortex. Tau

aggregates were noted in deep brain structures as well as in the cerebellum in aged animals. These data suggest that cognitive impairment is clearly dissociated from overt AD-like pathology in the Rhesus non-human primate and that functional neuronal impairment rather than extensive structural pathology and degeneration underlies the cognitive deficits in these animals.

P1.215

On the role of intra-collicular excitatory connections in the generation of horizontal, oblique and vertical saccades: a Human behavioural study

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In neural models of saccade generation, lateral interactions between neurons in the topographic motor map of the Superior Colliculus play a major role in determining saccade metrics. By reshaping the activity profile that initially arises from visual stimulation, short-distance excitatory and long-distance inhibitory interactions favor the emergence of a main peak of activity, which determines in turn where the eyes move. Thus, when two spatially-proximal stimuli are displayed simultaneously in the same hemifield, the eyes land at an intermediate location between the two stimuli (i.e. the Global Effect) because short-distance excitations resume the two initially active peaks into one intermediate peak. Recently, we provided Human behavioural evidence for this assumption; we showed that the global effect cancels out when the spatial separation between two stimuli presented on the horizontal meridian exceeds a threshold distance of about 1mm of collicular surface, as previously suggested by electrophysiological investigations in the monkey [Casteau & Vitu 2009]. Here, we tested whether the same threshold distance generalizes to stimuli presented on vertical and oblique axes. In separate blocks of trials, a singleton peripheral target (to be fixated) was presented on one of 6 possible axes, including the horizontal and the vertical meridian. The target was presented either in isolation, or simultaneously with a less eccentric distractor displayed on the same axis. Both the eccentricity of the distractor (2 and 4°) and the angular separation between distractor and target (1° to 7°) were manipulated.
On no-distractor trials, saccades were relatively accurate, irrespective of the presentation axis. On distractor trials, results varied depending on the presentation axis. In the horizontal condition, the eyes were deviated towards the distractor (i.e. the global effect). In the vertical condition, a global effect was present only when the separation between the stimuli was very small. In oblique conditions, the pattern was intermediate. A way to reconcile these findings with the lateral-interaction hypothesis is to assume that short-distance excitatory connections within the motor maps are mainly oriented along the representation of the horizontal meridian.

P1.216

Evaluation of cognitive function of two extracts of rubia peregrina using open field test on the rats

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Herbal medicine is increasingly used by general population. Nowadays In Africa medicinal and aromatic plants are used in traditional medicine . *Rubia peregrina* perennial shrub of *rubiaceas* family with medicinal proprieties. The whole plant is prescribed in folk medicine for the treatment of anemia

blood diseases, and the treatment of inflammations. The Aim of the present study is the evaluation of locomotors activities using the open field test on the rat under two concentrations of the ethanolic and water extract of *rubia peregrina*'roots. Results showed that the both extracts decreased the locomotors activities and then the number of crossing (peripherals and centrals) lines, and increased the time of immobility and the grooming behavior with both concentrations 500mg/kg and 800mg/kg, respectively. Conclusion**:** the ethanolic extract and water extract have an effect in locomotors activities on the rats nevertheless The ethanolic extract decrease locomotors activities more than water extract. Some constitutions of *rubia peregrina* maybe affect dopaminergic system

P1.217

Do stereotypes cue comprehension of speaker's ironic intent in autism and schizophrenia?

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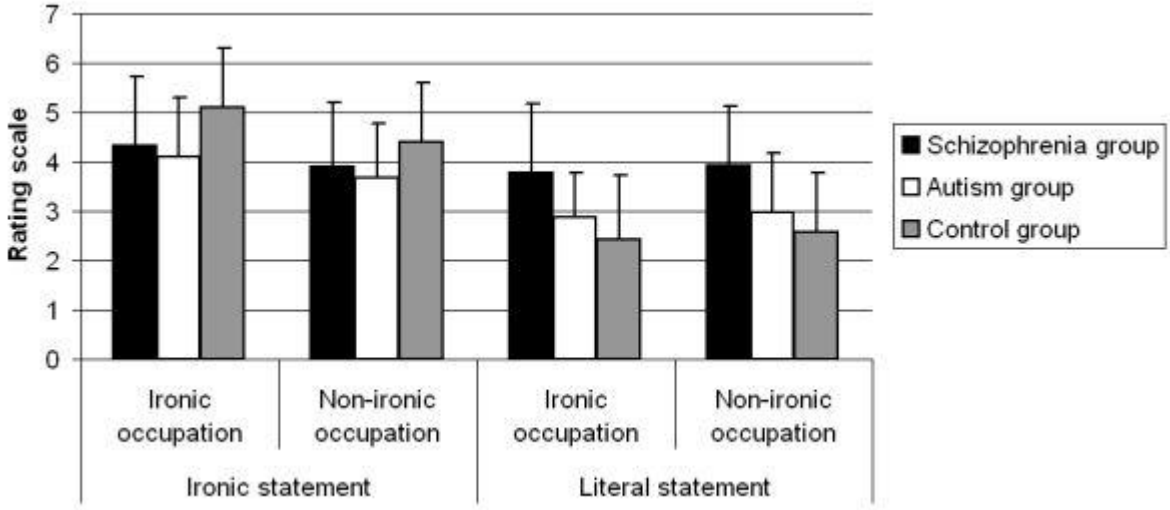
Individuals with autism spectrum disorders (ASD) and individuals with schizophrenia (SZ) have in common to be impaired in their social and communication abilities. Their inability to infer intentions and beliefs of others has been supposed to result from their inability to use contextual information. However, recent findings have shown that individuals with ASD are sensitive to stereotypes on gender, race and age. It was also suggested that several factors such as level of incongruity between context and speaker's utterance, prosody, or character's features set in the stimulus situation cue the comprehension of the ironic intent among healthy subjects (Ivanko and Pexman, 2003).
The goal of this study was to determine whether individuals with ASD and individuals with schizophrenia (SZ) exhibit the same patterns of performance on a task assessing if stereotypes (type of speaker's occupation) cue comprehension of ironic intent.
Thirty SZ individuals, sixteen adults with ASD and fifty matched healthy participants were recruited. Participants were asked to read 48 stories in which the speaker's occupation had been manipulated according to 3 conditions of occupation: occupation that cues ironic intent, occupation that does not cue ironic intent and no occupation.
Participants were asked to judge on a 7-point scale (1=not at all, 7=extremely) if the speaker was ironic, if he was mocking someone and if he was polite. The first two questions were used to assess attribution of mental states to the speaker while the last question was used to assess social perception.
Main results seem to show that both SZ participants and ASD participants were not sensitive to stereotypes (type of speaker's occupation) by contrast to healthy participants. However, while ASD participants performed like healthy participants judging irony and mockery, SZ participants gave answer at random (cf. Figure 1). There was no difference between the three groups for social perception.



[Figure 1]

Figure 1. Level of mockery of statement (ironic, literal) for each type of occupation (ironic, non-ironic) according to the type of participant (SZ, ASD, HC)

P1.218

Light induced release of endogenous oxytocin in the amygdala following its promoter driven expression of channelrhodopsin

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The neuropeptide oxytocin (OT) is synthetized in neurons of the supraoptic and paraventricular nuclei of the hypothalamus. This neuropeptide is known for its peripheral effects, controlling parturition and lactation in mammals, but also for its central effects, orchestrating social behaviours, enhancing trust and attenuating fear. However, the sites and the cellular structures of OT release within the forebrain remain unknown. Here we gained genetic access to hypothalamic OT neurons in the rat by synthesizing recombinant adeno-associated virus under the control of OT promoter. These AAVs, injected in some hypothalamic nuclei, allowed us to study the connectivity of fluorescent OT neurons in the brain and to control their activity by optogenetic means and blue-light stimulation. We found OT fibers, and particularly axons, in many forebrain regions, including the central amygdala (CeA), a structure critically involved in OT-mediated suppression of fear and associated autonomic responses. In the CeA, we visualized monosynaptic axonal projections of magnocellular OT neurons and recorded OT-dependent electrophysiological responses to localized blue-light stimulation of channelrhodopsin-2-expressing OT fibers. Thus, the behavioural and autonomic effects of OT are mediated by its central release from long-range axonal projections.

P1.219

Comparison of two treatment strategies on stress-induced impairment of fear extinction in rats: pharmacological approach and deep brain stimulation

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Difficulties to extinguish conditioned fear and difficulties to maintain fear extinction are considered as being involved in chronicity of post-traumatic stress disorder (PTSD) and PTSD relapse, respectively. Developing treatment strategies that facilitate extinction learning and memory in animal models is therefore of particular interest. Extensive evidence indicates that previous exposure to both acute and chronic stress can affect extinction learning (e.g., Rodriguez Manzanares et al., 2005; Akirav and Maroun, 2007) and memory (e.g., Miracle et al., 2006; Garcia et al., 2008). Pharmacological treatments have been mostly used to prevent such effects (e.g., Rodriguez Manzanares et al., 2005; Akirav and Maroun, 2007). Non-pharmacological techniques, such as deep brain stimulation of the hippocampus (HPC) in rats, are also being investigated as a treatment facilitating extinction memory (Farinelli et al., 2006; Deschaux et al., 2011). To further explore this issue, we compared acute effects of spadin, a peptide that produces antidepressant-like response through its action in the HPC (Heurteaux C. et al., 2006), and effects of low-frequency stimulation of the ventral HPC, considering lesions of this region reduce anxiety (Barkus et al., 2010). Male Wistar rats were submitted to a single footshock administration (1 mA, 5 sec), which was followed by situational reminders. This stress procedure was validated by higher levels of freezing to an aversive auditory stimulus and anxiety-like behavior in an elevated plus maze, as compared to non-shocked rats. This stress procedure also altered extinction of context conditioned fear, which was acquired in a different environment with 5 trials (0.6 mA, 1 sec each). Spadin and low-frequency stimulation of the ventral HPC failed to reverse this effect, but facilitated retention of extinction in rats that were not exposed to the previous stress procedure. Next step will be to evaluate the effects of the cotreatment since deep brain stimulation approach, which is now used for many psychiatric disorders, such as major depression (Hamani et al., 2010) and obsessive-compulsive disorder (Marazziti and Consoli, 2010), may also be useful for treating both PTSD chronicity and relapse when targeting appropriate area of the HPC.

P1.220

Linking social and vocal brains: social withdrawal prevents a proper development of the central primary auditory area in a female songbird

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Direct social contact and social interaction affect both speech development in human infants and song learning in songbirds, and are required in order to maintain perceptual abilities. However, the processes involved are still poorly known. In the present study, we tested the hypothesis that social withdrawal would prevent the proper development of a central auditory area, using an established animal model of vocal development, a songbird. Based on our knowledge of European starlings' vocal behaviour and development, we raised young female starlings with peers and adult male tutors only. This ensured that these females would show neither social bond with nor vocal copying from males. Electrophysiological recordings performed when these females were adult revealed perceptual abnormalities: they presented a larger auditory area, a lower proportion of specialized neurons and a larger proportion of generalist sites than wild-caught females, whereas these characteristics were similar to those observed in socially deprived (physically isolated) females. These results confirmed, and added to, earlier results for males, suggesting that the degree of perceptual deficiency reflects the

degree of social withdrawal. To our knowledge, this report constitutes the first evidence that the lack of social interactions can, as much as physical separation, alter the development of a central auditory area.

P1.221

Age dependent spatial memory impairment is associated with altered histone H3 and H4 acetylation in mice

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Normal aging is associated with impairments in hippocampus-dependent memory tasks and transcriptional deregulation of gene expression through altered histone acetylation. Indeed, post-translational modifications *via* histone acetylation is considered as a crucial mechanism controlling the transcription of genes required for long-term memory. We investigated whether normal aging affects the patterns of histone H3 and H4 acetylation in mice subjected to a 1 day-massed spatial training in the water maze. In this aim, adult and aged C57BL/6 mice were sacrificed at different time-points after learning and acetylated H3 (AcH3) and H4 (AcH4) levels were measured in hippocampal CA1 region. Spatial learning in adults produced significantly increased AcH3 and AcH4 levels 1-3h post-training. In aged mice, impaired learning was associated with significantly greater AcH3 but lower AcH4 levels at 1h post-acquisition, relative to adults. These data suggest that an imbalance between AcH3 and AcH4 might underlie altered expression of memory/plasticity-related genes. Next, adult and aged mice received intra-CA1 injection of Trichostatin A (TSA, histone deacetylase inhibitor) immediately post-acquisition and memory retention was assessed during a 24h-probe test. TSA-injected adults displayed histone hyperacetylation and enhanced memory retention. In contrast, restoring CA1 AcH4 levels *via* TSA injection in aged mice failed to alleviate spatial memory deficits. Because TSA did not rescue altered CREB phosphorylation seen in aged mice, our data support that a steady-state balance between histone acetylation as well as intact CREB signalling are required for induction of memory/plasticity-related genes.

P1.222

Updating the Valence of a spatial goal: are the medial prefrontal cortex and the dorsal hippocampus involved?

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Recent electrophysiological unit-recording data suggest joined coding of goal location by the dorsal Hippocampus (dHpc) and medial Prefrontal Cortex (mPFC) (hok et al. 2005) The present work ask how these two anatomically connected structures, are involved in updating the valence of a goal zone during spatial navigation. In the continuous spatial navigation task, Long Evans rats had to cross a non-cued area, the goal zone (GZ), to release one pellet in the openfield (appetitive condition). Once the learning was completed, the valence of GZ was reversed as crossing the GZ triggered an aversive stroboscopic light (aversive condition) instead of pellet release. During this session, mPFC or dHpc were temporary inactivated by bilateral injections of muscimol. Sham animals were injected with vehicle. During the aversive session, the decrease of GZ crossings observed for all groups (mPFC, dHpc, Sham) revealed that the valence of GZ was correctly updated. On the next day, updating retention was tested in the appetitive version, by measuring the latency of the first GZ cross. On this

retention test, mPFC animals quickly crossed the GZ, whereas the Sham and dHpc animals delayed their first GZ crossing. These results suggest that the mPFC and the dorsal dHpc are not required to update the valence of a goal zone, but that the mPFC is necessary for the long-term retention of this updating.

P1.223

Emotional valence encoding in amygdala circuits

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Although the amygdala is known to be involved in learning and memory of both positive and negative affects, the neuronal processing of opposite emotional valences in amygdala circuits remains poorly understood. In particular, little is known about how the amygdala processes and learns to predict appetitive stimuli.
In order to unify our understanding of aversive and appetitive associative learning, we developed a behavioral paradigm allowing for conditioning opposite emotional valences on a purely Pavlovian basis. In this paradigm, repeated pairings of an initially neutral stimulus (the conditioned stimulus: CS, i.e. tone), with an emotionally salient event (the unconditioned stimulus: US, i.e. foot-shock or intra-oral infusion of sucrose) lead to valence-specific conditioned responses upon subsequent CS presentations (i.e. freezing or orofacial movements).
In combination with single-unit recordings in the amygdala, this procedure allows us to study the neuronal substrates of appetitive and aversive learning and to determine whether or not memories of different emotional valences share the same circuits in the amygdala. Eventually, we expect that integrating data from appetitive and aversive paradigms will lead to a general model of emotional valence processing in the amygdala.

P1.224

Effects of hippocampal and prefrontal tetanic stimulations on re-emergence of extinguished auditory-cued conditioned fear following a sub-conditioning procedure

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A large body of evidence indicates that extinction of auditory fear pairing involves new learning that inhibits the expression of conditioned fear responses (CFR) rather than erases the fear memory. Indeed, extinguished CFR can spontaneously recover with the passage of time, or can be reinstated by the reinforcer alone.
Post-extinction exposure of rats to a sub-conditioning procedure can evoke conditioned fear, which may correspond to fear return and/or fear learning potentiation. The aim of the present study was to clarify this issue and examine effects of tetanic stimulation of the hippocampus (HPC) and medial prefrontal cortex (mPFC), two brain regions implicated in post-extinction modulation of conditioned fear. Rats were initially submitted to 5 tone-shock pairings with either a 0.7-mA or 0.1-mA shock. Tone-evoked freezing was observed only with the higher shock intensity, indicating that the 0.1-mA shock corresponded to a sub-conditioning procedure. All conditioned rats underwent fear extinction with 20 tone-alone trials. When retrained with the sub-conditioning procedure, they displayed again tone-evoked freezing, except when the initial tone was unpaired or a new tone was paired with the

0.1-mA shock, demonstrating fear return rather than fear learning potentiation. We also found that HPC and mPFC tetanic stimulations, applied 24 hours after the sub-conditioning procedure, similarly reduced this fear return. However, mPFC inactivation abolished temporary HPC tetanus effect, whereas HPC inactivation did not interfere with mPFC tetanus effect. These data confirm our previous findings and provide the nature of HPC-mPFC interactions in post-extinction modulation of conditioned fear. According to our previous study (Deschaux et al., 2010), conditioned fear following a sub-conditioning procedure should correspond to fear return rather than fear learning potentiation.

P1.225

Familiarity and recollection are crucially involved in implicit and explicit face recognition: a new ERP study insight

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In our everyday life, we are able to involuntary recognize familiar faces independently from the meeting context. Recognition of known faces can occur implicitly or explicitly. Measuring event related potentials has been fundamental to understand the temporal dynamics of face processing. Explicit memory tests require to deliberated select or retrieve previously studied faces whereas implicit ones are designed to activate memory without requiring subjects to engage voluntary recognition process. However, despite numerous researches on face recognition, one might observe discordant results. The aim of this study was to figure out how famous face recognition is processed according to context conditions (explicit and implicit) and if the two underlying cognitive processes interact or take place independently in the brain.
In order to address this issue, we used a high EEG resolution set up combined with Partial Least Square statistical analysis. Fourteen healthy subjects performed a gender categorization (implicit condition) and a recognition task (explicit condition) on famous and unknown faces. For the first time, our study shows clear distinct differences between explicit and implicit famous face recognition processes within N400 and P600 component time windows. The implicit recognition effects were reflected by a posterior N400 effect, whereas the explicit one by a frontal N400 effect. Within P600 time window, both conditions elicited a parietal P600 effect which was found earlier, larger and longer in the explicit condition. A frontal P600 effect was also found specific of the implicit condition. Posterior N400 effect reflect an automatic use of memory of faces devoid to contextual information whereas the parietal P600 effect is related to the retrieving of associated information to faces directed by strategic process. These results show that posterior N400 and parietal P600 effects underlie face recognition memory processes that are crucially involved in implicit and explicit conditions. Posterior N400 effect is linked to familiarity-based recognition whereas the parietal P600 effect is linked to recollection based-recognition. Our spatial and temporal results support distinct neural generators, reinforcing dual models of recognition memory.

P1.226

Opposite effects of corticosterone on fear memories in dorsal vs. ventral hippocampus

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Numerous studies indicate that high release of corticosterone (CORT) can alter memory consolidation for emotional events. The hippocampus, both required in memory consolidation and key targeted brain structure for CORT may mediate this deleterious effect of CORT. However, recent data have shown

that stress, as well as CORT, promotes and inhibits synaptic plasticity in the ventral and dorsal hippocampus (vHPC vs. dHPC), respectively. Consequently, we hypothesized that the effects of intra-hippocampal CORT on memory consolidation may be dependent on the hippocampal sector targeted. We have thus compared the effects of CORT injections into the dHPC and vHPC on the consolidation of an aversive conditioning either to a simple tone (tone as predictor of a footshock) or to the context (context as predictor of the footshock). Intra-hippocampal injections of CORT abolished the selection of the right predictor of the aversive stimulus. Specifically, injections of CORT into the dHPC produced a deficit in contextual conditioning and induced a conditioning to the tone in mice yet objectively submitted to the predicting *context* situation. Reciprocally, injections of CORT into the vHPC produced the exact opposite pattern of responses: a deficit of tone conditioning associated with an increased conditioning to the context was observed in mice yet submitted to the predicting *tone* situation.
In addition, injections of the glucocorticoid receptors (GR) agonist dexamethasone into the dHPC and vHPC mimic the opposite effects of CORT on the conditioned fear responses.
In conclusion, supporting a functional dissociation between the dHPC and vHPC our study unveils deleterious and opposite GR-dependent effects of CORT on fear memory as a function of the hippocampal sector targeted.

P1.227

Bilateral lesion of the mesostriatal dopaminergic pathway is involved in the orofacial and posterior handpaws static mechanical allodynia

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Parkinson patient suffer both motor and non motor symptoms. Pain is one major non motor symptom in Parkinson's disease (PD) (Ford 2000). To investigate the role that mesostriatal dopamine system plays in pain processing, partial bilateral 6-OHDA lesion to the substantia nigra was used as a PD model in rat. We examined static allodynia behavior in both inferior and superior handpaws and within the orofacial area of 6-OHDA-denervated rats. The application of non nociceptive von frey filament (1g) at the infraorbital skin or the 6g filament at posterior hindpaws revealed a static allodynia (widespread symptom of neuropathic pain for which mechanisms are still poorly understood). A significant threshold decrease in the withdrawal response was observed in the posterior but not the anterior handpaws. Interestingly, the orofacial region shows also a pain response behavior that corresponded to static mechanical allodynia. This is the first time a static mechanical allodynia has been demonstrated in 6-OHDA lesioned mesostriatal dopamine system. This study may lead to understanding the mechanism by which the mesostriatal dopaminergic system promotes pain process in PD.

P1.228

Membranar mineralocorticoid but not glucocorticoid receptors of the hippocampus mediate the rapid glucocorticoid effects on memory retrieval

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The study was aimed at determining the type of glucocorticoid membranar receptors (mineralocorticoid or glucocorticoid receptors, MR or GR) involved in the rapid effects of stress in memory retrieval. For this purpose we used a behavioural task:“the spontaneous delayed alternation”. These experimentations were performed on C57 bl/6 male mice, aged of 7 months. Our previous results showed that an acute stress (3 successive unavoidable electrical footshocks: 0.9 µA during 10 ms) administered 15 min before the test phase produced an impairment of spatial “episodic-like” memory (Chauveau et al, 2008;Tronche et al, 2010).
In experiment 1, to assess the role of glucocorticoids on the behaviour, animals were injected ip with metyrapone (a corticosterone synthesis inhibitor at the dose of 35 mg/kg, 30 min before stress. The results show that metyrapone restores spontaneous delayed alternation performance. Moreover, microdialysis study showed that metyrapone inhibited the stress-induced intrahippocampal corticosterone rise.
In experiment 2, corticosterone-bovine serum albumin (cort-BSA, which cannot cross the cell membrane) was bilaterally administered into the dorsal hippocampus (dHPC) 15 min before the test session. The results show that administration of cort-BSA mimics the effects of stress on spontaneous delayed alternation.
In experiment 3, the GR antagonist RU-486 or the MR antagonist RU-28318 were bilaterally injected into dHPC 15 min before the injection of cort-BSA. The MR antagonist blocks the rapid cognitive effects of cort-BSA. On the contrary, the GR antagonist had no effect.
In experiment 4, the MR antagonist RU-28318 was bilaterally injected into dHPC 15 min before the acute stress. The test session occurred 15 min or 60 min after the acute stress delivery. The results show that the MR antagonist restores the performance on memory retrieval in stressed animals. Nevertheless, these beneficial effects are not present when the test session occurred 60 min after stress.
In summary, the present study provides the first evidence for the involvement of intrahippocampal mineralocorticoid membranar receptors in the rapid effects of stress on memory retrieval.

P1.229

Effect of different lesions of mesencephalic dopaminergic system on motivational and cognitive functions: insights into neuropsychiatric disorders in Parkinson's disease

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Beyond the classical triad of motor symptoms observed in Parkinson's disease (PD), behavioural and cognitive disturbances are also commonly observed, including apathy (which is defined as a decrease in goal directed behaviours), anxiety and depression. Interestingly, these non-motor symptoms are frequently observed in parkinsonian patients with deep brain stimulation of the subthalamic nucleus (DBS-STN). A relationship with the postoperative reduction of the dopaminergic (DA) drugs unmasking apathy as a disease-related symptom has been recently suggested (Thobois et al., 2010). However, the precise pathophysiology of these non-motor symptoms remains unknown.
The present study aims to identify the different subregions of the mesencephalic DA system implicated in these neuropsychiatric symptoms. We have therefore performed bilateral and partial 6-OHDA lesions of DA mesocorticostriatal system in rats and evaluated their behavioural consequences.
We show that only a lesion of the lateral part of the ventral tegmental area and the substantia nigra compacta, that preserves the motor function, generates a hypodopaminergic phenotype with increased anxiety, depressive-like behaviour and a decrease in goal directed behaviour reminiscent of apathy. Importantly, this phenotype can be reversed by DA agonists (L-DOPA or ropinirole). This animal model shed light on the anatomical-functional substrates underlying neuropsychiatric disorders such as apathy in PD.
Thobois S, Ardouin C, Lhommee E, Klinger H, Lagrange C, Xie J, et al. Non-motor dopamine withdrawal syndrome after surgery for Parkinson´s disease: predictors and underlying mesolimbic denervation. Brain 2010; 133: 1111-27.

P1.230

Cognitive dysfunctions and cerebral plasticity in mice after chemotherapy: Potential protective role of drug adjuvants

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Evidence is emerging that cancer and treatments can induce cognitive impairments such as deficits of visual and spatial memories, and slowing of psychomotor processing speed, now referred to as "*chemofog*". The objective of the current project is to explore the direct impact of chemotherapy on cognition by means of animal models. Thus, we investigated the effect of a chronic administration of 5-fluorouracil (5-FU) or a 5-FU/oxaliplatin combination in young and aged mice, on emotional reactivity, spatial learning, learning flexibility and object recognition memory. 5-FU neither altered anxiety- and depressive-like behaviors, nor spatial learning and memory performances, in both young and aged mice. However, 5-FU-treated mice were impaired in the cognitive flexibility-dependant task in the Morris water-maze test, and exhibited a more pronounced preference for the novel object in the object recognition test, suggesting a hyper-reactivity to novelty. Moreover, 5-FU reduced the number of BrdU-labelled cells in the neurogenic area hippocampus, and induced alteration of metabolic activity in selective brain regions. Because of the different modalities of chemotherapy administration, we investigated the impact of adjuvants, *i.e.* saline versus glucose, associated to 5-FU or to a 5-FU/oxaliplatin combination. Glucose protected mice against chemotherapy-induced cognitive dysfunctions and neurogenesis inhibition. *In vitro*, increasing concentrations of 5-FU and oxaliplatin evoked direct neurotoxicity on neural stem cells cultured as neurospheres. When glucose was co-administered, the observed quiescent state of neurospheres likely protected from deleterious effects of chemotherapy.In conclusion, our study demonstrates that a chronic administration of 5-FU provokes a selective long-term impairment of behavioral flexibility and object recognition memory performances whatever the age. These cognitive alterations are associated to selective metabolic modifications in cerebral regions involved in the tasks used, and may be consecutive to the reduced number of adult generated hippocampal neurons.

P1.231

Role of CB1 receptors in the emotional consequences of repeated social stress

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We investigated the role of the endocannabinoid system in the emotional consequences of repeated social stress by using constitutive/conditional CB1 receptor mutant mice. C57Bl6/N mice, constitutive CB1 knock-out mice, and conditional CB1 mutants lacking CB1 receptors either from cortical glutamatergic neurons or from GABAergic neurons were daily exposed to social defeat (according to a method slightly modified from the one we published previously: Berton et al., 1998; Berton et al., 1999). Thereafter, control and stressed mice were examined for several behavioural (anxiety, cued fear expression, behavioural despair, sucrose consumption) and neuroendocrine (adrenal weights) indices. A first series of experiments indicated that social stress increased anxiety (as assessed in the

elevated plus-maze), fear expression on recall (cued fear conditioning), despair, and adrenal weights. Further, social stress increased the central levels of the endocannabinoid 2-arachidonoyl-glycerol after the last, but not the first, defeat. In keeping with this result, control and stressed C57Bl6/N mice were pretreated with the CB1 receptor antagonist rimonabant before each stress session. This treatment increased within- and between-session fear expression during recall in stressed mice, but did not affect to a major extent the other behavioural/neuroendocrine responses to stress. Beside intrinsic influences of constitutive and conditional CB1 mutations on some of these responses, it was found that the lack of CB1 receptors decreased fear expression on recall in stressed, but not in control, mice. Further, the selective mutation of CB1 receptors in cortical glutamatergic neurons decreased fear expression in stressed animals, compared to control animals. An opposite result was found in mice lacking CB1 receptors from GABAergic neurons.
The full deletion of CB1 receptors increases fear expression, an effect partly accounted for by the deletion of CB1 receptors from cortical glutamatergic neurons. In addition, CB1 receptors located in cortical glutamatergic neurons or in GABAergic neurons modulate in an opposite manner the expression of fear after repeated stress.

P1.232

A new paradigm to explore interactions between emotion and visual processing

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Emotional processing helps to preserve the viability of species and individuals. Hence, it can interact with and influence our cognitive functions. Here we explore the interactions with visual grouping. Visual grouping is intermediate between the extraction of visual primitives in the primary visual cortex and object recognition. We focus on the mechanisms allowing to group or separate visual items from one another. Such mechanisms are involved in the way objects are selected for attention, and it is plausible that emotion affects such selection. The goal of this study was thus to create a paradigm allowing to investigate if and how emotions affect visual grouping. We worked with the grouping factor connectedness in a paradigm derived from the literature. Five figures are displayed on a row and among them, two adjacent figures are identical. Two bars linking figures by pairs yield two types of targets: 'within-group pairs' (targets connected) and 'between-group pairs' (targets unconnected belonging to different pairs). Subjects have to decide whether the pair is composed of two squares with sharp or rounded edges. The originality of the paradigm is that we added the factor emotion. Superimposing each figure on a picture with an emotional valence (negative or positive), led to target pairs that could either be located on two pictures with the same or with two different emotional valences. In addition, we chose four different types of pictures (objects, animals, faces, social scenes). Like in previous studies using similar paradigms, connected targets were perceived faster than unconnected targets, thus revealing the benefit of visual grouping. Our study reveals supplementary a significant interaction between emotion, connectedness and picture types. When the background contains positive animal pictures, the effect of grouping is enlarged. If the background contains friendly faces, this effect is reversed, as if positive emotion incites subjects to distinguish faces from one another.
These results suggest that emotional stimuli influence the organisation of our environment qualitatively, at least for a subset of visual items. The paradigm created here might be useful to deepen our understanding of the interaction between emotion and visual processing.

P2.001

Drebrin A expression is altered after pilocarpine-induced seizures: time course of changes is consistent for a role in the integrity and stability of dendritic spines of hippocampal granule cells

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We used a pathophysiological model of temporal lobe epilepsy induced by pilocarpine in adult rats in order to assess the in vivo role of drebrin A (DA), one of the major regulators of F-actin. This model

displays a dynamic reorganization of the glutamatergic network including neo-spinogenesis, morphogenesis, and neo-synaptogenesis associated with an aberrant sprouting of granule cell axons in the dentate gyrus. This reactive plasticity contributes in dentate granule cell hyperexcitability that could lead to the emergence of recurrent spontaneous seizures. We investigated the hippocampal DA expression changes in pilocarpine animals using immunohistochemical, Western blot and in situ hybridization analyses. We showed that DA immunoreactivity was decreased in the inner molecular layer and in the hilus of the dentate gyrus, at latent stage, when spinogenesis and morphogenesis occur. Western blot analysis confirmed these overall hippocampal decreases of DA protein expression. At chronic stage, when newly formed glutamatergic synapses are being established, the levels of immunolabeling for DA in the hilus and the inner molecular layer were similar to control rats. This recovery is likely due to the increase of DA mRNA in perikarya of hilar and granule cells. Interestingly, our data showed that the changes pattern of labeling for Bassoon, a specific marker for presynaptic active zone, in the IML of pilocarpine-treated animals paralleled those found for DA at all time points examined. Furthermore, our double and triple immunofluorescence studies showed that the recovery in DA levels in the inner molecular layer occurred within the dendritic spines involved in glutamatergic active synapses of presumed granule cells. Altogether, our results indicate that in vivo DA is not critical for spinogenesis and morphogenesis but instead is consistent with an involvement in synaptic structural integrity, stabilisation and function. Thus, DA appears as a novel modulator of reactive synaptic plasticity associated with epilepsy.

P2.002

Embryonic midbrain dopaminergic neurons begin to release dopamine in the striatum just before birth

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Midbrain dopaminergic (mdDA) neurons send axons to the developing striatum at embryonic stages in the rat (from E17; Van den Heuvel *et al*., 2008) but when they become functional is not known. Using dynamic two photons imaging coupled to mathematical analysis of large neuronal ensembles, patch clamp recordings and amperometry in vitro in tyrosine hydroxylase (TH)-GFP mice, we investigated whether and when embryonic and young postnatal (E16-P7) mdDA neurons spontaneously generate immature network patterns and release dopamine in the striatum upon stimulation.
TH-positive fibers expand from the ventrolateral striatum at E16 to the whole striatum at E18-P0. Biocytin-filled mdDA neurons send axons outside the mesencephalon already at E16 and show a significant increase of their somato-dendritic tree at P0. Embryonic and young postnatal (E16-P7) mdDA neurons spontaneously generate the same types of immature patterns as cortical (Crépel *et al*., 2007, Allène *et al.*, 2008) and striatal (personal results) networks: intrinsically generated, TTX/nifedipin-sensitive and synaptic blockers-insensitive Ca^{2+} spikes and Ca^{2+} plateaus, followed at P3 by the first synapse-driven pattern, synchronized Ca^{2+} spikes. In whole cell recordings, spontaneous spiking begins around birth and overshooting Na spikes are elicited in 45% of mdDA neurons at E18 and in 55% at P0. In parallel, calcium-dependent DA release in response to local striatal stimulation is totally absent at E16, begins at E18 and is two times higher at P0.
Therefore, dopamine signals may modulate the development of the mouse striatal network shortly before delivery (E18) and after birth.

P2.003

Genetic profiling of adult olfactory bulb neurogenesis

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In mammals, neural stem cells located in the subventricular zone (SVZ) of lateral ventricles generate OB neurons throughout life. During differentiation, they divide asymmetrically thereby self renewing and generating transient amplifying progenitors. These give rise to neuroblasts which migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB), the place of their final differentiation. Dorso-lateral position of SVZ stem cells determines final position and neurotransmitter phenotype of their progeny. We aim at elucidating the genetic control the adult neurogenic process. For this purpose we have performed several high resolution molecular screens to identify mRNAs expressed during defined steps of neurogenesis. Several candidate molecules, like the transcription factor Vax1 or the Wnt downstream effector Gli3 are currently functionally analyzed.
In addition, we started to investigate the temporal and spatial expression of microRNAs (miRNAs) in the system using deep sequencing of microdissected tissue. By this method we identified several miRNA clusters showing differential expression depending on spatial origin or differentiation stage.
To demonstrate the functional importance of microRNAs during olfactory bulb neurogensis we blocked miRNA production in SVZ stem cells. This was achieved by in vivo electroporation of a Cre-recombinase expressing vector in a Dicer conditional KO mouse line. Furthermore, we have constructed microRNA cluster gain-of-function vectors. These vectors are currently electroporated in periventricular stem cells of post-natal mice to analyze their function in neurogenesis in vivo.
In parallel, we are comparing bioinformatically the microRNA and mRNA screens. These analyses is expected to allow the identification of specific target genes of miRNAs regulating neuronal differentiation.

P2.004

Development of cortical neuroblasts and their projections following grafting into the damaged adult motor cortex

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Damage to the adult motor cortex leads to severe and frequently irreversible deficits in motor function. Cell transplantation is a promising therapeutic strategy for the treatment of brain injury. The effectiveness of cortical transplantation depends on the capacity of grafted cells to develop appropriate neuronal phenotype and re-establish specific connections. In our study embryonic (E14) motor cortical tissue from enhanced green fluorescent protein (eGFP)-expressing mouse were grafted into the damaged motor cortex of adult mice. Cellular composition of grafts and development of their efferent innervation was determined at 2, 4, 7 and 14 days after transplantation. Two to 4 days following transplantation, grafts appeared as small tissue blocks lying to the lesion wall and were mainly composed of neuroblasts. At day 4, the first eGFP+ fibers leaving the transplant were present in the adjacent host cortex. At J7, eGFP+ fibers were mainly distributed in the adjacent motor and somatosensory cortices. Few axons were found in the corpus callosum and the dorsal caudate putamen (CPu). By 7 to 14 days, an increasing number of grafted cells expressed the neuronal nuclei antigen (NeuN), a mature neuronal marker, and the two main subtypes of cortical neurons, GABAergic interneurons and glutamatergic projection neurons, were identified respectively by using antibodies against GABA and the COUP TF1-interacting protein 2 (CTIP2, a layer 5/6 cortical marker). At day 14, dense bundles of radiating neurites occurred within the ipsilateral motor, somatosensory cortices and cingulum. The density of GFP axons was considerably increased both ipsi and contralaterally in the cortex and CPu. Fast developing fibers were identified within the thalamic and subthalamic regions, cerebral peduncle and, in some cases, at pontine nuclei level. In addition to appropriate projections, ectopic projections were sometimes observed in the septal nucleus, hippocampus and accumbens nucleus.
Our data show that cortical neuroblasts transplanted into damaged adult motor cortex (i) differentiate into mature neurons with appropriate neuronal phenotypes and (ii) develop projections to most of the cortical and subcortical targets normally innervated by motor cortex as early as two weeks after grafting.

P2.005

A novel cell-based fluorescent method to monitor activity-dependent BDNF secretion from living neurons

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Brain-derived neurotrophic factor (BDNF) has emerged as a major messenger for activity-dependent brain development and synaptic plasticity. BDNF can be secreted from both axon and soma/dendrite. Although discussed, a new paradigm has emerged, whereby the net effect of BDNF will depend on the extracellular proBDNF/mBDNF ratio which is itself controlled by the pattern of synaptic activity. Thus to understand the biological functions of BDNF it is essential to reveal the dynamic and processing of BDNF secretion from living neurons.
Here, we have developed and validated a new method to monitor the activity-dependent secretion of BDNF from “donor” neurons, allowing identification of the site, form, processing and effects of secreted BDNF on identified “recipient” cells. This approach exploits the fact that secreted BDNF is internalized by the target cells after binding to its receptors. We have constructed a bicistronic cDNA plasmid coding for both cherry-tagged BDNF and the non-releasable protein, GFP under the control of two distinct Ubiquitine promoters (BDNF-Ptracer). Time lapse analysis revealed that two days after transfection with the BDNF-Ptracer, the transfected BDNF-Cherry/GFP expressing neurons were surrounded by BDNF-cherry positive, but GFP negative. These newly BDNF-cherry positive neurons (hereafter referred to as “recipient” target cells) were not observed when the cultures were transfected with control cherry/GFP plasmids. The number of “recipient” cells as well as their level of fluorescence was reduced in cultures treated with TrkB-IgG, a scavenger of BDNF or with tetrodotoxin, which decreased synaptic activity. We further show that this approach can be used to detect the form and processing of BDNF that has been released by the transfected “donor” neurons. Finally, we have collected preliminary data showing that this approach can be used in organotypic slices cultures and in acute brain slices following *in vivo* expression.
To conclude, we have developed a novel approach to study activity-dependent secretion of BDNF based on internalization of secreted BDNF by the target “recipient” neurons. This method provides spatio-temporal information about BDNF secretion as well as information about the form and processing of secreted BDNF.

P2.006

Synaptic basis for experience-dependent plasticity of intracortical microcircuits in the mouse barrel cortex *in vivo*

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Experience-dependent plasticity of neocortical microcircuits is thought to play a key role in learning and memory formation. In the mouse barrel cortex, which receives sensory input from the mystacial whiskers, removing all but two neighboring whiskers causes the cortical representations of the spared whiskers to merge within the cortical map. Previous studies have suggested that such cortical plasticity is the result of Hebbian-like, activity dependent, strengthening of coactive inputs onto common neuronal targets. An increasing number of studies indicate that, in addition to the fast modification of pre-existing synapses, slow structural changes such as dendritic spine and axonal bouton growth could be involved in barrel map plasticity.
To investigate the synaptic circuit modifications underlying barrel map plasticity, we performed *in vivo* whole-cell recordings in layer (L) 2/3 of the C2 barrel column before and after trimming all but the C1

and C2 whiskers (dual whisker experience, DWE). In control mice, deflection of the C2 (principal, PW) or C1 (surrounding, SuW) whisker evoked compound EPSPs, consisting of a high probability “early EPSP” (eEPSP), and of a low probability “late EPSP” (lEPSP), presumably reflecting intra- and transcolumnar excitatory synaptic connections respectively. Repetitive pairing of PW-evoked EPSPs and back propagating action potentials led to a selective, postsynaptic NMDAR-dependent increase (postNMDA-LTP) in the amplitude of PW-evoked eEPSPs. Pairing of SuW-evoked eEPSPs and lEPSPs failed to induce potentiated responses. Interestingly, postNMDA-LTP of PW-evoked eEPSPs was not occluded after at least two days of DWE, but the probability of the occurrence of PW and SuW-evoked lEPSPs was significantly increased.
Our data indicate that the Hebbian-like merging of whisker representations upon DWE could entirely rely on the strengthening of transcolumnar L2/3-L2/3 excitatory projections, independently of the potentiation of vertical intracolumnar L4-L2/3 excitatory projections.

P2.007

Glial and neuronal plasticity in the hypothalamus after prolonged water deprivation in the desert rodent Meriones Shawi

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Supraoptic (SON) and paraventricular (PVN) nuclei are part of the hypothalamus-neurohypophysial system, they constitute the main source for vasopressin and they represent also obvious examples of activity-dependent neuroglial plasticity. In certain physiological conditions such as dehydration are accompanied by a structural remodelling of the neurones, their synaptic inputs and their surrounding glia. In the present work, adult Meriones Shawi (rodent adapted to desert life) are used as an animal model. Using GFAP and vasopressin expressions as indicators successively of astrocytes and neuronal activations, the effect of prolonged episode of water deprivation on the SON and PVN, hypothalamus nuclei were examined. We studied the immunoreactivity of GFAP and vasopressin in various hydration states (total deprivation of drinking water for 1 and 2 months compared to hydrated animals). Prolonged dehydration produces an important decrease of GFAP immunoreactivity in both SON and PVN after 1 and 2 months of water restriction. This decrease is accompanied by increased vasopressin immunoreactivity following the same periods of water deprivation. These findings may explain a real communication between vasopressin neurons and their surrounding astrocytes, thus the retraction of astrocytes and their processes is accompanied by an enhancement of vasopressin neurons density and their projecting fibers in response to this osmotic stress situation. Furthermore, these data could open further investigations concerning the possible involvement of the communication between astrocytes and neurons vasopressin in both PVN and SON in the regulation of Meriones hydrous balance and resistance to dehydration.

P2.008

Paradoxical increase of hippocampal neurogenesis in serotonin-deficient mice

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Recent theories have linked the effects of antidepressants to increased brain plasticity, in particular to increased adult neurogenesis. Selective serotonin re-uptake inhibitors increase adult neurogenesis in the dentate gyrus of the hippocampus , while serotonin neurotoxins reduce neurogenesis. However, the consequences of chronic serotonin depletion, as is expected to occur in depression, is not known. Here, we examined adult hippocampal neurogenesis in two genetic models with chronic serotonin

depletion: Pet1 knock-out mice, which lack fully developed serotonergic neurons in the raphe, and VMAT2 conditional knock-out mice, which lack the vesicular monoamine transporter specifically in serotonergic neurons. In both models, baseline cell proliferation was unchanged. We then quantified the survival of newborn neurons. While 50 to 70% of newly generated cells in the SGZ die normally within four weeks after birth, cell survival was significantly increased (two- to four-fold) in the serotonin-depleted animals. This marked increase in survival is not a developmental effect, since animals injected with the serotonin synthesis inhibitor PCPA during the first two postnatal weeks did not show increased survival of newborn neurons in the adult hippocampus. In contrast, chronic treatment of wild type adult mice with PCPA did increase survival of newborn hippocampal neurons. The newborn cells in serotonin-depleted mice appear to progress normally through the different maturation steps to become neurons, as shown by co-localization studies of BrdU with mature/immature neuronal markers like calretinin, calbindin, doublecortin, and neuN. Administration of the 5-HT1A agonist, 8-OHDPAT normalized the changes in neurogenesis in the Pet1 KO mice. Overall, these results indicate that serotonin chronic 5-HT depletion increases the survival of newborn neurons in the hippocampus and that this effect is mediated by lack of activation of 5-HT1A receptors.

P2.009

Changes in input resistance following induction of long-term synaptic depression

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Synaptic plasticity is not the exclusive mode of memory storage, and persistent regulation of voltage-gated ionic channels also participates to information storage. Long-term changes in neuronal excitability have been reported in several brain areas following learning. In central neurons, long-lasting synaptic modifications such as long-term potentiation (LTP) and depression (LTD) are associated with synergistic changes in postsynaptic neuronal excitability. Synergistic potentiation in synaptic and intrinsic excitation may eventually lead to hyper-excitable brain circuits if they are not compensated. In CA1 neurons, large LTP is associated with a decrease in input resistance (IR) and intrinsic excitability, indicating the presence of a continuum linking synergistic and homeostatic intrinsic plasticity following LTP induction (Campanac et al. J Neurosci. 2008). We examined here whether such as continuum also exists on the LTD side.
CA1 pyramidal cells were whole-cell recorded in hippocampal slices obtained from P15-20 rats. Excitatory post-synaptic potentials (EPSPs) were evoked at 0.1 Hz by the stimulation of the Schaffer collaterals. The apparent IR tested with hyperpolarizing pulses was monitored. LTD was induced by 3-5 episodes of low frequency (3 Hz, 5 min) stimulation. IR and the EPSP amplitude-slope relationship (A/P) were decreased following the first stimulation episode inducing moderate LTD (~20%). In contrast, they were increased following the fourth and fifth stimulation episodes producing large LTD (~50%). These changes were blocked by 1 µM ZD7288 suggesting that these modifications involve the regulation of the hyperpolarization-activated cationic h-current. Next, we tested the role of glutamate receptors. In the presence of the NMDA receptor antagonist D-AP5, IR was found to be increased following the first 3 Hz episode. In conclusion, moderate levels of LTD are associated with a NMDA receptor-dependent regulation of h-channel activity that is responsible for the decrease in both IR and A/P. Our work provides the first demonstration for the presence of a continuum linking synergistic and compensatory changes in excitability following LTD induction. Thus, synergistic and homeostatic plasticities are combined within a single learning rule.

P2.010

Human embryonic stem cells as a tool to monitor human neurogenesis in vitro and screen for neurogenesis-promoting agents

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Neuro-regenerative medicine is based on the hypothesis that neurogenesis may be re-introduced into the adult brain to counteract the neuronal loss associated with neurodegenerative diseases. Consequently, there is an increasing need for accurate in vitro models which allow high throughput screening of potential drugs with a positive effect on human neurogenesis. Human embryonic stem cells (hESC) do not only represent a wonderful hope for cell-based replacement, they are also a unique tool to model early developmental events in particular the very first step of neurogenesis. This has dramatically increased the possibility to screen for therapeutical effects of chemicals on generation of newly form human neurons.
We have focused our work on using hESC to develop tools to monitor many aspects of human neurogenesis *in vitro* in formats amenable to High Content Screening (HSC). We have developed a protocol which allows the isolation of a highly neurogenic neural stem cells population (NSC) from hESC which share several features with NSC found in foetal and adult brains. We have then developed, miniaturized and automated several tests which allow the quantification of the proliferation and the terminal differentiation of these progenitors into functional neurons into an HCS format. We have started using these tools to screen for small molecules with known biological target for a positive effect on neurogenesis.

P2.011

In vivo corticostriatal spike-timing-dependent plasticity in enriched environment rats

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Striatum, the main input nucleus of basal ganglia, process information from the whole cerebral cortex. The corticostriatal plasticity is involved in sensory-motor learning and cognitive processes. We have previously reported the existence of a bidirectional spike-timing-dependent plasticity (STDP), a Hebbian learning rule, in corticostriatal slices (Fino et al., J. Neurosci., 2005). Here, we investigated *in vivo* corticostriatal STDP in rats. The oro-facial area of the somatosensory cortex was electrically stimulated while intracellular recordings of the medium spiny neurons were performed in the striatum, on anesthetized (chloral hydrate) rats. Paired stimulations between cortical or facial cutaneous stimulus (pre-stimulation), and intracellular suprathreshold depolarisation (post-stimulation) consisted of 100 stimuli at 1Hz. Results were taken into consideration for a minimal post-protocol time of 45min, up to more than one hour. A synaptic plasticity was observed in a vast majority (80%, n= 70). With pre-post pairings, a long-term depression (LTD) was induced in most of the cells, in a time window of 5 to 150ms. With post-pre pairings, a LTD was mostly elicited (5-120ms), while a few neurons displayed a long-term potentiation (LTP), in a short (10-20ms) and large (70-10ms) time windows. Thus, as we mainly observed a unidirectional STDP, we tested different experimental conditions that could unveil bidirectional STDP. Namely, corticostriatal STDP was assessed in rats maintained in enriched environment for 2 months. The probability to induce LTP with post-pre pairings was significantly increased. Furthermore, with light anaesthesia and painless contention, the occurrence of LTP was even more favored. These results indicate that corticostriatal STDP occurs *in vivo* with different characteristics than *in vitro*. Indeed, the time window of plasticity induction is much larger *in vivo*, which could allow a modulation of the coincidence detection of cortical input in a wide time range.

P2.012

Mechanisms of neuronal plasticity during learning and memory at single-neuron resolution

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The biology of memory is among the most fundamental questions in modern neuroscience. In recent decades, detailed studies have advanced our understanding of how memories are formed. However, further progress has been limited by the sparse encoding of memories, since only a minority of neurons participate in storing a particular experience

Recent data indicate that ~25% of lateral amygdala neurons exhibit changes in firing rates and synaptic plasticity during fear conditioning. At the molecular level, ~25 % of lateral amygdala neurons also express the immediate early-gene Arc after fear conditioning, consistent with the known function of Arc in memory consolidation, and highly suggestive that Arc transcription occurs selectively in neurons of the fear memory trace. Therefore, we used the Arc-dVenus reporter mice to visually-identify these neurons in situ. Our goal is to characterize the mechanisms of plasticity occurring selectively in neurons activated during fear learning. Through patch-clamp recordings of dVenus-labelled cells compared to their non-dVenus neighbours, we aim to identify the electrophysiological signature of individual neurons responsible for memory encoding and maintenance. Active properties of Arc+ versus Arc- neurons are investigated, as well as synaptic inputs and short-term plasticity. Further, the Arc-dVenus mice permit a unique ability to examine the precise anatomical location, morphology, and gene expression profile of individual neurons in a fear memory network.

P2.013
VEGF modulates the synaptic transmission during embryonic development

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The nervous and the vascular system share similarities and are closely connected. They are both highly branched and form a ramified network that spread in the whole organism. A few studies, aiming at identifying the effect of the pro-angiogenic key factor Vascular Endothelial Growth Factor (VEGF) on neuronal activity, have been conducted in the adult central nervous system. However, it is not known whether VEGF acts on neuronal networks during their ontogeny. In the present study, we asked whether VEGF directly interacts with the neural network during development. Using an electrophysiological approach, we focused on the effect of VEGF on embryonic spinal lumbar motoneurons (MNs). Our results demonstrated that VEGF acts on the synaptic transmission by specifically increasing the frequency of the GABA/glycinergic events. Interestingly, we found that this modulatory effect occurs during a developmental time-window and is present at early developmental stages. Further studies are required to identify which VEGF receptor(s) and signalling pathway are involved in this regulatory effect. Taken together, our data emphasize a new role for VEGF in the functional maturation of the neuronal networks. Blockade of VEGF expression or activity may play an important role in early MNs degeneration.
**Bikfalvi A. & Branchereau P. are Co-senior authors*.

P2.014
Role of proBNDF secretion in the development of GABAergic synapses

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Over the past decade it has been established that brain-derived neurotrophic factor (BDNF) plays a critical role in activity-dependent processes such as synapse development and plasticity. BDNF is synthesized from large precursor proteins that are proteolytically cleaved either intracellularly or extracellularly to yield mature proteins. Until recently, only mature BDNF (mBDNF) was considered biologically active. This view was challenged by findings showing that exogenous proBDNF induces apoptosis in peripheral neurons and facilitates long-term depression (LTD) in the hippocampus. The expression of proBDNF is developmentally regulated, and the extracellular proBDNF/mBDNF ratio is

under the control of neuronal activity. Despite the importance of activity-regulated proBDNF expression for brain development and neuronal function and the opposed biological effects of proBDNF and mBDNF, few studies have addressed the importance of proBDNF in synaptic plasticity. In this study, we sought to analyze the impact of proBDNF on GABAergic synapse development. To this end we used a combination of molecular, cellular and electrophysiological techniques in cultured hippocampal neurons and in hippocampal organotypic slice cultures. We first demonstrated that endogenous release of proBDNF leads to a rapid decrease in $GABA_A$ receptor ($GABA_AR$) cell surface expression followed by a cytoplasmic diminution of total $GABA_ARs$. We then provided evidence that exogenous proBDNF reduces synaptic GABAergic transmission. This morphological and functional reduction of GABAergic synapses triggered by proBDNF was blocked in the presence of an antagonist of the proBDNF receptor p75 and rescued by exogenous application of mBDNF. We further demonstrated a direct regulation of the $GABA_AR$ gene by the proBDNF/p75 signaling pathway. proBDNF causes a decrease in transcripts level, suggesting that transcriptional regulation of $GABA_ARs$ is altered. Our study reveals that proBDNF is a potent regulator of GABAergic transmission during neuronal development.

P2.015

Increase in polysialyltransferase gene expression during mnesic processes in adult rat hippocampus

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Neural cell adhesion molecule (NCAM) is frequently associated with polysialic acid (PSA), and its function is highly dependent on polysialylation through activity of STX and PST enzymes. PSA-NCAM plays an important role in synaptic plasticity in the hippocampus. Learning and memory neural substrates could reside in the ability of synapses to undergo long-term changes in efficacy on widespread brain neural networks (Hebb's theory). Long-term potentiation (LTP) is a major candidate for such synaptic changes leading to a stable long-term memory.
In our study, we assess the involvement of STX and PST enzymes in LTP and memory in adult rat. Firstly, we investigated whether unilateral LTP induction in *dentate gyrus* (DG) *in vivo*, triggered NCAM polysialylation by STX and PST produced in the hippocampus. Secondly, we investigated STX and PST expression pattern during learning and memory processes using a hippocampus-dependent olfactory task. Using semi-quantitative RT-PCR, we found that levels of STX and PST mRNA significantly increased from the onset of hippocampal LTP and remained high during the maintenance of DG-LTP for at least 4 hours. This rapid increase in polysialyltransferase gene expression occurred in both hippocampi, probably resulting from bilateral LTP induction by strong unilateral high frequency stimulations distributed on the median perforant path. Thus, LTP triggers interhemispheric molecular changes in the hippocampal networks. During the behavioural training, we assessed STX and PST mRNA level in the hippocampus. Results showed a significant increase of PST expression in relation to the learning stage of animals and no change in STX expression.
This study is the first to describe the effects of LTP induction and maintenance on polysialyltransferases expression *in vivo*. Our findings indicate that hippocampal synaptic remodelling requires NCAM polysialylation. Moreover, results form our LTP and behavioural studies strongly suggest two distinct roles for STX and PST enzymes: while STX is probably involved in the proliferation of neural progenitor cells, PST could play a key role in synaptic plasticity of mature neural networks.

P2.016

Cell-specific urotensin II-related peptide gene expression reveals the occurrence of a common cis and trans transcriptional regulatory mechanism during motoneuron cell differentiation

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The urotensin II (UII) and UII-related peptide (URP) genes are expressed by motoneurons of the spinal cord and brainstem in vertebrates. Previous studies have shown that UII and URP gene expression in motoneurons starts during neuronal tube development and is concomitant with motoneuron lineage establishment in rat and chicken. The aim of this study was to decipher the transcriptional mechanisms involved during ontogenesis to drive the specific expression of urotensin II genes in motoneurons. Using the chick URP promoter as a model, we first identified by in *ovo* electroporation a 4-kb URP promoter, able to drive motoneuron-specific LacZ gene expression in chick embryo. Progressive 5' deletions of this fragment allowed us to identify a 500 bp fragment sufficient to direct specific LacZ gene expression in motoneurons. By using bioinformatics, we identified within this fragment two modules involved in the regulation of the homeobox gene Hb9, which is expressed selectively by motor neurons in the developing CNS. Transgenic analysis showed that the deletion of each module leads to a partial loss of the LacZ expression, while the deletion of both is followed by a total loss of the signal. On the contrary, the fusion of the two modules in an artificial promoter allowed the recovery of LacZ expression in motoneurons. In the Hb9 gene, these regions bind a complex of transcription factors including bHLH proteins and LIM-HD factors. The co-expression, in the whole spinal cord, of the 4-kb promoter and of a chimeric protein which mimics the complex bHLH-LIM-HD led to an ectopic LacZ expression, outside the motoneuron domain. Altogether, these results indicate that motoneuron-specific URP gene expression may be achieved using the combination of two modules which could bind a protein complex containing bHLH and LIM-HD factors. The similarity observed between the cis and trans-regulatory mechanisms of the Hb9 and URP genes indicates the occurrence of a common transcriptional program mediated by a set of specific factors acting at a certain stage of motoneuron differentiation. We propose that URP gene, like Hb9 gene, may be involved in specific steps following the establishment of newly differentiated motoneurons, to consolidate and maintain post-mitotic motoneuron identity.

P2.017

Role of pleiotrophin during the ontogenesis of mouse cerebellum

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Cellular migration with differentiation and proliferation are the three main steps of the development of the central nervous system. These processes are under control of various actors such as neurotransmitters, neuropeptides and extracellular matrix molecules. Among them, the pleitrophin (PTN) has been described as a pro-migratory and a pro-differentiating secreted cytokine. In rodent cerebellum, PTN is mainly localized in the external granule layer (EGL) and the molecular layer (ML) of immature cerebellum with a high expression during the first postnatal week. These data suggest that PTN could have a role during the crucial phase of cerebellum postnatal development. So we first undertook to characterize the profile of expression of the PTN system in mouse cerebellum. Our

results confirmed the presence of PTN in the EGL and ML from P0 to P12. Its expression increases during the first 4 postnatal days before decreasing until adulthood. We also localized the two PTN receptors RPTPξ and Syn3 in Purkinje cells and immature granule cells during the first postnatal week. The cerebellar level of RPTPξ reaches with a peak at P0 and P12 while Syn3 expression increases from P0 to P4 and then decreases slowly until adulthood. The presence of RPTPξ and Syn3 during cerebellum ontogenesis suggests that PTN could contribute to the formation of the cerebellar cell layers by acting directly on the immature granule cells or indirectly via the Purkinje cells. In order to test this hypothesis, we investigated the effect of PTN on cultured immature granule cells by time-lapse video-microscopy. Our results show that PTN significantly stimulates the migration of granule cells through Syn3 and/or RPTPξ in vitro. However, in vivo injections of PTN at the surface of the cerebellar cortex induce an atrophy of Purkinje cells and an exacerbated apoptosis of differentiated granule cells.
Altogether, these results indicate that PTN controls the migration of immature granule cell migration but also the development of the Purkinje cell trees and the apoptosis of differentiated granule cells. All these effects are associated to a precise spatio-temporal profile of PTN expression during the postnatal ontogenesis of the mouse cerebellum.
Supported by Interreg 4A AdMiN and LARC-Neurosciences.

P2.018

Impaired hippocampal long-term depression and defective spatial learning and memory in Scribble1 conditional knock-out mice

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Scaffold proteins at the post-synaptic density regulate the strength of synaptic activity by organizing neurotransmitter receptors and other associated signaling proteins, which are required for normal learning and memory. Scribble1 (Scrib1) is a scaffold protein that belongs to the LAP (leucine-rich repeats and PDZ domains) proteins family, with 16 leucine rich repeats and 4 PDZ (PSD-95/Dlg/ZO-1) domains. Our previous work with a spontaneous mouse model for Scrib1 has identified an important role for the protein in synaptogenesis and actin polymerization. Here we used conditional knock-out mice presenting a specific loss of Scrib1 in the forebrain to extend and complete our understanding of Scrib1 role(s) in the central nervous system. Mice lacking Scrib1 in principal neurons exhibited impairment in spatial learning and memory in the Morris water maze test compared to wild type ones. We also observed a decrease in spine density and altered dendritic morphology of hippocampal CA1 pyramidal neurons. Additionally, these neurons had normal probability of glutamate release but an overall decrease in basal synaptic transmission. When tested for synaptic plasticity, CA1 pyramidal neurons revealed an altered synaptic depotentiation whereas long-term potentiation was normal. Our *in vitro* results show a decrease in AMPAR endocytosis in neurons in which Scrib1 was downregulated, which could explain these electrophysiological characteristics. Importantly, this puts forward a functional link between synaptic depotentiation and learning and memory processes. Finally, these results suggest an original and critical role for Scrib1 in AMPAR endocytosis that affects synaptic function and plasticity, dendritic morphology and higher cognitive functions.

P2.019

The pH-dependent inhibition of native $GABA_A$ receptors by HEPES and related buffers masks the physiological sensitivity of $GABA_A$ receptors to protons

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Artificial pH buffers such as HEPES are extensively used to control extracellular pH (pH_e) to investigate the effect of H^+ ions on $GABA_A$ receptor ($GABA_AR$) function. We studied the effect of pH_e on the currents induced by GABA or $GABA_AR$ agonists under conditions where pH_e was controlled with bicarbonate or different concentrations of artificial pH buffers. Changing HEPES concentration from 1 to 20 mM at constant pH_e strongly inhibited the currents induced by submaximal GABA applications on cultured neurones from spinal cord dorsal horn (DH), dorsal root ganglia, and cerebellar granule cells (GC), increasing by two fold the EC_{50} for GABA in DH neurones and GC. Neither glycine- nor glutamate-induced currents were inhibited by HEPES. Submaximal $GABA_AR$-mediated currents were also significantly inhibited by 20 mM PIPES, MOPS, TRIS or imidazole. PIPES and HEPES, both piperazine derivatives, similarly inhibited $GABA_AR$, whereas the other pH buffers had significantly weaker effects and MES had no effect. HEPES-induced inhibition of submaximal $GABA_AR$-mediated currents was not modified by diethylpyrocarbonate, a histidine-modifying reagent, and was therefore not related to the H^+- induced potentiation involving histidine 267 of the β subunit. HEPES-induced inhibition of submaximal $GABA_AR$-mediated currents was not modified by flumazenil, and was therefore not involving the benzodiazepine binding site on $GABA_ARs$. This inhibition was independent of the membrane potential, of intracellular Cl^- concentration and of HCO_3^- ions, but depended on pH_e. With the physiological HCO_3^-/CO_2 buffer, changing pHe from 6.8 to 7.8 by saturating the solutions with different CO_2/O_2 mixtures had no effect on submaximal $GABA_AR$-mediated currents. However, such changes in pH_e in the presence of 20 mM HEPES had a strong effect on these currents. Thus, inhibition of $GABA_ARs$ by HEPES depended on pH_e, leading to an apparent H^+-induced inhibition of DH $GABA_ARs$ that did not reflect to the pH sensitivity displayed by these receptors in both low and physiological buffering conditions.
These data indicate that protonated HEPES was responsible for this inhibition.

P2.020

Maturation of synaptic plasticity in the postnatal prefrontal cortex of Reelin Haploinsufficient Mice

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The extracellular matrix protein reelin is a large secreted glycoprotein present in the central nervous system. Reelin binds to different family of cell surface receptors : the very-low-density lipoprotein receptor (VLDLR) and the apoliprotein E receptor 2 (ApoER2), and to cell adhesion molecule of the integrin family (α3β1). Reelin plays a crucial role in the development of laminated structures where it participates to migration, neuronal positioning and dendrite outgrowth. In the postnatal brain, reelin regulates several aspects of synaptic maturation. We have previously shown that reelin controls the maturation and the homeostasis of NMDA receptors in vitro.
To investigate functional and morphological consequences of reduced levels of reelin ex vivo, we studied the maturation of glutamatergic synapses in the prefrontal cortex (PFC) of the Heterozygote Reeler Mice (HRM). In a longitudinal study, we combined electrophysiological and imaging approaches to characterize synapses between layer 2/3 and layer 5/6 in PFC slices. We found that HRM display a transient lower synaptic strenght due to a delay in the maturation of AMPAR/NMDAR ratio. NMDA-dependent Long Term Potentialisation is completely abolished in HRM during early postnatal stages compared to wild type mice. We speculate that the negative impact in developmental events due to a decrease in reelin content could lead to abnormal functioning of the PFC.

P2.021

PICK1-dependent reorganisation of endogenous AMPARs during chem-LTP in cultured hippocampal neurons

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The expression of long-term potentiation (LTP) is governed largely by modulation of postsynaptic ionotropic glutamate receptors, especially through regulated trafficking of specific AMPA receptor subunits to the cell surface. AMPA receptors (AMPARs), which are tetramers of GluA1 to GluA4 subunits, mediate rapid excitatory synaptic transmission. The presence of the edited GluA2 subunit critically determines AMPAR properties. AMPARs that lack the GluA2 subunit are permeable to calcium, exhibit rapid kinetics and high conductance, and are inwardly rectifying.
The transient expression of calcium-permeable GluA2-lacking AMPARs at the postsynaptic membrane has been proposed as a mechanism for initiating and stabilizing the long-term changes in synaptic transmission that underlie LTP. While these observations indicate that the regulation of GluA2-lacking calcium-permeable AMPARs is important for input-specific long-term synaptic plasticity, the molecular processes that mediate switching between calcium-impermeable GluA2-containing AMPARs (CI-AMPARs) and calcium-permeable GluA2-lacking AMPARs (CP-AMPARs) remains unknown in cultured hippocampal neurons. We demonstrate that glycine-induced chemical LTP causes transient changes in surface AMPAR subunit composition in cultured hippocampal neurons. At both the postsynaptic density and at extrasynaptic sites, we observe a rapid incorporation of endogenous GluR2-lacking AMPARs after 5 min and a late insertion of GluR2-containing AMPARs after 20 min following chem-LTP induction. These results are in agreement with a previous report showing the transient incorporation of CP-AMPARs and their replacement by GluA2 containing AMPARs ~25 min after pairing-induced LTP. Our data also indicate that a proportion of surface AMPARs are internalised and may contribute to the newly inserted pool of AMPARs in response to the LTP-inducing stimuli. Based on our results we suggest a mechanism in which PICK1 regulates incorporation of CP-AMPAR by retaining GluA2 containing AMPARs away from the synapse.

P2.022

Anti-apoptotic effect of the opioid remifentanil in the developing mice brain

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Remifentanil can be used during cesarean section under general anaesthesia, especially in pre-eclamptic parturients, and neonatal intensive care units. Clinical remifentanil formulation Ultiva contains glycine as vehicle (3.6 mM Glycine/50 µM remifentanil). Its use in a context of potential prematurity and the doubts emitted about the innocuousness of opioids in the NEOPAIN study[1] strongly leads to explore this morphinic agent during development. It is known that opioids can modulate NMDA receptor (NMDA-R) activity and induce hyperalgesia. Furthermore, NMDA-R is involved in excitotoxic lesions genesis in newborn. We previously showed that NMDA exerted a dual effect on immature neocortex: pro-necrotic in deep mature layers, and anti-apoptotic in superficial immature layers[2]. We evaluated the potential necrotic and apoptotic effects of remifentanil, alone or associated with glycine (Ultiva), in mice developing brain. Acute cerebral slices from postnatal day 2 mice were treated with the different compounds in basal (aCSF) or excitotoxic conditions (NMDA).

Ultiva (10-240 µM) had no impact on necrotic death. In contrast, 50 µM Ultiva significantly reduced caspase-3 activity, Bax and cleaved caspase-3 contents. Immunohistochemical data showed that neuroprotective effect of Ultiva targeted the neocortex. Ultiva's effect was reversed by the µ antagonist naloxone and the NMDA antagonist MK801. In presence of 12.5 µM NMDA (ineffective dose), association of 25 µM remifentanil and 1.8 mM glycine exerted a synergic inhibitory effect on apoptotic death whereas it was devoid of necrotic action. Furthermore, 50µM remifentanil and 12.5 µM NMDA, without glycine, significantly reduced apoptosis. The present data indicate that Ultiva had no pro-necrotic effect but exerted an anti-apoptotic action in immature mouse brain, in basal as well in excitotoxic conditions. The Ultiva's action involved activation of µ and NMDA receptors. Considering the suspicion about the safety of anaesthetics in neonates, these results reassure as for the use of Ultiva in a neonatal context. *Supported by "la Fondation Motrice", the University of Rouen, INSERM, ANR, FEDER, Région Haute-Normandie and the LARC Neuroscience network.*
1 Anand, KJ et al., Lancet 2004; 2 Desfeux A et al., Cereb Cortex 2010

P2.023

Persistent post-lesion cell body response in a sub-population of bulbospinal respiratory neurons after chronic cervical spinal cord hemisection

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After an injury in the CNS, the ability of injured axons to regrowth may in part depend on the level of the cell body response to injury. Thus, the persistence or not of such cell body response during the weeks and months following the injury may determinate the level of spontaneous anatomical plasticity and the efficiency of reparative strategies applied after a chronic CNS injury. Therefore, we analyzed, after a chronic cervical C2 hemisection, the expression of some markers of post-lesion neuron response, NO synthase, c-jun and HSP27, known to be induced after a PNS injury and to play a role in axon growth. The analysis was realized on the bulbospinal respiratory neurons in the medulla, and, for comparison, in the gigantocellularis nucleus. One month after spinal hemisection, NO synthase appeared in a subset of axotomized respiratory neurons, among 25% of them. This same neuron sub-population, one month post-injury, was also characterized by the co-localisation of the 3 analyzed markers, although HSP27 and c-jun were already expressed earlier in these neurons (at 7 days post-injury). None of these markers were detected in the other axotomized respiratory neurons. A similar pattern was also obtained 3 months post-injury and for the other analyzed region. This study shows that a subset of medullary respiratory neurons remains responsive after a chronic spinal injury. Such long-lasting cell body response may play a role in long-term respiratory pathways plasticity and reactivation.

P2.024

Folate deficiency impairs hippocampal progenitor differentiation and synaptic plasticity through alterations of cytoskeleton-related proteins

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Dietary deficiency in methyl donors like folate (vitamin B9) is associated with elevated levels of homocysteine, a risk factor for various neurodegenerative diseases. Folate deficiency has been shown

to influence the normal development of the brain, with impaired cognitive functions involving the hippocampus.
We studied the consequences of folate deficiency on hippocampal neuronal progenitors, the H19-7 cell line, which is known to display the properties of embryonic neurons. Folate deficiency leads to the disruption of the one-carbon metabolism, with an increase of homocysteine concentration and impaired neuronal proliferation, differentiation and synaptic functions.
The ability of neurons to polarize is crucial for the process of neurite growth, axon emergence and synaptic plasticity. Cytoskeletal proteins involved in the formation of microtubules and neurofilaments are determinant factors of neuronal polarity. We studied the effects of folate deficiency on these proteins and our results showed a disruption of the cytoskeleton and a lack of cell polarization. Analyses by Western blot and immunocytochemistry revealed significant abnormalities in the expression and localization of the cytoskeletal components. Co-immunoprecipitation experiments showed that the aggregation and subsequent degradation of actin, a component of neurofilaments, was a result of the binding of homocysteine to this protein.
Regarding the potential use of neuronal progenitors in regeneration strategies, our observations suggest that the folate status should be considered in order to improve cell survival and plasticity.

P2.025

Hippocampus morphological abnormalities and anterior commissure dysgenesis in *SCHIP1* mutant mice

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SCHIP-1 was initially described as a component of axon initial segments and nodes of Ranvier, two regions enriched in voltage-gated Na^+ channels, which are essential for the generation and propagation of action potentials in myelinated fibers (Martin et al., 2008). SCHIP-1 is highly enriched in the brain and six isoforms, encoded by the same gene, have been identified so far.These isoforms differ by their N-terminal part but share a C-terminal domain including a ~40-residue leucine zipper predicted to adopt a coiled-coil conformation.
To shed light on the possible role of SCHIP-1 *in vivo* we generated mutant mice in which the exon 10 of the gene was deleted, using a three-lox recombination strategy. Deletion of exon 10 was predicted to introduce a premature stop codon in the conserved C-terminal domain, common to all SCHIP-1 isoforms. *SCHIP1* mutant mice had mild age-dependent abnormalities of peripheral myelinated fibers. In contrast they displayed early social interaction deficits, stereotyped movements, and behavioral abnormalities sometimes associated with autism-spectrum disorders. Brain gross anatomy analysis revealed dysgenesis of the anterior commissure, a bipartite tract interconnecting olfactory structures/anterior piriform cortex and posterior piriform cortex of the temporal lobes. Defects in the anterior commissure were already detectable at P0, before myelination, suggesting a role for SCHIP-1 in axon outgrowth and/or guidance. Adult mutant mice also showed abnormal compaction of the pyramidal cells of the hippocampal CA3 region at the entrance to the dentate gyrus, which is likely to result from defects in hippocampal development. These defects migth be cell-autonomous or/and rely on defaults in axonal projections from the dentate gyrus toward the hippocampal CA3 pyramidal cell layer. In order to further characterize these defects, we started to analyse hippocampal organization in mutant mice during development by performing specific immunolabelings for the different cell sub-types. We also initiate *in situ* hybridation experiments to determine *SCHIP1* expression pattern during development and in adulthood. Preliminary results showed that *SCHIP1* is expressed at the level of the CA3 pyramidal cell layer and in the dentate gyrus from P0 to adulthood.

P2.026

Pool-specific regulation of motor neuron survival by neurotrophic support

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The precise control of motor neuron death and survival following initial innervations of skeletal muscle targets is a key step in sculpting a functional motor system, but how this is regulated at the level of individual motor pools remains unclear. Hepatocyte growth factor (HGF) and its receptor Met play key developmental roles in both muscle and motor neurons. We generated mice (termed "*Nes-Met*") in which *met* is inactivated from mid-embryonic stages onward in the CNS only. Adult animals showed motor behavioral defects suggestive of impaired innervations of pectoral muscles. Correspondingly, in neonatal spinal cords of the same mice we observed death of a discrete population of *pea3*-expressing motor neurons at brachial levels. Axonal tracing using *pea3* reporter mice revealed a novel target of *pea3*-expressing motor neurons: the *Pectoralis Minor* muscle. In *Nes-Met* mice, the *Pectoralis Minor* muscle was initially normally innervated but, in parallel with death of motor neurons in the corresponding motor pool, became progressively denervated. Therefore, HGF/Met signaling is required for survival of *Pectoralis Minor* motor neurons during late embryogenesis. In contrast, the survival of neighboring Met-expressing motor pools was not affected in mutant mice. Our results demonstrate the exquisite degree to which outcomes of signaling by receptor tyrosine kinases are regulated on a cell-by-cell basis. They also provide a model for one way in which the multiplicity of neurotrophic factors may allow for regulation of motor neuron numbers in a pool-specific manner.

P2.027

Bidirectional actions of secreted BDNF on the efficacy of developing GABAergic synapses

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Network construction is a bidirectional process whereby neurogenesis, neuritic outgrowth and synaptogenesis are balanced by neural death, neurite retraction and synaptic pruning. The brain-derived neurotrophic factor (BDNF) has emerged as a major target-derived messenger for activity-dependent development and synaptic plasticity. Like many peptide hormones or growth factors, BDNF is synthesized as a large precursor (proBDNF) that is subsequently cleaved to generate the mature form (mBDNF). Until recently, the mBDNF was considered to be the sole secreted and biologically active ligand. Recent data have shown however that a significant proportion of BDNF is secreted as a precursor, thereby inducing diametrically opposite cellular responses in neuronal cultures. Whether proBDNF is secreted during development and how secreted proBDNF affects developing neuronal network remain unknown.
In a previous study we found that ongoing glutamatergic synaptic activity triggers a long-lasting potentiation of GABAergic activity (LLP_{GABA-A}) in acute newborn rat hippocampal slices. LLP_{GABA-A} induction required the secretion of BDNF and subsequent activation of specific TrkB receptor by the mBDNF (Kuczewski et al., J Physiol, 2008). In the present study we explore the possibility that locally secreted proBDNF might elicit alternative action on developing GABAergic synapses.
To this aim, we have studied the effect of bath applied aprotinin, a membrane impermeable plasmin inhibitor that prevents the extracellular proteolysis of proBDNF. We found that aprotinin, prevents the induction of LLP_{GABA-A} in CA3 pyramidal neurons and uncovers a long lasting depression of GABAergic synaptic activity (LLD_{GABA-A}). LLD_{GABA-A} was not observed in slices treated with the $p75^{NTR}$ receptor antibody or with the TAT-pep5, a $p75^{NTR}$ signalling inhibitor.
These results suggest that

1) in the developing brain, a significant proportion of BDNF is secreted as precursor, subsequently cleaved to yield mBDNF in physiological conditions and that
2) the accumulation of endogenous proBDNF depresses developing GABAergic activity acting on $p75^{NTR}$.

P2.028

The G protein coupled receptor BAI3 might regulate neuronal morphogenesis and synaptogenesis through the Rac1 signaling pathway

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Neuronal morphogenesis and synaptogenesis constitute key steps in the formation of a functional neuronal network. Abnormalities in these processes could lead to neurodevelopmental diseases such as autism or schizophrenia. However, the molecular mechanisms controlling these events are not yet fully understood. A new family of G protein coupled receptors, the Brain Angiogenesis Inhibitor (BAI) receptors, has been identified in synaptic preparations. One member, the BAI3 receptor, was detected in parallel fiber/Purkinje cell (PC) synapses purified from mice cerebella. To investigate the function of BAI3 in the brain, we are using two transgenic mouse lines, which specifically express in PCs either a truncated form of BAI3 coupled to GFP (SCT), or full length BAI3 receptor with a GFP insertion in its cytoplasmic domain (FLT). Immunohistochemistry performed on cerebellar tissues from SCT and FLT transgenic mice showed that the transgenes are expressed in PCs from postnatal day 10 and targeted to dendritic spines. Quantitative analyses of the extension of climbing fiber innervation on PCs showed a significant increase of this territory in FLT mice compared to wild type (WT), suggesting that expression of this transgene leads to changes in the innervation of PCs *in vivo*. In parallel, we are investigating the signaling pathway tethered to BAI3 either in heterologous (HEK293) cells or in cerebella from SCT and FLT transgenic mice. Our preliminary results suggest that, as was shown previously for BAI1, an intracellular partner of the BAI3 receptor is the protein ELMO1 known to regulate Rac1 signaling. Rac1 is a small GTPase known to control dendritic morphogenesis and synaptogenesis through its regulation of the actin cytoskeleton. We are now investigating a possible role for BAI3 in these processes by studying the morphology of neuronal and non-neuronal cell lines transfected with WT or modified forms of BAI3. Given the potential genetic link between BAI3 and some symptoms of schizophrenia, our study will allow us not only to reveal key roles played by this receptor in the formation of a functional neuronal network, but will also provide new insights in the study of neurodevelopmental disorders.

P2.029

Schwann cell c-Jun regulates glial support for survival and regeneration of PNS neurons after injury

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The ability of neurons to survive and regenerate after injury or disease depends strongly on their surrounding glial cells. The Schwann cells, the glial cells of peripheral nerves, allow much more effective neuronal regeneration to take place than their counterparts in the CNS. Therefore, identification of Schwann cell regulators of nerve response to injury and pathology is critical for understanding and helping the process of neuronal survival and regrowth. We recently identified the transcription factor c-Jun as one of these regulators (Mirsky and Jessen 2008 Glia Vol 56 No.16 Pages 1552-1565).
We show here that c-Jun controls the extensive change in Schwann cell gene expression involved in the generation of the denervated cell of injured nerves. Transcriptome analysis of the distal stump of sciatic nerves 7 days after transection in mutant and control mice allowed us to identify 172 genes which are abnormally regulated in the absence of c-Jun in Scwhann cells after injury. The most promising candidates have been further studied in the nerve in response to injury and in purified cultured Schwann cells in response to c-Jun expression. The corresponding proteins constitute candidate glial regulators of the neuronal response to injury since the absence of c-Jun in Schwann cells after nerve injury leads to dramatic impairment in functional recovery and axonal regeneration. This is associated with a striking loss of dorsal root ganglion sensory neurons following Wallerian degeneration. Using microfluidic chambers, we were able to further demonstrate that c-Jun controls direct interactions between axons and denervated Schwann cells which support axonal regeneration. Finally, we used adenoviral infection to enforce c-Jun expression in Wlds nerves in vivo and showed that c-Jun is sufficient to generate a growth-supportive nerve phenotype.

P2.030

From neural stem cells to brain tumors: a genetic analysis

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Increasing evidence suggests that stem cells are at the origin of many brain tumors in human. Neural stem cells (NSCs) are characterized by the ability of self-renewal and unlimited proliferation and multi-lineage differentiation. The asymmetric division of NSCs has to be tightly regulated as defects can lead to tumorigenesis. Indeed, in *Drosophila*, mutations in genes that are involved in the asymmetric division of NBs, such as *brain tumor* (*brat*), *numb*, or *prospero* (*pros*), result in the formation of brain tumors. However, the molecular mechanisms that control the self-renewal and differentiation of NSCs are poorly understood.
We use ***Drosophila*** as a model system to study the genes involved in the proliferative cell division of brain NBs. The central brain of *Drosophila* contains about 100 lineages, each generated by an identified NSC that is called Neuroblast (NB). Using a NB-specific Gal4 driver (*insc-Gal4 and wor-Gal4,ase-Gal80*), we carry out a transgenic UAS-RNAi analysis of the roles of key developmental control genes encoding cell fate determinants in the normal and abnormal proliferation of NBs. We employ a genome-wide neuroblast screen to search for new candidate tumor suppressor genes and explore their tumorigenic and metastasic potential by transplantation into host w^{1118} flies.
We show that knockdown of *numb* or α-*adaptin* or *ap2-sigma* in NBs using targeted RNAi transgenics results in overgrowth in the central brain of *Drosophila*. Following transplantation, overproliferating brain tissue induces tumors and metastasis into the host flies. By contrast knockdown of *PP4*-RNAi does not induce overproliferating brain tissue and tumors after transplantation. We are currently analyzing the tumorigenic and metastatic potential of knockdown and loss-of-function of these and other candidate genes (obtained from our RNAi screen) involved in neural proliferation in brain NBs and testing their possible roles in enhancing or suppressing tumor formation.

P2.031

Are group II metabotropic glutamate receptors involved in Schaffer collateral LTP?

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The roles of group I and II metabotropic glutamate receptors (mGluRs) for the induction of LTD are well characterized. However, earlier studies reported that (1S,3R)-ACPD, an agonist of both group I and group II mGluRs, induces LTP in CA1 pyramidal cells (Bashir et al., 1993; Bortolotto et al., 1994; Breakwell et al., 1996). In these studies, bath application of (1S,3R)-ACPD resulted in slow onset potentiation of the EPSP evoked in CA1 by Schaffer collateral stimulation in rat hippocampal slices. The objective of our study is to evaluate the contribution of group II mGluRs in this form of LTP. We find that bath application of (1S,3R)-ACPD (10 mM, 20 min) induces slow onset potentiation of EPSPs recorded in CA1 pyramidal cells in acute hippocampal slices of mouse (28.7 ± 2.7%, n=11/14, P< 0.0001). To determine whether the activation of group II mGluR is required for this form of LTP, we bath applied (1S,3R)-ACPD in presence of LY341495 (3 mM, applied 20 minutes before (1S,3R)-ACPD perfusion until the end of the recording), a selective group II mGluR antagonist. Under these conditions, (1S,3R)-ACPD failed to induce slow onset potentiation of EPSPs (28.7 ± 2.7% vs -1.5 ± 4.5%, n=3/3, p=0.0128). In conclusion, activation of group II mGluR is necessary for ACPD-induced LTP in CA1 pyramidal cells.

P2.032

Role of glia in chronic neuropathic and cancer pain

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Nowadays, glia are thought to contribute to chronic pain. We recently investigated whether spinal glial activation correlates with the apparition of pain symptoms in two different models of chronic pain in rats (see poster by Ducourneau et al): a bone cancer pain model induced by injecting MRMT-1 rat mammary gland carcinoma cells into the right tibia and a peripheral neuropathic pain model obtained by right sciatic nerve ligation. We found that although chronic pain induced by spinal nerve ligation is correlated with spinal astrocytic and microglial activation, this is not necessarily true for chronic pain induced by bone cancer, suggesting that glia play different roles in the development and maintenance of chronic pain in these two models.
Here we investigated the effect of functional inactivation of glia upon pain symptoms in these two models. Mechanical allodynia and hyperalgesia were quantified using von Frey hairs and incapacitance using dynamic weight bearing. Intrathecal administration of a single dose (2 nmoles in 5 µL vehicle) of the glia metabolism inhibitor fluorocitrate did not reduce mechanical allodynia and hyperalgesia at day 21 in rats receiving intra-tibial injections of MRMT-1 cells as well as at day 14 in neuropathic rats. We are currently investigating the effects of chronic administration of fluorocitrate in the two models. Preliminary results suggest that chronic treatment with the microglia inhibitor minocycline (daily 50 mg/kg IP) reduced pain symptoms in neuropathic rats. Finally, since astrocytes have been shown to contribute to glutamatergic NMDA receptor-mediated transmission through d-serine release, we investigated whether pain symptoms are NMDA receptor dependent in the two models. Intrathecal administration of a single dose (25 nmoles in 5 µL vehicle) of the NMDA receptor antagonist AP-5 significantly reduced mechanical allodynia and hyperalgesia in cancer rats as well as in neuropathic rats. Further investigation will determine whether glia play a role in chronic neuropathic and cancer pain through NMDA receptor modulation.

P2.033

tPA processing of ADAMTS4 ensures the degradation of neurocan, promoting axonal regeneration and functional recovery after compressive spinal cord injury in rats

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The establishment of glial scar in response to injuries of the central nervous system (CNS) creates a dense physical barrier around the lesion site as well as a chemical barrier due to the release of chondroitin sulfate proteoglycans (CSPG) by reactive astrocytes. This inhibitory environment mainly contributes to the failure of axons to regeneration leading to persistent functional impairments. Compressive spinal cord injury (SCI) in rats causes an acute activation of astrocytes within the spinal cord leading to a persistent glial scar at least 3 weeks later. Reactive astrocytes synthesize and release large amounts of the main inhibitor of tPA (tissue Plasminogen activator), PAI-1 (type 1 Plasminogen Activator Inhibitor), into the extracellular space. We postulate, in the present study, that the tPA/PAI-1 axis may have an influence on secondary damages occurring after SCI, especially on axonal regeneration and glial scar. To address this question, we have first investigated the role of tPA in primary mixed cultures of medullary neurons and astrocytes. Our results evidence that tPA leads to a reduction of the glial reactivity and subsequent promotion of neurites growth. Then, we tried to determine the molecular and cellular mechanisms by which tPA may promote neuritic growth. *In vitro,* we evidenced that tPA is regulating the activity of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS4) known to control degradation of CSPGs such as neurocan. Accordingly*, in vivo*, the administration of tPA, 24h after the injury, leads to a reduced glial reactivity/scar and also favours the processing of the pro-ADAMTS4 into its active forms. Finally, we evidenced that active ADAMTS4 may be responsible of a degradation of neurocan, then improving axonal regeneration and functional recovery after SCI. This study highlights the relevance of tPA as a key actor during SCI.

P2.034

Prenatal exposure to methylphenidate may alter the adult brain rewarding functions in male rats

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Methylphenidate (MPH) is the gold standard medication for attention-deficit/hyperactivity disorder (ADHD). This disorder affects 5% of children and frequently persists throughout adulthood. Since now, little is known about the effects of MPH at the earliest stages of brain development. As MPH is a psychostimulant, we investigated the reward system reactivity (RSR) from 21 postnatal days to adulthood in a rat model mimicking an MPH prenatal exposure during the first trimester of pregnancy. For this aim, MPH (10 mg/kg/day, s.c.) or its vehicle (saline) was injected to female Wistar rats from the 13th to the 20th day of gestation. The RSR of the male progeny was evaluated in both groups in basal condition and after a single or chronic exposure to cocaine (15 mg/kg per day or during 5 days i.p., respectively). This was assessed via the observation of locomotor activity and c-fos immunoreactivity that are altered by drugs of abuse.
Only the adult data of this work will be presented as interesting results were observed.
(i) Both MPH or saline prenatally exposed adult rats did not display distinct locomotor activities in basal conditions.

A single cocaine injection induced a significant and comparable increased locomotor activity in both groups ($p < 0.015$). However, a 5 days treatment with cocaine induced a sensitization of the locomotor effects in the MPH group ($p=0.001$) while the control animals did not show any significant difference when compared to the acute condition ($p=0.081$).
(ii) Moreover, a single cocaine injection significantly enhanced c-fos immunoreactivity MPH animals vs controls in regions belonging to the reward network ($p=0.006$ and 0.023 in the shell of the nucleus accumbens and the cingulate cortex).
No difference between MPH and control groups was observed in non limbic regions ($p=0.417$ and 0.298 in the dorsal striatum and core of the nucleus accumbens).
Such preliminary findings suggest that a higher sensitivity of the MPH group vs controls to the effect of cocaine that could be due to an altered RSR of adult male rats prenatally exposed to MPH. Further experiments evaluating dopamine release in limbic regions of the reward network will be conducted in this model to test this hypothesis.

P2.035

Mutation of the MAP1B-related gene *futsch* affects synaptic organization and function at the *Drosophila* neuromuscular junction (NMJ)

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Structural Microtubule-associated proteins (MAP) are known to control the microtubule cytoskeleton organization, stabilization and function. Hence, they play an important role in neurite growth during development. However, their role in synapse differentiation and function has scarcely been studied. The *Drosophila* gene *futsch* is the homologue to the vertebrate MAP1B microtubule-associated protein (Hummel *et al*., 2000). It is largely concentrated in the nervous system, and colocalizes with the microtubule cytoskeleton at the *Drosophila* NMJ, a synaptic terminal consisting of a string of varicosities. *futsch* mutants were reported to have fewer but bigger varicosities at this synaptic terminal (Roos *et al*., 2000). However, the precise consequences of the loss of Futsch on subcellular organization of synaptic components within the varicosities were never studied. Here we show that Futsch is important for the organization of vesicles and active zones which has consequences vesicules release at the *Drosophila* NMJ.

P2.036

EB3 stably links microtubules to ankyrin G in the axon initial segment

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The Axon Initial Segment (AIS) plays a key role in maintaining the molecular and functional polarity of the neuron. The relationship between the AIS architecture and the microtubules (MTs) supporting axonal transport is unknown. Here we identify a direct and specific interaction between the AIS scaffold protein ankyrin G (ankG) and the microtubule plus-end binding (EB) protein EB3. AnkG concentrates and stabilizes EB3 along MTs in the AIS, contrasting with the role of EB3 as a dynamic MT plus-end tracking protein (+TIP). In addition, EB3 participates in AIS stability, and AIS disassembly leads to a cell-wide up-regulation of EB3. Thus, EB3 coordinates a molecular and functional interplay between ankG and the AIS MTs that supports the central role of ankG in the maintenance of neuronal polarity.

P2.037

Involvement of phosphorylation/dephosphorylation mechanisms in the intracellular trafficking and activation of the potassium/chloride co-transporter KCC2 in the neonatal rat

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GABA and glycine, the major inhibitory neurotransmitters in the adult spinal cord play a key role in neuronal plasticity. Their effect mediated by $GABA_A$ and glycine receptors can be excitatory, depending on the chloride concentration of the target cell. This is mainly regulated by the neuron-specific potassium-chloride co-transporter, KCC2, and the ubiquitous sodium-potassium-chloride co-transporter, NKCC1. These transporters extrude and intrude chloride ions, respectively. We investigated the plasticity of inhibitory synaptic transmission, in particular that of cation-chloride co-transporters, during development and after spinal cord injury in neonates. We showed that during development, the expression of KCC2 increases while that of NKCC1 decreases especially when the effect of inhibitory postsynaptic potentials on the resting membrane potential switches from depolarization to hyperpolarization. After neonatal spinal cord transection, the expression and intracellular trafficking of KCC2 are affected and this is correlated with a loss of transporter function. Phosphorylation/dephosphorylation mechanisms play a pivotal role in the regulation of cation-chloride co-transporters. The conventional view is that net dephosphorylation activates KCCs. KCC2 is subject to phosphorylation by serine/threonine and tyrosine kinases at sites within the C-terminal cytoplasmic domain. There is considerable interest in how its functional expression is controlled and particularly by phosphorylation/dephosphorylation events. We combined the analysis of KCC2 expression with that of its phosphorylation state during development and after neonatal spinal cord transection.
Our results suggest that tyrosine and serine phosphorylations may be involved in addressing and stabilizing the protein in the plasma membrane whereas threonine phosphorylation appears to play a role in its functional activation.

P2.038

Spatio-temporal regulation of the sumoylation machinery in the rat Central Nervous System

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Small Ubiquitin-like MOdifier protein (SUMO) is a key regulator of the nuclear function. However the role of this post-translational modification outside of the nucleus is still largely unknown, particularly in the Central Nervous System. Here, we report that the levels of SUMO-modified proteins as well as the amount of sumoylation and desumoylation enzymes are temporally and spatially regulated during rat brain development.
Using brain fractionation experiments at various developmental stages, we find that the nuclear and cytosolic expression levels of SUMO substrates are maximum early in the development and then decrease slowly towards the adult stage suggesting a role for the SUMO pathway in either neuronal proliferation, neuronal differentiation and synaptogenesis. The nuclear and cytosolic expression levels of sumoylation and desumoylation enzymes follow the same pattern with higher protein amount expressed early in the development. Interestingly, while the overall level of SUMO substrates is decreasing during brain development, there is an increased expression of both sumoylation enzymes and SUMO substrates in the synaptic compartment that reaches a maximum between birth and the adult stage.
Furthermore, using immunocytochemistry on primary cultures of rat hippocampal neurons, we show that there is a synaptic redistribution of sumoylation and desumoylation enzymes during neuronal maturation, indicating a potential role for the sumoylation machinery in synaptic communication and/or plasticity.

P2.039

A multicolor approach to explore lineage and cellular interactions in the developing nervous system

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Neuronal circuits of the mammalian central nervous develop through a sophisticated spatiotemporal choreography. Lineage tracing techniques marking a unique progenitor (e.g. retroviral injection) or a population of cells (e.g. genetic fate mapping) have allowed for major advances in understanding neural stem cell development. Approaches with a single type of label are however limited as they fail to spatially resolve adjacent progenitors, their lineage and their interrelationship. To overcome this limitation and characterize the ontogenesis of neural structures with increased precision, we are developing a lineage tracing approach to reliably track more than one individual clone in a given animal. To establish this 'multiclonal' approach, we are using and further improving the Brainbow labeling strategy which relies on Cre/*lox* recombination to trigger expression of random combinations of 3-4 spectrally distinct fluorescent proteins (such as CFP, YFP and RFP) in a cellular population. Those color labels can be used to simultaneously mark multiple adjacent cortical precursors in mouse embryos and track their progenies. In order to unambiguously distinguish individual groups of clonally related cells, we are generating Brainbow transgenes expressing an expanded palette of fluorescent labels. In addition, we are refining expression strategies to restrict both the timing and spatial extent of the multicolor labeling. We validate these approaches in the mouse embryo using inducible Cre mouse lines and in utero electroporation. This lineage tracing methodology will allow for resolving adjacent clones, comparing their migratory behavior and fate and analyzing their interactions at various steps of cortical development. We anticipate that our work will open avenues to gain further insights on neural development and potential tracks to unravel the pathophysiological bases of neurodevelopmental disorders.

P2.040

The immune molecule CD3zeta and its downstream effectors ZAP-70/Syk mediate ephrin signaling in neurons and regulate glutamatergic synaptic content

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Several proteins are expressed in both the immune and nervous systems, but their nonimmune functions in the brain remain poorly understood. In the immune system, the transmembrane adaptor protein CD3z is associated with cell-surface receptors in hematopoeitic cells and is involved in antigenic recognition. In the nervous system, CD3z has recently been shown to be expressed in neurons and to play a key role in dendrite development and glutamatergic synaptic plasticity. However, the upstream signals that might recruit and activate CD3z in neurons are still unknown. In this study, we identify ephrinA1-dependent EphA4 receptor activation as an upstream regulator of CD3z. In young cultured neurons, stimulation with ephrinA1 induces the translocation of both CD3z and its activated effector molecules, ZAP-70/Syk tyrosine kinases, to EphA4 receptor clusters. EphrinA1-induced growth cone collapse, a strong repulsion event implicated in neurite outgrowth and guidance, was abrogated in CD3z$^{-/-}$ neurons and was markedly reduced by ZAP-70/Syk inhibition. Given the functional importance of ephrin/Eph signalling in synaptic functions, we investigated whether ephrin stimulation regulated CD3z distribution in mature cultured neurons. We found that ephrinA1,

known to cause dendritic spine retraction, induced a redistribution of synaptic CD3z clusters to intradendritic small aggregates. Gain- and loss-of function experiments are under investigation to further study the contribution of CD3z in ephrinA1-induced synapse regulation. To begin the characterization of $CD3z^{-/-}$ synapses, biochemical and immunohistochemical analyses were performed in WT and $CD3z^{-/-}$ mice brain. Levels of NR1 and NR2A NMDA glutamate receptor subunits as well as CamKII, a protein kinase involved in synaptic plasticity, were significantly reduced in the postsynaptic density fraction of $CD3z^{-/-}$ mice compared to WT. Interestingly, NMDA receptor and CamKII expression levels showed no significant change in the whole membrane fraction, suggesting that the lack of CD3z affects synaptic accumulation of NMDA receptors and CamKII without interfering with their cellular expression levels. Altogether, our data suggest that CD3z regulates synaptic molecular homeostasis by relaying ephrin/Eph signaling.

P2.041

Quantification of input density in acute cerebellar slices; relevance to endocannabinoid signaling

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The endocannabinoid system is expressed a most central synapses. In the cerebellum, endocannabinoids mediate the synaptically-driven suppression of excitation (SSE) induced by high frequency firing of parallel fibers (PFs). We previously showed that this endocannabinoid signaling only occurs when stimulating a set of spatially clustered (dense) synaptic inputs. Here, we measured the input density in acute cerebellar slices, and we quantified the spatial input dependency of endocannabinoid signaling. Transgenic mice expressing the a calcium indicator selectively in granule cells were used to detect the action potential-evoked calcium rise in activated PFs. Stimulation-evoked fluorescence changes provided an image of the beam of activated PFs whose section was determined. The density of activated PFs was derived from the number of activated PFs in the beam, which was calculated using the averaged fluorescence change evoked by the stimulation of single PFs. We show that, on average, 30-50% PFs respond to stimulation in sagittal slices, and twice more in transverse slices. The density of activated PFs varied by a factor of 20 from slice to slice preparation. Although the SSE correlated with the density of activated PFs, consistently with our previous qualitative finding, the SSE was detectable for input densities as low as 1 PF per μm^2. We conclude that input density 10 times below the maximum achievable experimentally is enough to evoke SSE.

P2.042

Blocking CREB function in adult mice impairs memory, enhances LTD and leads to learning-induced spine collapse in CA1 pyramidal neurons

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Contextual memory formation is correlated with synaptic plasticity and structural modifications of dendritic spines within the hippocampus. The molecular mechanisms controlling these experience-driven neuronal alterations are still not well understood. In this study, we investigated how chronically blocking the function of the CREB pathway in adult mice alters synaptic function and learning-induced

spine formation of CA1 pyramidal neurons. Using transgenic mice expressing a doxycycline-regulated dominant-negative mCREB, we observed an impairment of memory in the contextual fear conditioning task (CFC) and a shift of synaptic plasticity towards a weakening of synaptic strength in hippocampal CA1 pyramidal neurons. Paradoxically, while mCREB expression did not affect dendritic spine number and morphology in CFC naïve mice, it led to the collapse of dendritic spines after learning. WT mice increased both thin and mushroom spine densities after CFC, but blocking CREB function prevented the increase in mushroom spine density and led to a significant loss of thin spines. Finally, mCREB expression perturbed learning-related actin dynamics, providing a mechanistic underpinning for the observed spine collapse. Turning off mCREB expression with doxycycline reversed the behavioral, synaptic plasticity and spine dynamics phenotypes. These results show that the CREB pathway shapes physiological synaptic strength and learning-dependent spine dynamics in neural circuits sustaining memory formation.

P2.043

Disruptions of spatacsin and/or spastizin functions lead to motor neuron axonal outgrowth defects in the Danio rerio

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The hereditary spastic paraplegias (HSP) are clinically and genetically heterogeneous inherited neurological disorders characterized by the degeneration of the cortico-spinal tract leading to a progressive spasticity of lower limbs. A common form of autosomal recessive HSP associates various additional signs to this spasticity: mental retardation and/or cognitive deficits, neuropathy, cerebellar ataxia and an abnormal brain MRI with thin *corpus callosum* (TCC) and white matter hyperintensities. Two genes, SPG11 and SPG15, coding for the spatacsin and the spastizin proteins respectively, have been identified as responsible for most cases of these complex HSP. Mutations in *SPG11* and *SPG15*, most leading to abnormally truncated proteins, suggest that both diseases are due to loss of protein functions. Strikingly, HSPs linked to *SPG11* and *SPG15 show* an identical associated phenotype and both genes have similar patterns of expression. This suggests that spatacsin and spastizin are involved in a common pathway, although their functions are still unknown.
Here we used the *Danio rerio* (zebrafish) to explore these functions during development. We first demonstrated that the expression profiles of both genes are similar and that their mRNA are ubiquitously expressed from the first developmental stages to adulthood with a higher level in the brain. Morpholino-based knock-down of spatacsin and spastizin resulted in a frequent curly tail phenotype associated with reduced or abolished motility. Moreover, motorneurons and oligodendrocytes fluorescent strains revealed that the phenotype was due to aberrant motor neuron axonal outgrowth.
To test whether the spatacsin and spastizin proteins are involved in a common pathway, we co-injected SPG11 and SPG15 morpholino at doses that did not markedly result in a phenotype when either morpholino was injected individually. The co-injected embryos presented with curly tails and reduced locomotor activity suggesting that their motor neuronal tract may be disrupted.
Together, these data suggest that *SPG11* and *SPG15* act in a common pathway involved in the development of spinal motor neurons. This model should help us to decipher both spatacsin and spastizin functions and may represent a powerful tool for therapeutic approaches.

P2.044

Studying circuit architecture and postnatal rearrangement in the calyceal brainstem projection using Brainbow mice

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Neuronal populations form complex wiring patterns which are typically refined during development. Present efforts in circuit studies aim to trace connectivity and investigate wiring/rewiring during development with single-cell resolution. We study the architecture and postnatal development of the binaural circuit in the mouse brainstem. In this projection, which serves for sound localization, neurons located in the cochlear nucleus (CN) extend large axons to the medial nucleus of the trapezoid body (MNTB) where they form giant synapses called calyces of Held.
While one-to-one connectivity prevails in this projection, some studies suggest that a subpopulation of multiply innervated cells may exist in the developing MNTB. To demonstrate the existence and investigate the significance of such multiple innervations, we use the Brainbow multicolor labeling strategy: In Brainbow mice, combinations of fluorescent proteins randomly attributed to neurons allow us to resolve juxtaposed calyceal inputs based on their color. Analyzing brainstem sections, we unambiguously identify a small fraction of multiply innervated MNTB cells before and after onset of activity (hearing). While this portion of MNTB cells appears steady, changes in the relative areas of axonal contact in development indicate that postnatal rearrangements are occurring at the level of individual calyceal synapses.
The origin of inputs converging on a same MNTB cell remains unknown. Tracing axon branches back to their neuronal source or branchpoint requires a large volume, high resolution imaging strategy. However light scattering limits imaging depth, and solving this issue with serial sectioning leads to tissue loss. We bypass these problems using a new tool capable of generating continuous confocal image stacks spanning several millimeters. Ultimately we seek to provide a fine-scale map of all calyces of Held in the MNTB and to individually track every CN axons to its origin. A complete wiring diagram would allow quantitative analysis of many aspects of circuit organization including topography, branching patterns and the degree of convergence/divergence between two connecting populations. Such connectomic data is essential for a full understanding of the structural underpinnings of neural function.

P2.045

Influence of N-cadherin adhesion on microtubule recruitment and dynamics during neuritogenesis

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Deciphering the mechanism underlying neuritogenesis, which leads to the formation of a functional neuronal network, is of primary importance in neurobiology. The neuritogenesis, from neurite initiation to extension involves a multitude of processes among which cytoskeleton remodeling and neuronal plasma membrane adhesion to the surrounding environment are major events. Well known key actors of these processes are N-cadherin (Neural cadherin) and actin which are part of a multi-molecular complex linking the cytosketon, the cell membrane and the surrounding environment driving neuritogenesis. Another key actor of neuritogenesis would be the microtubules (MTs). However, while their involvement in neurite initiation has been demonstrated, their role in the elongation and their relationship with actin and cadherin is still unclear and subject to controversy.
In this presentation we will report on our recent findings regarding the relationship between N-cadherin, actin cytoskeleton and MTs during neuritogenesis. Using a non neuronal model system, we evaluated the recruitment and dynamics of MTs in C2 cells spread on N-cadherin substrates,

mimicking cell-cell contacts, or fibronectin substrates. Our results show, in the context of cadherin adhesion
(i) a reduction of MTs penetration within adhesion areas,
(ii) a decrease of the recruitment of the +TIPs proteins EB1 and EB3 and
(iii) the reduction of the speed of MT growth.
Moreover, the perturbation of the actin networks alleviates these three effects. Surprisingly, in primary hippocampal neurons, a significant increase in MT dynamics in the context of cadherin adhesion was observed. We are currently investigating the effect of perturbations of N-cadherin adhesion and actin on MT dynamics during neuritic initiation and extension.
Thus our results establish a clear functional cross-talk between cadherin adhesion, actin and MTs organization. However, the controversy remains with N-cadherin contacts reducing the microtubules growth and penetration in non-migratory myogenic cells while increasing their growth in elongating neurites. We suggest that the MT increased dynamics induced by N-cadherin adhesion is a key regulator of neurite elongation.

P2.046

Alteration of short-term plasticity at GABAergic synapses in the globus pallidus of dopamine-depleted rats

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The globus pallidus (GP) receives two major GABAergic inputs arising from the striatum and local axon collaterals. Previous studies have shown that activation of presynaptic D2-like receptors modulates evoked transmission at striato-pallidal (STR-GP) synapses and that chronic dopamine depletion leads to an enlargement of STR-GP synapses in the GP suggesting an hyperactivity of this pathway in Parkinson's disease. On the other hand, the properties and function of lateral inhibition in the GP are poorly understood, but a balanced inhibition between STR-GP and pallido-pallidal (GP-GP) pathways seems to be critical for normal information processing by GP neurons.
The aim of this study was to test the impact of chronic dopamine depletion on GABAergic synaptic transmission in the GP. Using patch-clamp whole-cell recordings in brain slices, we first characterized IPSCs kinetics, short-term plasticity profile and modulation of GP GABAergic synapses by administration of the specific dopamine D2 receptor agonist quinpirole. Typical STR-GP synapses displayed short-term facilitation (ratio 3.1 at 10Hz) and were modulated by quinpirole whereas GP-GP synapses were governed by short-term depression (ratio 0.8 at 10Hz) and were insensitive to the D2 receptor agonist. Additionally, minimal stimulation also revealed facilitating and depressing responses corresponding to striatal dendritic and pallidal somatic synapses, respectively. After chronic dopamine depletion, GP-GP synaptic transmission was boosted. The amplitude of evoked IPSCs at GP-GP synapses were increased without change in short-term plasticity dynamics. Strikingly, synaptic transmission at STR-GP synapses was unaffected at low frequency in parkinsonian animals. These results suggest an imbalance between STR-GP and GP-GP synaptic transmission after dopamine deprivation which might contribute to the aberrant GP neuronal activity observed in Parkinson's disease.
Support: This project is founded by the ANR agency (grant ANR-JCJC 0087). CM has a postdoctoral fellowship from the Basque Government.

P2.047

Deciphering the molecular interaction between sodium channel Nav1.8 and ankyrin G

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In the central nervous system (CNS), the accumulation of voltage gated sodium channel, Nav1 (Nav1.2 and Nav1.6) at the axon initial segment (AIS) results from a direct interaction between the AIS motif of Nav1 and ankyrin G. Importantly, this interaction is regulated by protein caseine kinase 2 (CK2) phosphorylation. Sequence analysis indicates that the Nav1.8 hold a motif close to the conserved AIS motif found in the Nav1 mainly expressed in CNS. Since Nav1.8 was found in the Node of Ranvier of certain types of fibers we addressed the question as to whether Nav1.8 possesses all the information required for clustering at the AIS.
Using surface plasmon resonance, we found that Nav1.8 interacts with the membrane-binding-domain of ankyrin G (Kd= 1nM) and that this interaction is weakly regulated by CK2 phosphorylation. When the AIS motif of Nav1.8 was fused to the potassium channel Kv2.1, the resulting chimeric protein (Kv2.1-Nav1.8) was clustered at the AIS of transfected primary hippocampal neurons. Moreover, over-expression of Kv2.1-Nav1.8 acted as a dominant negative and displaced the endogenous Nav1. Finally, we observed by GST pull down assay that AnkG from rat brain interacts with the AIS motif of Nav1.8.
Altogether, these results indicate that Nav1.8 carries all the information required for clustering at the AIS and that its accumulation is not regulated by protein kinase CK2, unlike CNS Nav1. As several studies have shown that Nav1.8 is ectopically expressed in some neuronal populations of the CNS in multiple sclerosis and in mouse models of multiple sclerosis, the ability of Nav1.8 to interact with ankyrin G may contribute to the pathological aspects of multiple sclerosis.

P2.048

Distinct coincidence detectors govern the corticostriatal spike timing-dependent plasticity

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The corticostriatal pathway is the main input of basal ganglia, an ensemble of sub-cortical interconnected nuclei involved in learning of contextual cognitive and motor sequences related to environmental stimuli. Striatum receives glutamatergic inputs from the whole cerebral cortex and relays the integrated cortical information towards the basal ganglia output nuclei, through which it operates a selected activation of behavioral effectors. As learning and memory is underlying by long-term synaptic efficacy changes, the corticostriatal plasticity provides a basic mechanism for the function of basal ganglia in procedural learning. Here, we focused on the spike timing-dependent plasticity (STDP), a Hebbian synaptic learning rule, considered as the first law of synaptic plasticity. We reported the existence of a robust spike timing-dependent plasticity (STDP) at corticostriatal synapses. Compared to STDP observed in other mammalian brain structures, corticostriatal STDP displayed a reversed orientation: paired postsynaptic followed by presynaptic (post-pre) stimulations induced LTP while pre-post sequences triggered LTD. To better characterize the corticostriatal STDP, we have then explored which receptors and pathways were involved in the induction of both forms of plasticity. Classical models for STDP propose NMDA receptors as the unique coincidence detector. Here, we show that corticostriatal STDP depends on distinct molecular coincidence detectors. Specifically, LTP relies on a single coincidence detector based on the postsynaptic NMDA receptor, while LTD requires distinct coincident detectors: the phospholipase Cb (PLCb), the inositol-triphosphate receptor (IP_3R)-gated calcium stores and the diacylglycerol lipase (DGLa). Furthermore,

we found that PLCb activation is controlled by group-I metabotropic glutamate receptors, type-1 muscarinic receptors and voltage-sensitive calcium channels activities. Activation of PLCb and IP_3Rs leads to robust retrograde endocannabinoid signaling mediated by 2-arachidonoyl-glycerol and cannabinoid CB1 receptors. Therefore, LTP and LTD induced by STDP at corticostriatal synapses are mediated by independent signaling mechanisms, each one being controlled by distinct coincidence detectors.

P2.049

Delayed synaptogenesis by the β-amyloid peptide in the adult brain

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Clinical hallmarks of Alzheimer´s disease (AD) have been related to extracellular deposits of β-amyloid peptide (Aβ) in the cerebral cortex and hippocampus as amyloid plaques. Aβ peptide is the cleavage product by beta and gamma secretase of a bigger transmembrane protein known as the amyloid precursor protein (APP). Although AD was described more than 100 years ago, its pathogenic mechanism remains unclear. In humans and animal models of AD, cognitive decline becomes evident much earlier than amyloid plaque formation. There is also evidence of dendritic spine abnormalities in a variety of mental retardation syndromes.
In this work we propose that Aβ peptide affects synapse formation, and this mechanism is responsible for the early cognitive alterations seen in AD. We used adult neurogenesis to study the effects of Aβ on *de novo* synaptogenesis by retrovirally traducing APP and/or reporter proteins in newborn neurons of the adult mice hippocampus.
In vivo cell-autonomous overexpression of different forms of APP affected the way in which these neurons develop. Electrophysiological recordings to monitor their function and integration showed altered synaptic connectivity. Passive properties evaluation also exhibited less mature phenotype of the neurons expressing amyloidogenic constructions. Surprisingly, confocal analysis of dendritic spine density did not evidenced differences between neurons expressing APP and control.
Further experiments will lead us to a better understanding of these alterations as well as the mechanisms underlying these developmental changes induced by the cell-autonomous expression of APP.

P2.050

Maf proteins in low-threshold mechanoreceptor neuron development

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Low-threshold mechanoreceptor neurons (LTMs) of the dorsal root ganglia (DRG) are essential for touch sensation. They form highly specialized terminations in the skin and display stereotyped projections in the spinal cord. Functionally defined LTMs depend on neurotrophin signaling for their postnatal survival and functioning, but how these neurons arise during development is unknown. We showed recently that specific types of LTMs can be identified shortly after DRG genesis by unique expression of the MafA transcription factor, the Ret receptor and coreceptor GFRalpha2, and find that their specification is Ngn2 dependent. The Ret ligands GDNF and Neurturin specifically maintain MafA+ neurons in explant cultures of embryonic DRG. In mice lacking Ret, these LTMs display early differentiation defects, as revealed by reduced MafA expression, and at later stages their central and

peripheral projections are compromised. Moreover, in MafA mutants, a discrete subset of LTMs display altered expression of neurotrophic factor receptors. To further investigate the role of MafA in LTM development we have introduced a membrane bound GFP cassettte into the MafA locus such that its expression controlled by Cre recombinase. Results from the analysis of these mice will be presented. Overall, our results provide evidence that genetic interactions involving Ret and MafA progressively promote the differentiation and diversification of LTMs.

P2.051

Adult neural stem cells resist to high dose radiation but their niche does not sustain neurogenesis

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Neural stem cells (NSC) reside in the subventricular zone (SVZ) ensuring the continuous production during adulthood of new neurons which integrate in the olfactory bulbs (OB). Exposure to ionizing radiation, however, has been reported to provoke long term impairment of neurogenesis in the SVZ. We have shown recently that neurogenesis is almost ablated by preferential irradiation of SVZ, profoundly reducing new neurons in the OB and having consequences on olfactory memory (Lazarini and coll. PLoS One 2009).
Here, we examined the mechanisms of neurogenesis reduction after irradiation of the adult mouse brain and depicted the SVZ niche. Proliferation and neuroblast production in the SVZ, and their integration in the OB, were deeply decreased for several months after whole brain exposure to 15 Gy (γ-rays). Surprisingly, we showed that most of NSC resisted to such a high irradiation dose and remained quiescent for long term in the SVZ. In addition, NSC from irradiated mice were viable in culture generating neurospheres with characteristics similar to NSC obtained from non-irradiated mice (i.e., long term proliferation and multilineage differentiation). The SVZ niche is thought to play a major role in the regulation of neurogenesis. When NSC from normal GFP mice were enriched by flow cytometry then grafted into SVZ from non-irradiated mice, they recreated a neurogenic niche producing neuroblasts that migrated toward OB. By contrast, NSC grafted into the SVZ of irradiated brain, did neither proliferate nor sustain neuroblast production and GFP-cells integrated in OB presented an abnormal GFAP expression. Beside, we showed that cytokines of the TGFβ pathway were persistently increased in irradiated SVZ pointed to a functional impairment of the NSC niche. Modifications of the TGFβ signalling and their implication in the inhibition of neurogenesis after brain irradiation will be discussed.

P2.053

The renal v-ATPase a4 subunit is expressed in a subpopulation of human anaplasic oligodendrogliomas and pilocytic astrocytomas

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Gliomas are the most frequent human brain tumors. The WHO histological classification distinguishes astrocytomas (grade I to IV), oligodendrogliomas and oligoastrocytomas (grade II and III). In addition to histological parameters, a precise diagnostic may require the identification of molecular markers. V-ATPases are proton pumps which acidify various intra-cellular organelles. Different isoforms of v-ATPase a-subunit are expressed with specific tissular distribution and subcellular localization. The a4 isoform, encoded by the ATP6V0A4 gene, is only expressed in proton-secreting cells in kidney and epididymis.
By quantitative RT-PCR, we demonstrated that several malignant gliomas have a high expression of a4, which is not expressed in epileptic brain biopsies. Expression of a4 allowed us to characterize a sub-population of grade III oligodendrogliomas (40 % expressing a4). In these oligodendrogliomas, no correlation with the co-deletion of chromosomes arms 1p and 19q, a molecular marker of oligodendroglial tumors, could be demonstrated.
To look for a role played by a4-containing v-ATPases, we used the Hs683 cell line, a human oligodendroglioma cell line expressing a4. By transfection with a4-targeted siRNAs, we decreased a4 expression, and analyzed consequences for drug-resistance, invasiveness, or resistance to acidic extracellular pH. No changes in these different tests were demonstrated.
We recently found a4 expression in 56% benign pilocytic astrocytomas. This expression is associated with fusion of the KIAA1549 and BRAF genes. Since ATP6V0A4 is localized on chromosome 7q33-34 just downstream of KIAA1549, a4 expression could result from a disorganization of regulatory sequences.
In conclusion, we established expression of a4 as a new marker of anaplasic oligodendrogliomas and pilocytic astrocytomas.

P2.054

$VEGF_A$ protects against glutamate-induced excitotoxicity on the deep layer VI of the immature cerebral cortex of mice neonates

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In industrialized countries, cerebral palsy affects 2.5 ‰ of preterm and term infants. At a neurochemical level, the massive release of glutamate constitutes a major process leading to excitotoxicity and brain lesions. Previous studies revealed that, in $^{\delta/\delta}VEGF_A$ transgenic mice, glutamate-induced brain lesions are exacerbated suggesting that $VEGF_A$ could play a protective action against excitotoxicity. Using cultured cortical brain slices, the aim of the present study was to further characterize the intracellular mechanisms associated with VEGF-induced protection against excitotoxicity in neonates. Glutamate induced a strong increase of necrotic cell death in the deep cortical layer VI and a decrease of apoptotic death in superficial layers II-IV. When administered alone, $VEGF_A$ had no effect on both apoptotic and necrotic deaths. In contrast, $VEGF_A$ abolished the glutamate-induced necrosis observed in layer VI. While MEK and PI3-K inhibitors had no effect on the protective action of $VEGF_A$, L-NAME, a pan inhibitor of NOS, abrogated the effect of $VEGF_A$ and exacerbated the excitotoxic action of glutamate. Neuroprotective effect of $VEGF_A$ was also blocked by LNIO and NPLA, two inhibitors of constitutive NOS, while AGH, an iNOS inhibitor, had no effect. Nitrites measurements, electron paramagnetic resonance spectroscopy and immunohistochemistry indicated that glutamate was a potent inducer of NO production *via* activation of nNOS in the cortical layer VI. Glutamate also modified the level of nNOS Ser1412 phosphorylation and the protein-protein interactions between nNOS and NR2B. *In vivo* injections of nNOS siRNA promoted excitotoxicity and mimicked the effects of LNIO and NPLA. Calcimetry experiments performed on brain slices revealed that $VEGF_A$ reduced the massive calcium influx induced by glutamate in layer VI and this effect was blocked by L-NAME. These findings demonstrate that, in deep cortical layers of mice neonates, *i)* glutamate increase nNOS activity, *ii)* contrasting with mature brain, glutamate-induced NO production

is neuroprotective and *iii)* $VEGF_A$ exerts an anti-necrotic effect *via* a NO-dependent mechanism. *This work was supported by grants from the University of Rouen, Inserm, ANR, the Region Haute-Normandie, the French Research Ministry, ELA and FEDER.*

P2.055

An extended binding pocket allows the design of a potent and selective orthosteric agonist of subtype-4 metabotropic glutamate receptor with anti-Parkinsonian properties

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Over the past few years, metabotropic glutamate receptors (mGluRs) have appeared as promising target to restore the abnomal neurotransmission within the basal ganglia that is observed in Parkinson disease, with a special interest on the subtype 4 of mGluRs. Indeed, mGlu4 modulates both glutamatergic and GABAergic neurotransmission in basal ganglia and decreases the parkinsonian symptoms in animal model of Parkinson disease. However, the development of subtype selective orthosteric drugs has proven to be difficult for these receptors. In the present study, we present an alternative way for providing subtype selective orthosteric ligand for mGluRs. Taking advantage of the existence of a second binding pocket located nearby the orthosteric binding pocket of mGlu4, we design the first potent and selective orthosteric agonist of mGlu4. The present article describes the pharmacological profile of LSP4-2022 on heterologous systems using two different functional assays. Then, combining site-directed mutagenesis and molecular modelling, the molecular determinant of binding and selectivity which delineate the binding pocket of the compound are depicted and demonstrate that this new ligand is binding across the two binding pockets. We demonstrate that LSP4-2022 inhibits neurotransmission in cerebellar slices and produces an anticataleptic effect in an animal model of Parkinson disease. The discovery of a second binding pocket in mGluRs and consequently the development of *in vivo* active, selective orthosteric ligands exploiting this exosite opens interesting perspective to develop selective orthosteric among mGluR subtypes.

P2.056

The puzzling problem of glutamate transport for DA neurons: when too much glutamate uptake becomes also toxic

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By transporting both glutamate and cysteine, the neuronal excitatory amino acid transporter EAAT3/EAAC1/SLC1A1 regulates glutamatergic transmission and supplies neurons with precursors of the main brain antioxidant, glutathione. This last function may be crucial for dopamine (DA) neurons that degenerate in Parkinson's disease. Indeed, we had previously found that EAAT dysfunction induced by L-trans-2,4-pyrrolidine dicarboxylate (PDC), a substrate inhibitor of EAATs, is preferentially toxic for these neurons, as the subsequent decline in antioxidant defenses increased their vulnerability

to NMDA receptor-mediated excitotoxicity. Here, we further investigate the interactions between PDC and glutamate-induced toxicity in immature (4 day old) and mature (8 day old) mesencephalic cultures. Glutamate (500 µM) was not toxic for immature DA neurons, indicating that they do not rely on the cystine/glutamate exchanger, system xc-, for their antioxidant defenses, even in immature stages. On the contrary, glutamate was toxic for mature DA neurons and for both immature and mature non DA neurons. In immature non DA neurons, glutamate-induced cell loss was independent of excitotoxicity and of oxidative glutamate toxicity, as not being protected by glutamate antagonists or antioxidants. It was prevented by inhibiting glutamate transport through either EAATs (by PDC or DL-threo-β-benzyloxyaspartic acid) or system xc- (by 4-carboxyphenylglycine), but not by the combination of both treatments. In mature mesencephalic cultures, glutamate excess was primarily excitotoxic. The protective effect of glutamate transport inhibitors on glutamate toxicity was no longer observed in mature non DA neurons, but surprisingly appeared in mature DA neurons. These data suggest that a moderate glutamate transport is required for the viability of immature non DA and mature DA neurons in mesencephalic cultures, and that both excessive glutamate transport and complete blockade are toxic for these cells. This may be linked to oxidative deamination of glutamate into alpha-ketoglutarate, a TCA cycle intermediate, by glutamate dehydrogenase as both inhibition and excessive activation of this enzyme have been found to be toxic for DA neurons.

P2.057

An orphan class C GPCR associated protein complex

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In the field of the G protein-coupled receptors (GPCRs), a special interest has focused on orphan receptors (about 90) these last years, in order to discover their ligand, roles and activity. However, a lot of them are still difficult to de-orphanize and study. Indeed, it happened that recently, studying the function of some of the orphan receptors showed they have likely no ligand but associate to other proteins to contribute or regulate their function.
In parallel, it has been clearly demonstrated these last years, that GPCR are part of large protein complexes that control their function and activity. Thus, the identification of the receptor protein partners, their interaction dynamics and the consequence of their association are today a challenge for the GPCR field community, because the formation of complexes changes the molecular and cellular properties, the physiology and then the possible therapeutic targeting of the receptors.
GPR158 is a class C orphan receptor, the ligand and coupling of which are still unknown, and as such, it has to be studied from another point of view. Indeed, its large C-terminal domain (548 residues long) led us to the hypothesis that it could be a platform for protein complexes. Thus, identifying the proteins able to interact with its C-terminal domain could give information about the role of the orphan receptor. Actually, there are motives that could be involved in protein interaction, and could interfere with the G protein activity. This could indicate that either the receptor can couple and regulate its own coupling to G proteins, or regulate the G protein coupling of receptors in the microenvironment. Moreover, we observed a strong interaction with an RGS protein, which is also a key player in the regulation of the G protein pathways. In the present work, we describe molecular determinants of the interaction with these receptor partners, and explore some functional consequences.

P2.058

Morphology of mouse hippocampal excitatory terminals lacking the Vesicular Glutamate Transporter VGLUT1

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Excitatory synaptic vesicles (SVs) are equipped with Vesicular Glutamate Transporters (VGLUTs) responsible for filling them with neurotransmitter. While the essential function of VGLUTs as neurotransmitter carriers has been well established, the evidence for additional cell-biological functions of VGLUTs is more controversial.
The three VGLUT isoforms expressed in the rodent brain show approximately 70 % sequence identity and were found to possess virtually identical bioenergetics and pharmacology of neurotransmitter loading. However, VGLUT1-3 display differential expression patterns throughout development and at adult stages and therefore define three glutamatergic systems, that may display differences in synaptic physiology and pathology. Together, VGLUT1 and VGLUT2 mark all classical glutamatergic neurons. In addition, VGLUT1 and -2 were recently shown to bind differentially with synaptic partners like Endophilin A1.
In this context, we used an array of electron microscopy, and biochemical approaches to reinvestigate the morphological defects observed in VGLUT1 knock out mouse hippocampal neurons.

P2.059

Glioblastoma vascularization: cause or consequence of tumor development?

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Glioblastoma multiforme (GBM) is characterized by very poor prognosis. Due to their outstanding vascular density, angiogenesis is often seen as a privileged therapeutic target against these tumors, but clinical trials show moderate success. Better understanding of the interactions that tumor cells establish with blood vessels is therefore needed, for example to clarify whether angiogenesis is responsible for accelerated tumor development or whether angiogenesis is simply a consequence of tumor cell proliferation.
Answering this question requires a dynamic study with repeated observations of the same tumor over several weeks. Whereas techniques were so far lacking, we have recently developed a protocol based on two-photon microscopy to visualize the *in vivo* progression of GBM tumors grafted orthotopically into the brain of nude mice. Grafted GBM U87 cells were detectable due to their expression of GFP, while injection of fluorescent dextrans of variable molecular weights revealed both the morphology and the permeability of functional blood vessels at different distances from the tumor epicenter. We observed that tumor growth induced a major centripetal remodeling of brain blood vessels into irregular and tortuous branches. These tumor blood vessels showed a clearly compromised Blood Brain Barrier, as injected fluorescent dextrans leaked into the brain parenchyma with an amplitude inversely proportional to their molecular weight.
Quantification of the dynamics of tumor growth and the dynamics of tumor vascularization over several weeks indicated that regions of highest tumor cell proliferation didn´t match with regions of highest vascular density. No correlation was indeed found between tumor proliferation and vascular density or plasticity. The relative independence of tumor development with regard to blood supply was further confirmed *in vivo* by the modest and transient effect of complete angiogenesis blockade on tumor growth. These data were confirmed histologically by the presence of strong BrdU immunostaining

irrespective of the status of tumor vascularization. Altogether our data suggest that angiogenesis is not the major player of GBM tumor development.

P2.060

A mesenchymal-like Zeb1^{+} niche harbors dorsal radial GFAP^{+} stem cells in the spinal cord

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In humans and rodents the adult spinal cord harbors neural stem cells located around the central canal. Their identity, precise location, and specific signaling are still ill-defined and controversial. We report here on a detailed analysis of this niche. Using microdissection and glial fibrillary acidic protein (GFAP)-green fluorescent protein (GFP) transgenic mice, we demonstrate that neural stem cells are mostly dorsally located GFAP(+) cells lying ependymally and subependymally that extend radial processes toward the pial surface. The niche also harbors doublecortin protein (Dcx)(+) Nkx6.1(+) neurons sending processes into the lumen. Cervical and lumbar spinal cord neural stem cells maintain expression of specific rostro-caudal Hox gene combinations and the niche shows high levels of signaling proteins (CD15, Jagged1, Hes1, differential screening-selected gene aberrative in neuroblastoma [DAN]). More surprisingly, the niche displays mesenchymal traits such as expression of epithelial-mesenchymal-transition zinc finger E-box-binding protein 1 (ZEB1) transcription factor and smooth muscle actin. We found ZEB1 to be essential for neural stem cell survival in vitro. Proliferation within the niche progressively ceases around 13 weeks when the spinal cord reaches its final size, suggesting an active role in postnatal development. In addition to hippocampus and subventricular zone niches, adult spinal cord constitutes a third central nervous system stem cell niche with specific signaling, cellular, and structural characteristics that could possibly be manipulated to alleviate spinal cord traumatic and degenerative diseases.

P2.061

A potent vasoactive peptide and its paralog regulate astrocyte activity through common and distinct mechanisms. Two biaised ligands for different functions?

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Urotensin II (UII) and urotensin II-related peptide (URP) are the endogenous ligands of a G protein-coupled receptor named UT. The central actions of these two vasoactive neuropeptides are currently unknown. Here, we have compared the mechanism of action of UII and URP on cultured astrocytes. Competition experiments showed the presence of very high- and high-affinity binding sites for UII, and a single high-affinity site for URP. UII and URP provoked a membrane depolarization accompanied by

a decrease of input resistance and generated an increase in cytosolic calcium concentration ($[Ca^{2+}]_c$) involving the PLC/IP_3 pathway that was pertussis toxin (PTX)-insensitive. The addition of EGTA reduced the peak and abolished the plateau phase whereas the T-type calcium channel blocker mibefradil totally inhibited the calcium response evoked by both peptides. However, URP and UII induced a mono- and biphasic dose-dependent increase in $[Ca^{2+}]_c$ and provoked short- and long-lasting Ca^{2+} mobilization, respectively. Similar mono- and biphasic dose-dependent increase in [^{3}H]inositol incorporation into polyphosphoinositides in astrocytes was obtained but sole the effect of UII was significantly reduced by PTX, although BRET experiments revealed that both peptides recruited G_o protein. Finally, UII induced a dose-dependent mitogenic activity on astrocytes, but not URP. Therefore, we described that UII and URP exert not only similar but also divergent actions on reactive astrocyte activity[a]. In tumorogenic high-grade astrocytomas, expressing mRNA encoding UT, URP and UII, we observed that UII and URP favor cell-cell adhesion at subnanomolar concentrations, whereas UII exhibits specific pro-migratory chemotaxic behaviors, at very low concentrations. Altogether, our data demonstrated that *i)* the vasoactive peptides UII and URP act through distinct mechanisms on astrocytes and *ii)* UII may be involved in an autocrine/paracrine invasive function, a process particularly deleterious in gliomagenesis (*see poster of Lecointre C*).

[a] Jarry *et al*. (2010) The vasoactive peptides Urotensin II and Urotensin II-related peptide regulate astrocyte activity through common and distinct mechanisms. Involvement in cell proliferation. *Biochem. J.,* **428**, 113-124.

P2.062

Function of Rho GTPase signaling during postnatal Purkinje cell differentiation

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By their capacity to control actin cytoskeleton dynamics, Rho GTPases and their regulators, GEFs and GAPs, play a key role in the embryonic development of the nervous system. In contrast, the role of Rho signaling in postnatal cerebral development is not well understood. Here we investigate the importance of Rho signaling during mouse cerebellar development, focusing on postnatal Purkinje cell (PC) differentiation. Purkinje neurons occupy a central and integrative position in the synaptic network of the cerebellum and have the most elaborate dendritic tree among CNS neurons, which develops remarkably in the first two postnatal weeks. Since Rho GTPases play an essential role in actin cytoskeleton dynamics, it is very likely that they are implicated in PC development.
In order to identify actors of Rho signaling that are implicated in this process, we compared gene expression profiles of all RhoGTPases and a number of RhoGEFs at various stages of PC postnatal differentiation (P3, P7, P15 and P20) using real-time quantitative PCR. As PCs represent only 3% of the whole cerebellum, we purify them by FACS-sorting taking advantage of a mouse strain that harbors GFP-expressing Purkinje neurons (Pcp2-GFP). Among the identified interesting genes, we are now focusing on one GTPase and one GEF whose expression is modulated dramatically during cerebellar development and which present distinct localizations in PCs. Preliminary data, using lentiviral vectors to knock down their expression in organotypic cultures, reveal strong dendritic spine defects, suggesting a role in PC differentiation.

P2.063

Effect of tPA on blood-brain barrier integrity during ischemic stroke: study on alpha 1 and alpha 2 chains of type IV collagen

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Ischemic stroke results from the occlusion of a brain vessel. Accordingly, the only approved acute treatment for ischemic stroke is thrombolysis through the injection of tissue type plasminogen activator (tPA). Unfortunately, the benefit from thrombolysis (in particular late thrombolysis) may be reduced by side effects of tPA on components of the neurovascular unit.
We first performed a microarray analysis in order to identify potential candidates responsible for the noxious sensitivity of the neurovascular unit to late thrombolysis. Among the genes with altered expression in a mouse model of thrombotic stroke (Orset ***et al***., 2007), we focused on type IV collagen and more specifically the alpha 1 and alpha 2 chains (COL4A1 and COL4A2). COL4A1 expression increases during ischemia whereas COL4A2 expression increases when late thrombolysis is performed with tPA. This observation might be particularly relevant, since mutations in COL4A1 gene induce cerebrovascular and small vessel diseases (Lanfranconi and Markus, 2010).
We next confirmed our microarray analyses by performing real time PCR. Moreover, we found an increase in COL4A1 and COL4A2 expression in a model of focal permanent ischemia by middle cerebral artery electrocoagulation, but not in a model without cerebral infarction, induced by a chronic hypoperfusion through carotid ligature.
In murine astrocyte cultures, we observed that tPA induces a decrease in COL4A1 and COL4A2 expression. In human endothelial cell cultures, tPA induces no changes. Type IV collagen expression under tPA treatment remains to be studied in other cell types to explain what happens during ischemic stroke. We also now intend to determine the effect of late thrombolysis on the metabolism of type IV collagen, as well as its impact on the integrity of the neurovascular unit.
Lanfranconi, S. & Markus, H.S. COL4A1 mutations as a monogenic cause of cerebral small vessel disease: a systematic review. Stroke 41, e513-8(2010).
Orset, C., Macrez, R., Young, A.R., Panthou, D., Angles-Cano, E., Maubert, E. et al. Mouse model of in situ thromboembolic stroke and reperfusion. Stroke 38, 2771-8(2007).

P2.064

A quantitative phosphoproteomic approach reveals differential phosphorylation of serotonin 2A receptors upon activation by hallucinogenic *versus* non-hallucinogenic agonists

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The serotonin (5-hydroxytryptamine, 5-HT)$_{2A}$ receptor is a primary target of psychedelic hallucinogens such as lysergic acid diethylamide (LSD), which reproduce some of the core symptoms of schizophrenia. An incompletely resolved paradox is that only some 5-HT$_{2A}$ receptor agonists exhibit hallucinogenic activity, whereas structurally related agonists with comparable affinity and activity do not. Using a quantitative phosphoproteomic approach combining stable isotope labelling by amino acids in cell culture (SILAC), phosphopeptide enrichment by hydrophilic interaction chromatography (HILIC)/immobilized metal affinity chromatography (IMAC) and high resolution mass spectrometry, we compared the phosphoproteome in HEK-293 cells transiently expressing the 5-HT$_{2A}$ receptor under three conditions: no-stimulation; exposure to the phenethylamine hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) and exposure to the non-hallucinogenic 5-HT$_{2A}$ agonist lisuride. Among the 5,996 identified phosphopeptides (FDR < 1%), 454 sites were differentially phosphorylated upon exposure to DOI *vs.* lisuride (ANOVA p value < 0.05). These include a serine residue phosphorylated upon exposure to DOI but not to lisuride and located in the i3 loop of the 5-HT$_{2A}$ receptor, a region important for receptor desensitization. Mass spectrometry analysis of immunopurified receptor further confirmed differential phosphorylation of this residue upon exposure to hallucinogenic (DOI and LSD) *vs.* non-hallucinogenic (lisuride and ergotamine) agonists. Correspondingly, exposure to hallucinogens induced a less pronounced desensitization of receptor-

transduced Ca^{2+}increases and Erk1,2 phosphorylation than exposure to non-hallucinogenic agonists. Moreover, mutation of the serine into aspartate (to mimic phosphorylation) reduced the receptor desensitization induced by non-hallucinogenic agonists. In conclusion, this phosphoproteomic analysis reveals that 5-HT_{2A} receptor stimulation by hallucinogenic *vs.* non hallucinogenic agonists induces contrasting phosphorylation patterns that may be related to their distinct behavioural responses. It also provides one of the first demonstrations of differential phosphorylation of a G protein-coupled receptor upon stimulation by "biased" agonists.

P2.065

Modulation of perisynaptic NMDA receptor activation by intra-cleft electric fields

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When a glutamatergic synapse becomes active, current flowing through open AMPA receptors induces intra-cleft electric fields that are strong enough to significantly accelerate or slow down glutamate diffusion depending on trans-membrane potential. To determine whether electric field-induced changes in glutamate diffusion may in turn affect receptor response, we analyzed glutamatergic transmission in NTS neurons using whole-cell recording.
We found that the NMDA component of miniature synaptic currents recorded in NTS neurons in the absence of magnesium ions was significantly increased by shifting holding potential to positive values. This effect of voltage could not be accounted for by residual magnesium block since responses induced by bath application of NMDA had linear current-voltage relationship. In addition, experiments involving AMPA receptor blockade by CNQX or short voltage step applications showed that currents passing through AMPA receptors were required. Analyzing the effects of synaptic electric fields by computer simulation revealed that NMDA receptor facilitation by depolarization was dependent on receptor location being stronger for perisynaptic than for synaptic receptors. Furthermore, NMDA receptor facilitation required a precise timing between depolarization and glutamate release suggesting a possible involvement in forms of synaptic plasticity that depend on spike timing.

P2.066

Effect of nicotine and alkaloids of tobacco on striatal dopamine terminals fonction: a study by intracerebral microdialysis in freely-moving rats

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Nicotine, the main alkaloid fund in tobacco plant, has been considered for a long time responsible for the development of addiction in mammals. Some studies have suggested that other molecules contained in the plant potentiate the behavioral effects of nicotine. Here, we hypothesized that these molecules could amplify the effect of nicotine on the activity of central dopamine (DA) neurons.
In this study, we have studied the effect of an extract of tobacco plant or nicotine on the efflux of DA and its metabolite DOPAC monitored in vivo by intracerebral microdialysis in the striatum of freely-moving Sprague-dawley rats. We used an extract containing, from a chemical separation procedure, the alkaloids including nicotine. Five to seven days after the implantation of a guide-cannula, a microdialysis probe (CMA 11, 4 mm length, 250 µm) was inserted into the striatum and perfused at a constant flow rate (0.5 µl/min) with an artificial cerebrospinal fluid. Samples, collected every 20

minutes, were analyzed for their DA and DOPAC content by high pressure liquid chromatography coupled to electrochemical detection.
The intraperitoneal (ip) injection of nicotine (0.5 mg/kg) slightly enhanced striatal DA extracellular levels (+20% above baseline levels) and merely affected DOPAC levels. The administration of the extract (0.5 mg/kg, ip) evoked a significant increase in DA extracellular levels. The effect picked 40 minutes after its injection to reach +65% above baseline values. It decreased slowly to reach values closed to those of the nicotine group. DOPAC extracellular levels were enhanced (+40%) till the end of the monitoring period. The effect of the extract was significantly higher compared to that of nicotine and saline injection for both DA and DOPAC.
In conclusion, we provide neurochemical evidence that an extract containing nicotine plus the other alkaloids is more efficient than a high dose of nicotine to increase DA transmission in the brain. A similar effect in the nucleus accumbens could mean a possible facilitation of the alkaloids on the motivational aspects of addictive behavior elicited by tobacco.
Hanane Kalkhi received funds from the GDRI and the Neuromed for the completion of this study.
Keywords: Tobacco extract, nicotine, microdialysis, dopamine.

P2.067

Delineation of $GABA_B$ tetramerization interface and functional consequence of oligomer destabilization

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The main inhibitory neurotransmitter of the adult brain, γ-aminobutyrate (GABA), acts on the ionotropic $GABA_A$ receptor and on the metabotropic $GABA_B$ receptor. $GABA_B$ receptor belongs to class C G-protein coupled receptors (GPCR), which also includes the glutamate, calcium sensing or sweet and umami taste receptors. Like most class C GPCRs, the $GABA_B$ receptor is dimeric, but has the originality to be an obligatory heterodimer: it is constituted of the $GABA_{B1}$ subunit that bears the agonists binding site, and the $GABA_{B2}$ subunit responsible for G-protein activation. In a recent study, we showed that, unlike metabotropic glutamate receptors, the $GABA_B$ receptor could form tetramers, ie dimers of heterodimers, with $GABA_{B1}$ as the central element of the oligo-heterodimers formation. Here, we propose to assess what is the interaction interface between two $GABA_B$ heterodimers. To that aim, we used chimeras to map which part of $GABA_{B1}$ is critical for the oligomer formation. Our results indicate that the extracellular domain of $GABA_{B1}$ is critical for the formation of higher order oligomers. To define more precisely the area involved in this interaction, we introduced next N-glycosylation sites in the protein and we monitored both the FRET signal between two $GABA_{B1}$ and the function of the mutant receptors. The data suggest that the mutation of a small area at the tip of the extracellular domain destabilizes the oligomers.

P2.068

Fibronectin type III-like domains of Neurofascin-186 are implicated in Gliomedin clustering at heminodes during myelination

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The distribution of ion channels within specific subcellular domains along myelinated axons ensures proper nerve conduction and is altered in multiple sclerosis and demyelinating peripheral neuropathies. At the node of Ranvier of myelinating fibers, the voltage-gated sodium channels are concentrated at the nodal gap and separated from the juxtaparanodal potassium channels by the paranodal axo-glial junctions. The immunoglobulin superfamily cell adhesion molecules (Ig-CAMs) play a crucial role in the organization of axonal subdomains. Indeed, the expression of NrCAM and Neurofascin-186 (NF186) precedes and mediates the voltage-gated sodium channels recruitment at the node. In the peripheral nervous system, Gliomedin (Gldn) expressed by Schwann cell microvilli induces the nodal clustering of NrCAM and NF186. The olfactomedin domain of Gldn has been implicated in interaction with nodal Ig-CAMs. However, the interacting modules of NrCAM or NF186 involved in Gldn association were unknown.
Here, we report that fibronectin type III-like (FnIII) domains present in both NrCAM or NF186 mediate their interaction with Gldn in pull-down and cell binding assays. Focusing on NF186, we characterized the FnIII domains 1 and 2 as the minimal interacting module to ensure association with Gldn. Interestingly, we observed that soluble FnIII domains of NF186 (FnIII-Fc) inhibited the Gldn clustering at heminodes, which are considered as precursors of mature nodes in myelinating cultures. In surface plasmon resonance assays, we determined a similar affinity of NrCAM and NF186 for Gldn (Kd ~ nM). Our study reveals the importance of FnIII domains of Ig-CAMs in the organization of nodes of Ranvier. Our project is to generate transgenic mice expressing NF186 deleted of the Ig domains and to analyze whether the FnIII domains would be sufficient to rescue the phenotype of NF186-null mice.

P2.069

Correlative microscopy: application for the study of chronic pain mechanism

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Correlative microscopy permits the detection of the same target by two microscopy approaches. The most common is the association of electron microscopy with light confocal detection, that allows combining high resolutive ultrastructure with a large population of fluorescently labelled neurons. We are currently developing correlative microscopy to investigate spinal mechanisms of disinhibition in pain conditions. We previously showed that GABAB inhibition is impaired in the dorsal spinal cord of a rat neuropathic pain model. One mechanism that may account for such disinhibition process relies on the redistribution of GABAB receptors in neuropathic pain conditions. We will apply correlative confocal electron microscopy to detail changes in GABAB receptor subcellular distribution in spinal cord neurons under chronic pain conditions.
For this purpose, we used primary spinal neurons isolated from 13.5 days embryos of a knock-in mouse model expressing GABAB1 subunit fused with GFP (B1-GFP). The method that we are currently exploring relies on the use of a commercially available bifunctional probe, the FluoroNanogold secondary antibody. It enables the detection of GFP with fluorescence and electron microscope by a singular secondary antibody linked to Alexa488 fluorochrome and 1.4nm nanogold particle.
Conditions of fixation have been set up before microscopy procedures. Then, B1-GFP-expressing neurons are monitored with confocal microscopy. In a second step, these neurons of interest are visualized and precisely localized on alphabet indexed glass coverslips with differential interference contrast. Ultra-small gold particles are silver-enhanced, and neurons are embedded in resin. Neurons that have been monitored with confocal microscopy are finally identified, and ultrathin sections are cut for observation.
Our study defined the experimental protocol that is best suitable for correlative microscopy. This approach allows documenting the GABAB receptor distribution relatively to synaptic contacts. In particular, the synaptic, or extrasynaptic localization of the receptor is detailed with electron

microscopy. It will be further applied to studying changes in the distribution of GABAB upon chronic stimulation of nociceptive pathways.

P2.070

Do polyunsaturated fatty acids induce epigenetic changes in rat neural stem cells?

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We isolated and grew neural stem cells/neural progenitors (NSC) from 1-day old rat pups issued from mothers fed either an n-3 polyunsaturated fatty acid (PUFA)-deficient,-balanced or -supplemented diet. We compared the ability of the cells to proliferate and differentiate in the absence or presence of PUFA.
The cells isolated from n-3 deficient pups proliferated consistently more slowly than cells isolated from n-3 balanced and n-3 supplemented pups. The differences in proliferation rates were noted up until 40 days of culture, and were highly significant.
The three cultures differentiated in a similar fashion when the growth factors were removed from the culture media, and the proportions of neurons, astrocytes and oligodendrocytes were unchanged. Yet, when the cells were able to differentiate under PUFA supplementation the deficient cells exhibited a higher degree of neuron maturation, as demonstrated by the increase in neurite lengths. The neurite extensions of neurons derived from supplemented pups were not modified in the presence of PUFAs.
We therefore examined the expression of 96 genes involved in cell growth and differentiation by means of TaqMan low density arrays.
17 genes were differentially expressed in proliferating cells issued from deficient or supplemented pups: EGF, EGFR, and PPAR alpha were more expressed in supplemented cells, while Cyclin D1, Neurog1 and Ngf were less expressed as compared to deficient cells.
Differences in gene expressions were also recorded in differentiating cells: Pax6, TLX and PPAR α were more expressed in supplemented cells.
The protein contents were also determined for some of the modified genes.
Because of the consistency and the stability of the differences between the cultures, we checked the level of total DNA methylation.
Therefore, omega 3 PUFA levels in the maternal diet can markedly modify NSC transcriptome and characteristics on a long-term basis.

P2.071

The vasoactive peptide Urotensin II induces chemotaxic migration or cell-cell adhesion during glioma development

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Urotensin II (UII) is considered as the most potent vasoactive peptide. UII and its receptor UT are highly expressed in cardiac and vascular tissues, and UII has been found to exert a potent

vasoconstrictor effect in various species. Although the genes encoding UII and its receptor UT are expressed in central nervous system, little is known regarding the function of the urotensinergic system in the brain. It has been shown that UII promotes neoangiogenesis from brain microvessels and acts as a chemoattractant, stimulating migration of human monocytes and endothelial progenitor cells. We have demonstrated the presence of functional UT in cortical glial cells as well as the expression of UII and UT in human glioblastoma cell lines. The aim of the present study was to identify the UT-associated pathways involved in the UII-mediating effects in glioma development. By Western blot, cytometry and immunohistochemistry, we showed that UT is expressed in glioma cell lines and fresh glioma explants. In an astrocytoma cell line, UII, inactive on proliferation and cell cycle, exhibited chemoattraction and dose-dependently stimulated cell migration. To investigate the effect of homogenous UII concentrations on collective cell migration, we used the cloning ring assay. We demonstrated by video-microscopy and cell tracking that, UII strongly inhibited cell motility of glioma cell lines and UT-expressing HEK293 cells, likely reinforcing cell-cell adhesion. This phenomenon is abolished in HEK expressing a truncated UT receptor lacking the 332-370 UT C-terminal domain. Together, these observations suggest a differential UII-evoked mechanism, *i.e.,* a gradient-induced directional cell migration (low concentration) and an homogenous UII high concentration responsible for cell-cell adhesion. To identify the mechanism of action of UT-associated different couplings on glioma migration, we investigated UT protein partners interacting with the 332-370 UT domain by the yeast two-hybrid strategy. Until now, several proteins have been clearly characterized, including Filamin A, a protein known to be involved in cell migration and/or adhesion. This work is currently extended through the comprehension of the exact mechanism of UII-mediated effect on migration and cell-cell adhesion.

P2.072

Assessing the importance of the anaplerotic cycle of glucose metabolism in the CNS *in vivo*: a combination of molecular and Magnetic Resonance Spectroscopy approaches

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It is now well accepted that the energy metabolism of neurons is more oxidative while astrocytes are more glycolytic. Hence, the enzymes involved in energy production are differentially expressed and regulated in these cells types. Among these enzymes, glutamine synthetase and pyruvate carboxylase are almost exclusively expressed in astrocytes. This specific gene expression location can be used as a tool to study the interaction of astrocytes and neurons in relation to brain energy metabolism. Very little is known about the function of the anaplerotic cycle in the astrocyte/neuron metabolic cooperation. We used a molecular approach to inhibit key enzymes involved in energy production and measured its effects with magnetic resonance spectroscopy (MRS) and autoradiography.
RNA interference (RNAi) technique was used to reduce pyruvate carboxylase expression. RNAi targeting the enzyme sequence was delivered in the cortex of rat by using a liposome transfection approach allowing efficient knock-down *in-vivo*. We measured by MRS the effects of the down regulation of the pyruvate carboxylase enzyme in the cortex. The spectra were acquired on a 9.4T using the SPECIAL sequence. We found variation of metabolites, in the hemisphere injected with the PC siRNA in comparison with the hemisphere injected with the non-interfering siRNA. The main changes measured were an increase in aspartate and lactate concentrations in the cortex, compared to the controlateral side.
In parallel to this NMR approach, we evaluated the knock down of this enzyme by mapping changes in glucose consumption by quantitative autoradiography. Interestingly we found an increase in glucose consumption in the cortex having less pyruvate carboxylase enzyme.
Using this combined approach to decrease expression of metabolic enzymes *in-vivo* in a cell-specific target and in combination with MRS analysis of energy metabolism we are now extending this approach to other enzymes involved in neuron-glia metabolic coupling.

P2.073

Molecular mechanisms regulating the membrane trafficking of KCNQ2/3 (Kv7.2/7.3) channels

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In neurons, KCNQ2 and KCNQ3 (Kv7.2/3) subunits are found at axon initial segments and nodes of Ranvier and play a crucial role in regulating neuron excitability (Devaux et al.,2004; Devaux 2010). Human mutations in *Kcnq2* and *Kcnq3* genes lead to benign neonatal familial convulsions (BNFC) and may affect the trafficking of these channels. Dictionnaire - Afficher le dictionnaireKCNQ2/3 subunits ÉcouterLire phonétiquemen possess a long cytoplasmic C-terminal tail involved in gating, assembly and trafficking (Haitin and Attali.,2008). However, the precise mechanisms regulating KCNQ channel expression are unclear. To decipher how KCNQ2/3 subunits are trafficked to the cell surface, we generated CD4 fusion proteins incorporating the C-terminal tails of KCNQ2 (CD4-Q2) or KCNQ3 (CD4-Q3).
We found that CD4-Q2 was retained in intracellular compartments, likely the endoplasmic reticulum (ER). By contrast, CD4-Q3 was readily addressed to the cell surface. We identified two motifs in the C-terminus of KCNQ2 that are responsible for the ER retention of CD4-Q2. Sequence alignment indicated that one motif is conserved in both KCNQ2 and KCNQ3. The second motif is specific to KCNQ2, and plays a complementary function. Indeed, both motifs need to be deleted to abrogate ER retention. In addition, our results indicated that ER retention of CD4-Q2 is dependant on the coat protein complex I (COPI). Cotransfection of CD4-Q2 with a dominant negative mutant of ARF1 (a protein involved in Golgi-ER transport) induced the relocation of CD4-Q2 in the Golgi. These findings indicate that KCNQ2 subunits possess two ER retention motifs in its C-terminus among which one is affected by human mutations. The retention of KCNQ2 subunits appear to be mediated by the retrograde COPI route from Golgi to ER. These motifs may be important for the trafficking of heterotetrameric channels to the cell surface and to axon initial segments.

P2.074

Eph/Ephrin forward signaling controls axon fasciculation by modulating microtubule dynamics

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Within the developing neuromuscular system motor and sensory axons form large bundles that travel along stereotypical routes to reach their target. Both pathfinding and fasciculation processes are finely regulated by a number of interaxonal adhesion proteins and by guidance molecules expressed in surrounding tissues. Eph receptors and their ligands the ephrins have been shown to participate in both axon guidance and fasciculation, however, the molecular mechanisms by which they do so remain elusive.
Here we used the sensory-motor system innervating the limb bud as a model and showed that genetic deletion of ephrin-B1 leads to defects in fasciculation of both motor and sensory axons. We further demonstrated, using the Cre-lox system, that ephrin-B1 acts non autonomously to regulate axonal fasciculation. Using an in vitro culture assay we found that activation of forward signaling induces growth cone collapse and neurite fasciculation in a Rho kinase-dependent manner. Moreover, we showed that forward signaling regulates the subcellular distribution of microtubule associated proteins including the novel microtubule associated protein ASAP.
Altogether our results suggest that Eph/ephrin signaling regulates axon fasciculation by modulating microtubules dynamics.

P2.075

Are oligodendrocyte precursor cells of the mature somatosensory cortex only progenitors?

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Oligodendrocyte precursor cells (OPCs) constitute the main source of myelinating oligodendrocytes during postnatal development and after demyelinating lesions. To explore if these cells may play other roles in addition to that of progenitors, we compared the intrinsic electrophysiological properties and the response to neuronal activity of OPCs between the second and fourth postnatal week in acute slices of the mouse somatosensory cortex.
Dramatic developmental changes underwent on potassium channel expression in layer V OPCs. The I-V relationship recorded with a KCl-based intracellular solution showed the characteristic outward rectification described for these cells in the second PN week, but a linear shape in the fourth PN week. Pharmacological analysis of the K+ current underlying the linear I-V curve in advanced developmental stages showed that it is partially blocked by 100 µM extracellular Ba2+, but relatively insensitive to TEA and 4AP. This K+ current was present in CsCl-based intracellular solution and partially blocked by either 200 µM quinine or acidification of the cytosol. The lack of effect of TEA and 4AP for voltage-gated K+ channels as well as the inhibition of the current by Ba2+, quinine and acidic pH is characteristic of a TWIK1 channel, a two-pore domain K+ channel. Post-recording immunostainings confirmed the presence of this channel in OPCs.
In the presence of ionotropic and metabotropic receptor antagonists, low-frequency extracellular stimulation consistently evoked a current in all recorded cells of the fourth postnatal week (but not in the second PN week). This current was abolished by the Na+ channel blocker tetrodotoxin (TTX), confirming that it was evoked by neuronal activity. Electrophysiological analysis showed that this current had very long-lasting kinetics, a highly depolarized reversion potential (20 mV) and no current amplitude variability. This slow current is probaly mediated by TWIK1 channel. Indeed, it was also sensitive to Ba2+ and intracellular acidic pH. Our results suggest that OPCs contribute to the K+ buffering through a TWIK1-like channel activity in the mature, but not in the young somatosensory cortex and point toward a new role for these cells in the adulthood.

P2.076

Novel fluorescent tools to characterize the central distribution of the rat V1b receptor involved in stress and anxiety

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Vasopressin (VP), besides its well-known peripheral actions, regulates many cognitive and behavioural functions, including stress and anxiety, *via* a central action. In rodents, the tissue localization of the VP receptors in the central nervous system remains poorly understood because of the lack of selective antibodies and specific radio-markers. Based on the vasopressin-derived peptides

previously synthesized in our group (Pena *et al*, 2007), we have developed new V1b-R selective ligands coupled to fluorophores chosen for their spectral emission and brightness. These probes were designed for detecting low levels of receptors in native tissues and have been previously characterized on human VP and oxytocin (OT) receptors in heterologous systems (Corbani *et al*, under publication).
In this study, we characterized the affinity and functional activation of phospholipase C or adenylate cyclase of the most promising analogues on the rat receptors of the VP/OT family (V1a-R, V1b-R, V2-R and OT-R) using stable cell lines transfected with either receptor subtype, or using membranes extracted from native tissues. Two ligands behaving as full agonists and exhibiting a highly selective V1b-R affinity (1-25nM) were selected and further tested for imaging the V1b receptors on transfected AtT20 cells. Since they provided convincing images in confocal microscopy, demonstrating the presence of V1b-R, these two fluorescent peptides were challenged on native V1b-R in primary cultures of rat pituitary or rat brain slices. Indeed, V1b sites were detected in these tissues including the hippocampus, known to be involved in the regulation of stress and anxiety among numerous other behavioural functions. Experiments are now in progress to determine the expression pattern of the V1b receptor within the rat brain and putative regulation under stressful conditions.
The Averroes program within the framework of the European program Erasmus Mundus supported this work.

P2.077

Characterization of glutamatergic vesicle acidification and refilling dynamics in hippocampal neurons

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The refilling of synaptic vesicles with neurotransmitters is potentially a rate-limiting step in neurotransmission. Biochemical investigations on isolated vesicles indicate that all vesicular transport depends on a proton electrochemical gradient ($\Delta\mu H^+$) generated by the vacuolar-type H^+-ATPase. $\Delta\mu H^+$ consists of two components: the membrane potential ($\Delta\Psi$) and the pH gradient (ΔpH), the ratio of which influences unevenly the vesicular uptake of different neurotransmitters.
In the process of vesicular refilling not only proton ions are of uttermost importance. Indeed, chloride ions allow isolated synaptic vesicles to increase ΔpH thus to acidify by collapsing $\Delta\Psi$, and influence vesicular glutamate transport velocity and final content. Recently, reconstitution of vesicular glutamate transporters in liposomes has led to two opposite models where chloride concentration influences the transport and storage of glutamate inside vesicles either through a chloride conductance in the transporter itself or through an allosteric regulation of the transport (Schenck et al., Nat. Neurosci. 2009; Juge et al., Neuron 2010). These studies strengthen the role, still ambiguous, of chloride in vesicular acidification and filling.
Yet, the dynamics of vesicle recycling in intact neurons are still largely unknown. Here we studied the kinetics of vesicular reacidification in live cultured hippocampal neurons using synaptopHluorin, the pH-sensitive variant of GFP (pHluorin) coupled to the luminal domain of synaptobrevin 2 as well as an antibody against the vesicular GABA transporter labelled with cypher5E, a pH-dependent variant of the cyanine dye Cy5. Utilizing Rose Bengal, an inhibitor of the vesicular glutamate transporters, we have investigated the relationship between vesicular glutamate refilling, the establishment of $\Delta\mu H^+$, and the initial intravesicular chloride concentration. Our results indicate that vesicular acidification is not an independent step preceding transmitter refilling but that in a preserved environment generation of $\Delta\mu H^+$ and neurotransmitter loading in newly endocytosed synaptic vesicles are strongly coupled, i.e. no proton gradient can be built up in the absence of transmitter transport.
MM is the recipient of a Marie Curie Intra-European Fellowship (FP7-IEF).

P2.078

Yif1B, a chaperone protein for the dendritic targeting of the serotonin 5-HT_{1A} receptor: from brain localization to knock-out mice

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The 5-HT_{1A} receptor (5-HT_{1A}R) is a G-Protein-Coupled Receptor whose localization is restricted to somas and dendrites of neurons. We characterized Yif1B, a 5-HT_{1A}R-interacting protein, as a chaperone protein crucial for its targeting to distal dendrites in primary cultures of neurons[1]. Yif1B, yet uncharacterized in mammals, belongs to the Yips family, implicated in *S. cerevisiae* in secretion and in intracellular vesicular traffic. We recently showed by immunolabeling and electron microscopy on rat brain sections that Yif1B is expressed in a vesicular compartment in somas and dendrites that partially overlap ER compartment and the somatic cis-Golgi apparatus. This result suggests that Yif1B cycles between ER and cis-Golgi apparatus. How Yif1B, a protein belonging to the intermediate compartment between ER and Golgi apparatus, controls the targeting of the 5-HT_{1A}R in dendrites, is under investigation.
The 5-HT_{1A}R plays an important role in the control of serotonergic neuron discharge. Thus, in order to investigate in vivo the role played by Yif1B in the function of the 5-HT_{1A}R, we generated a mutant mouse for the gene encoding Yif1B. We first generated a mouse in which the Yif1B gene was flanked by loxP sites (Floxed Yif1B), these mice express normal levels of Yif1B in brain. We bred Floxed Yif1B mice with CMV-Cre transgenic mice in order to obtain a constitutive Yif1B knock-out (KO Yif1B) mouse. Constitutive Yif1B KO mice are viable and do not express Yif1B protein. Yif1B KO -/- mice are not able to reproduce, Yif1B -/+ breed but their offsprings are less frequent and fewer in comparison with Floxed Yif1B mice. We are now characterizing consequences of Yif1B deletion in 5-HT_{1A}R dendritic localization and function.
[1] Carrel et al. (2008) Targeting of the 5-HT1A serotonin receptor to neuronal dendrites is mediated by Yif1B. *J. Neurosci*. **28**:8063-8073.

P2.079

Specific alanine-serine-cysteine (ASC) transporter subtypes mediate D-serine release and uptake in vivo

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D-serine is an endogenous co-agonist of glutamatergic N-methyl D aspartate (NMDA) receptors that binds to the NR1 subunit. It is synthesized by serine racemase in neurons and glials cells and has been implicated in several neurological and psychiatric disorders such as schizophrenia. However, the biochemical mechanisms that control D-serine extracellular concentration in vivo are still largely unexplored. ASC transporters are small neutral amino acid transporters that mediate alanine, serine, cysteine and threonine uptake. They function as amino acid antiporters and can mediate, in principle, D-serine uptake and release. They are expressed in the central nervous system as two major subtypes: alanine-serine-cysteine 1 (Asc1) and alanine-serine-cysteine-threonine 2 (ASCT2) transporters.
In this study, using focal D-serine injections near a specific D-serine microelectrode biosensor, we investigated D-serine diffusion in the extracellular space of the frontal cortex of anesthetized rats. The kinetics of D-serine clearance revealed a clear uptake component. This uptake was inhibited by infusing small neutral amino-acids that are substrates for ASC transporters. Interestingly, these amino acids also produced a large transient increase in the D-serine signal, indicating D-serine release through ASC transporters. We next determined the specific ASC transporter subtypes implicated in these effects. We interfered with ASCT2-mediated transport by infusion of asparagine, a specific ASCT-2 substrate. By competing with D-serine at ASCT-2 transporters, asparagine significantly

inhibited D-serine reuptake in vivo, but did not induce any detectable D-serine release. By contrast, Asc1 blockade with the specific inhibitor S-methyl-L-cysteine blocked both D-serine reuptake and D-serine release induced by L-serine. Therefore Asc1 transporters appear to mediate both D-serine reuptake and release whereas the ASCT-2 subtype is only implicated in D-serine reuptake.
These results identify small neutral amino acid transporters (Asc-1 and ASCT2) as key components in the regulation of D-serine extracellular levels in vivo. These transporters could provide important pharmacological targets for treating brain disorders related to NMDA dysfunction.

P2.080

Calcium-dependent regulation of SNARE-mediated membrane fusion by calmodulin

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Neuroexocytosis requires SNARE proteins, which assemble into trans complexes at the synaptic vesicle / plasma membrane interface and mediate bilayer fusion. Ca^{2+}-sensitivity is thought to be conferred by synaptotagmin, although the ubiquitous Ca^{2+}-effector calmodulin has also been implicated in SNARE-dependent membrane fusion. To examine the molecular mechanisms involved, we examined the direct action of calmodulin and synaptotagmin in vitro, using fluorescence resonance energy transfer to assay lipid-mixing between t- and v-SNARE liposomes. Ca^{2+}/calmodulin inhibited SNARE assembly and membrane fusion by binding to two distinct motifs located in the membrane-proximal regions of VAMP2 (K_D = 500nM) and syntaxin 1 (K_D = 2µM). In contrast, fusion was increased by full-length synaptotagmin 1 anchored in v-SNARE liposomes. When synaptotagmin and calmodulin were combined, synaptotagmin overcame the inhibitory effects of calmodulin. Furthermore synaptotagmin displaced calmodulin binding to t-SNAREs. These findings suggest that two distinct Ca^{2+} sensors act antagonistically in SNARE-mediated fusion.

P2.081

Innovative microbiosensors for *in vivo* brain glucose monitoring

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Implantable enzymatic biosensors are widely used for *in vivo* monitoring applications; in particular in neuroscience research to detect neurotransmitters and metabolites with outstanting temporal resolution. Enzymatic biosensors are typically constructed according to a common basic design in which an enzymatic layer is immobilized on a platinium (Pt) electrode. Various immobilization techniques are available such as glutaraldehyde fixation or enzyme entrapment in sol-gel. Nevertheless, these methods require the use of toxic components that limit their potential use in clinical applications.
Here, we report the successful fabrication of new microelectrode biosensors based on glucose oxidase immobilized using Poly(ethylene glycol) diglycidyl ether (PEGDE). PEGDE provides a simple, non-toxic alternative for the preparation of *in vivo* microelectrode biosensors. Such biosensors exhibited high sensitivity, a response time in order to seconds and were very stable. When implanted

in the cortex of anesthetized rats, they reliably monitored changes in brain glucose concentration following insulin administration followed by glucose injection.
This new fabrication process has been successfully extended to D- amino acid oxidase, lactate oxidase for respectively D-serine and lactate detection *in vitro* or *in vivo*. Its low-toxicity, simplicity of use and low cost should make it a reference technique for constructing stable and reproducible biosensors in the future.

P2.082

Cholinergic modulation of glutamatergic inputs on spinal motoneurons

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Spinal cord motoneurons integrate information from intraspinal and extraspinal afferences through multiple synaptic interactions. The integration of this information flow depends on the intrinsic regulation of excitability of these cells as much as on the interactions established by their different synaptic influences.
In the present work, I decided to address how specific activity from glutamatergic influences, presumably originating in the corticospinal tract, are modulated by cholinergic activity. An important cholinergic synaptic influence has been highlighted in recent years as an important modulator for motoneurons excitability. Nevertheless, the specific interaction of the cholinergic influence and glutamatergic afferents from different origins has not been addressed at the synaptic level.
My results show a selective modulation of AMPA but not NMDA-mediated glutamatergic activity at thoracic and lumbar spinal motoneurons when the cholinergic system is pharmacologically stimulated. Using the direct photolysis of MNI-glutamate as well as addressing the regulation of the neurotransmitter release, I could determine that the muscarinic modulation is occurring directly at the postsynaptic level.
These results suggest that the cholinergic inputs to motoneurons affect not only the excitability of the cells but can directly interfere with the incoming synaptic interactions thus changing the weight of specific afferents.

P2.083

Phosphinothricin modulation of inwardly rectifying K^+ channels increased excitability in striatal medium-sized spiny neurons

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Phosphinothricin (PPT) is the active component of a broad-spectrum herbicide. PPT inhibits the plant glutamine synthetase (GlnS) leading to a lethal accumulation of ammonia. GlnS is also a key enzyme in the glutamate/GABA-glutamine cycle in mammalian CNS where it allows neurotransmitter glutamate conversion into glutamine within astrocytes. Clinical and experimental studies report that an acute PPT exposition induces convulsions and memory impairment in mammals. However, this mechanism is not well understood.
Here, we address the role of PPT in the excitability of striatal medium-sized spiny neurons (MSNs). Horizontal brain slices (350 µm thick) were prepared from C57BL/6J mice. In current-clamp experiments, the firing properties of MSNs were then compared before and after addition of 100µM PPT for 10 min to the bath solution. PPT decreased the rheobase and increased the firing frequency

of action potentials. However, action potentials did not reveal significant differences in amplitude, half-amplitude duration or afterhyperpolarisation amplitude. In addition, PPT had no effect on the resting membrane potential (-89.1 ± 0.2 mV and -91.3 ± 0.3 mV, respectively, $p>0.05$). These effects on membrane properties were blocked by the NMDA receptor antagonist AP5 (50 µM). In voltage-clamp experiments, PPT inhibited the inwardly rectifying K+ channels channel currents (Kir). Finally, the direct inhibition of Kir channels with 40µM Ba^{2+} mimicked the effects of PPT application on neuronal excitability. All together, these results show that the herbicide PPT is a modulator of Kir channels in MSNs, through the activation of NMDA receptor. Thereby, Kir channels are potent regulators of the excitability of MSNs and reduced open probability of these channels would generate a powerful upregulation of neuronal output.

P2.084

Could the vasopressin and corticoliberin physiological synergism be related to the specific V1b and CRF1 receptors heterodimerisation?

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In native tissues, vasopressin (AVP) and corticoliberin (CRF) act in synergism via V1b and CRF1 receptors respectively. Indeed, they are involved in stress responses, mainly by regulating ACTH and catecholamine secretion. Moreover, new evidence suggests that these neuropeptides also play a role in anxiety by direct action in the brain via activation of V1b-R and CRF1. The aim of this study is to explore molecular mechanisms potentially underlying this synergism in the brain. HEK293 cells were co-transfected with V1b and CRF1 receptors and their coupling was evaluated: InsP3 for V1b-R, cAMP for CRHR1 respectively. It was confirmed that AVP potentiates the CRF-response and reciprocally that CRF potentiates the AVP-response. One explanation may be cross-talk amongst second messenger pathways. Interestingly, inhibiting this cross-talk using different inhibitors failed to totally block the potentiation, leaving of 30% residual synergism. Immunoprecipitation, BRET and chaperoning rescue experiments clearly showed a physical association of V1b-R and CRF1 suggesting that heterodimerization accounts for the observed residual synergism (Murat et al, submitted). In order to establish the specificity of V1b-R/CRF1 heterodimerisation, it was evaluated whether V1b-R could also associate with CRF2, and if CRF1 could associate with V1a-R or OT-R, other receptors also involved in stress and anxiety. HEK293 cells were co-transfected with receptors of each family. Dimerization and second messengers accumulation were monitored. The results demonstrated a lack of physical interaction between V1b-R and CRF2, as well as a complete loss of signalling synergism between these two receptors. The co-expression of CRF1 with V1a-R also indicates the same lack of interaction. These data strongly support a high degree of specificity in the association of V1b-R with CRF1, emphasizing their complementary role in stress and anxiety as heterodimers. Work supported by SERVIER (France).

P2.085

Setting up in-vitro models of the rat Blood Brain Barrier (BBB) and Blood Spinal Cord Barrier (BSCB): comparative analysis under pro-inflammatory treatment

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The central nervous system (CNS, brain and spinal cord) has very specific features: the vascular systems of the CNS which separate blood from the nervous tissue constitute highly impermeable physiological barriers known as the blood brain barrier (BBB) and blood spinal cord barrier (BSCB). These barriers protect efficiently the CNS from toxic molecules, viral and bacterial infections, but also restrict very efficiently the passage from blood to the nervous tissue of drugs or molecules with proven therapeutic potential. Research in neurobiology has enabled the characterization of new classes of very promising therapeutic agents mainly represented by large size molecules, peptides, recombinant proteins, fusion proteins, enzymes, cytokines and neurotrophic factors. Unfortunately, for the vast majority, these large molecules do not cross the physiological barriers. There is some evidence that barriers differ in the various structures of the CNS and one of our objectives is to better characterise, at the molecular level, the properties of the BBB and the BSCB in order to propose targeting strategies that allow preferential targeting of the brain or the spinal cord, particularly in situations of pathology and lesion. We have thus developed models of the BBB and BSCB *in vitro* and we compared the transcriptome of endothelial cells from the BBB and BSCB in control and in inflammatory conditions following exposure to pro-inflammatory cytokines. We find that endothelial cells from the brain and spinal cord respond differently to cytokines and that globally, inflammatory responses are enhanced in BBB cells relative to BSCB. We have confirmed expression of a number of pro-inflammatory markers using qPCR and immunocytochemistry and/or enzymatic assays. In particular we show strong induction of a number of matrix metalloproteinases and we show that proteinases affect BBB integrity *in vitro*.

P2.086

Acetylcholinesterase, a back door enzyme

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Acetylcholinesterase (AChE) rapidly hydrolyzes the neurotransmitter ACh at central and peripheral synapses to restore the excitability of the postsynaptic membrane of neurons and muscles. AChE inhibitors are used to attenuate some cognitive or functional deficiencies. The AChE active site is buried inside the molecule at the bottom of a gorge which is the only identified way for the opposite traffics of ACh and its hydrolysis products. The apparent discrepancy of this geometry with the AChE high activity has suggested that a back door (BD), distinct from the gorge entrance, must exist to permit a fast exit of the products and/or alternative ACh entry. This BD may also be involved in a new mechanism for regulation of AChE catalysis.
Our aim is to prove the existence of the BD and document its structure and functioning as a regulatory site. This will allow us to reconcile the geometry and activity of AChE, and define a new target site for more specific therapeutic drugs.
Our model is eel AChE (EeAChE) because 1) it associates in tetramers similar to those found in mammals and has a high activity suggesting an efficient BD, 2) we have inhibitors which bind EeAChE either at the gorge entrance or in the BD region; such ligands are not available for other AChEs, 3) preliminary structural results obtained in our team point to several residues as belonging to the BD region.
We designed a soluble wild type EeAChE (EeAChE-wt), produced it in HEK cells and purified it. Analysis of its structural and functional features, using biochemistry, pharmacology and enzymology techniques, showed that it is representative of natural EeAChE. Mutants modified on residues suspected to belong to the BD region were produced, purified and analyzed for homogeneity, stability and sensitivity to inhibitors. Four mutants display the expected properties while three others are partially misfolded and weakly active, suggesting that functional and structural determinants, respectively, were altered. We also produced EeAChE-wt in sufficient amounts to start a structural study. A primary X-ray dataset was collected. We are trying to improve the resolution and to crystallize

a mutant with a possibly enlarged BD. Our data will allow us to describe the BD of AChE at the structural and functional levels.

P2.087

Nerve growth cones as chemical sensors, filters and amplifiers

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Nerve growth cones (GCs) are chemical sensors that convert extracellular cues into oriented motion. Although families of guidance signals have been uncovered, the mechanisms by which GCs quantitatively process directional information are still poorly known, largely due to the limitations of standard guidance assays. Here, we probe the response of dissociated neurons to controlled gradients using novel shear-free microfluidic devices. By measuring and quantitatively modeling the polarization of $GABA_A$ chemoreceptors at the GC membrane, we analyze the amplification and filtering properties of nerve GCs during GABA directional sensing. We find that:
(i) GCs are able to non-adaptively amplify extracellular gradients, with a dependence on the ligand concentration determined by the saturable response of chemoreceptors,
(ii) GCs act as low-pass temporal filters with a cut-off frequency independent of stimuli conditions.
These experiments pave the way for an integrative approach of the GC response to complex spatiotemporal stimuli patterns, from a molecular to a systems-level.

P2.088

Molecular, histochemical and electrophysiological profiling of globus pallidus neurons *in vitro*

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The globus pallidus (GP) constitutes the central node in BG circuitery as it controls the pattern of activity of all other nuclei of the BG through its widespread projections. The GP is exclusively composed of GABAergic neurons classified into different groups according to morphological, neurochemical, and electrophysiology criteria. As these parameters have not been correlated in previous studies, our first objective was to combined electrophysiological characterization of GP neurons with molecular profiling to improve our knowlegde about these different neuronal populations. Using perforated-patch recordings, we found two types of GP neurons. The first group of neurons (type I), representing 2/3 of our recordings, were characterized by a regular discharge of action potentials (AP) with a moderate afterhyperpolarisation (AHP) and an early accommodation in their discharge frequency during injection of depolarizing current steps. The second group (type II) was characterized by silent or low firing frequency neurons, with a large AHP and a lack of accommodation during depolarizing current steps. To correlate these electrophysiological properties with specific markers of GP neurons, we combined whole-cell patch-clamp recording with
1) post-recording identification of biocytin-filled neuron and immunohistochemistry for parvalbumin (PV) the most express calcium-binding protein in the GP, or
2) cytoplasm harvesting to perform single-cell RT-PCR.
We found that type I GP neurons were more often immunopositive for PV (84 %) whereas type II GP neurons were mainly PV-negative (70 %). Single-cell mRNA profiling of type I GP neurons revealed

that these neurons expressed GAD67, PV and Nkx2.1 a transcription factor involved in the molecular specification of GP neurons, whereas type II GP neurons were characterized by the presence of GAD65, enkephalin, and NpasI (another transcription factor expressed by a subpopulation of GP neurones) mRNAs. These results suggest that there is a two main neuronal subpopulations in the GP, which can be distinguish by their molecular and electrophysiological profiles. Further experiments, will be needed to to elucidate the contribution of both GP subtypes in the functionning of the BG network.

P2.089

Lingo1, a protein involved in various neuro-developmental functions directly interacts with different domains of neurofibromin (Nf1), a negative regulator of ras: molecular studies and BRET experiments

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Neurofibromin is a large protein (280kDa) encoded by the NF1 gene. It is ubiquitously expressed at low levels with highest expression levels in CNS cells (neurons, Scwhann cells, and oligodendrocytes) and in leucocytes. Mutations of the NF1 gene are responsible of the most common genetic diseases, type 1 neurofibromatosis, which affects 1 in 3500 individuals. The phenotype of NF1 is highly variable, with benign (neurofibromas) or malignant peripheral nerve sheath tumors, CNS tumors (gliomas, astrocytomas) and cognitive deficits (40% of NF1 patients). Although the RasGAP (GTPase Activating Protein) activity of Nf1 is known to inhibit effector pathways of ras (Raf, Akt, Rho pathways), the different functions of Nf1 and the molecular mechanisms involved in their regulation are poorly understood. A better knowledge of the signalling network associated with NF1 should improve our understanding of the physiological functions of NF1 and help identify new therapeutic targets for NF1 treatment.
To address this issue, we performed a two-hybrid screen on a human brain cDNA library using the SecPH, the domain adjacent to the GAP domain of NF1, as bait. Among proteins identified interacting with the SecPH domain, Lingo-1 seemed to be particularly interesting. Lingo1 is a transmembrane protein exclusively expressed in the nervous system and known to inhibit several neuro-developmental mechanisms such as oligodendrocytes differentiation, myelinisation, neuronal differentiation and survival. We confirmed the interaction between the Sec-PH domain and Lingo1 by co-immunoprecipitation in HEK-293. We also evaluated the specificity of this interaction showing that the three human paralogs of Lingo1 (Lingo2, Lingo3 and Lingo4) do not interact with SecPH domain. Systematic analysis of all domains of Nf1 reveals that the GAP domain of NF1 also interacts with Lingo1. Moreover, using bioluminescence Resonance Energy Transfer (BRET), we showed that the interaction between these NF1 domains and Lingo1 is direct. The implication of these interactions on both Lingo-1 and NF1 functions are under progress.
In conclusion, Lingo1 identified as a new partner of neurofibromin constitutes an interesting target to understand signalling pathways involved in cognitive deficits of NF1 patients.

P2.090

Characterization of tyramine-β-hydroxylase in the nervous system of the cockroach *Periplaneta americana*

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Octopamine (OA) is an important neuroactive substance that modulates several physiological functions and behaviors of various invertebrate species. This biogenic monoamine, structurally related to noradrenaline, acts as a neurotransmitter, a neuromodulator or a neurohormone in insects (Roeder 2005 Annu Rev Entomol). The tyramine-β-hydroxylase (TBH) catalyzes the last step in OA biosynthesis and thus plays a key role in the regulation of synthesis and secretion of OA in neurons. The aim of this study was to investigate the TBH, expressed in the nervous system (NS) of the cockroach *Periplaneta americana*.
Here we report the molecular cloning of 5 full-length cDNA encoding TBH in the NS of *P. americana* (*PaTBH*) which could be the consequence of RNA editing events. Gene encoding this PaTBH was analyzed phylogenetically within a large cohort of homologous enzymes from insects. Tissue distribution of *PaTBH* was studied by RT-PCR and a cDNA clone was expressed in *Xenopus* oocytes in order to characterize TBH activity. Since OA was described as a stress hormone, the TBH activity was studied under normal and stress conditions with a novel ELISA assay using nerve chord extracts of adults of *P. americana*.

P2.092

Unpredictable chronic mild stress (UCMS) and neuroinflammation in various stress responsive regions of mice brain

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According to WHO, depression is the second most disabling disease worldwide which is expected to rise to number one by the year 2030. Its co-prevalence with vascular disorders particularly in advanced age has been underlined. Depression in old age is associated with poor responsiveness to conventional antidepressants and can also precede neurodegenerative illnesses. Both preclinical and clinical data show that inflammatory cytokines have a bidirectional relationship with depression. Some pro-inflammatory cytokines have been found to increase with age. Consequently neuroinflammatory effect of raised cytokine concentration can be implicated as a potential mechanism for the anhedonic effects of stress. In order to ascertain role of neuroinflammation in pathogenesis of depression, we examined effect of unpredictable chronic mild stress (UCMS) and lipopolysachheride (LPS) on the activation of microglia in various brain regions. UCMS is a validated model of depression in mice. A group of 31 mice were subjected to various mild stressors for 9 weeks with a control group kept in standard conditions. The mice were injected with bacterial lipopolysachheride 90 minutes before sacrifice. Transcardial perfusion with 4% PFA was done after anesthesia, brains were dissected out, sliced and analyzed for microglial activation by immunohistochemistry using rat anti-cd11b antibody as a marker of microglia. The surface area covered by microglia was calculated using histoab software. Our results strongly suggest that UCMS causes a depressive like behavior through neuroinflammation evident by the activation of microglia in frontal cortex, nucleus accumbens, caudate putamen and CA1 & CA3 regions of hippocampus. These regions are established to be implicated in animal's response to stress. The control mice injected with LPS also showed marked activation of microglia. In the stressed mice, however, LPS failed to cause an increase in the already activated state of microglia. This shows that microglia are activated to a maximum level when the animal is subjected to UCMS. An acute inflammatory agent like LPS cannot increase it anymore. Long term effects of stress on microglial particularly in aged mice can be a very interesting follow up for this experiment.

P2.093

Cognitive event-related potentials: towards a clinical application in psychiatry

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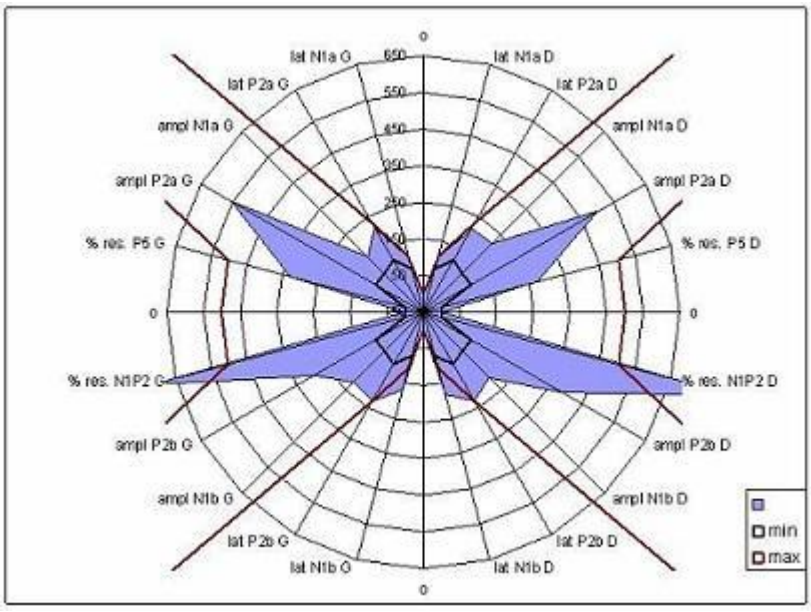
Psychiatric diseases suffer of a weak clinical neurophysiologic characterization. On-going EEG is generally used to eliminate organic pathologies and Event Related Potentials (ERPs) are mainly recorded in experimental designs, and rarely integrated in a routine examination, unlike evoked potentials in neurology.
For last 3 years, we have developed a ERPs examination proposed to every new patient in our psychiatry department. After validation of our protocol, we have introduced our “ERPs exam package” in clinical routine. A first difficulty consists in the signal-to-noise ratio for each patient, without any averaging between patients (“grand moyennages”) in clinical routine.
We propose a complete ERPs examination including: auditory P50 (sensory gating when listening to two successive audio clicks), auditory P300 (auditory oddball paradigm in 3 different conditions: passive condition, counting targets and motor response), auditory N400 (elicited by a semantic violation in ecological sentences) and Contingent Negative Variation (preparation to response to a stimulus).
Exploring latency, amplitude and topography for each ERP component, we obtain for each subject about 50 parameters and we are currently:
i) Researching the best way to resume the data and to present the ERP results in a clear, useful and short report using graphic representations, and
ii) Analyzing the relevant correlations between neurophysiologic parameters, and their correlations with clinical (psychometric) and therapeutic parameters.
Today, some striking differences appear between pathologies, and before any complete statistical evaluation (in preparation), we can observe that neurophysiologic cues are in agreement with clinical states of the patients.
Figure 1:
Graphic presentation (star) summarizing data of P50 ERP with gating (in blue: one patient's individual measures; literature minimums (black) and maximums (red) are shown to compare to patient's values).



[Figure 1]

P2.094

Changes of expression of vesicular glutamate transporters induced by high frequency stimulation of subthalamic nucleus

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Abnormal hyperactivity of glutamatergic transmission, as reported in dopamine-depleted conditions, has been suggested to play a key role in the expression of motor symptoms in Parkinson's disease (PD). Thus, high-frequency stimulation of the STN has been developed and became a powerful surgical approach for treatment of parkinsonian motor syndrome. However, the cellular mechanisms concerning its clinical effects remain unclear. Three vesicular glutamate transporters (VGLUT1, 2 and 3) were recently identified. These proteins are responsible for the uploading of glutamate into pre-synaptic vesicles and represent the first specific markers of glutamatergic neurons available. This study aims, by using immunoradioautography, to analyze VGLUTs expression within different structures of basal ganglia network and its potential changes induced by STN-HFS both in naive and in 6-OHDA-SNc lesioned rats. Lesioned rats exhibited lower levels of VGLUTs expression than naive rats (except for VGLUT1 in striatum, nucleus accumbens and cingular cortex). Moreover, STN-HFS induced a clear up-regulation of VGLUTs expression levels in all structures examined, thus restoring in lesioned animals an expression level of VGLUT2 similar to that detected in naïve rats. Overall, these findings confirm the glutamatergic dysregulation in dopamine depleted rats and strongly suggest the contribution of VGLUTs expression in STN-HFS mechanisms.

P2.095

Cinnabarinic acid, an endogenous metabolite of the kynurenine pathway, activates type-4 metabotropic glutamate receptors

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Cinnabarinic acid is an endogenous metabolite of the kynurenine pathway that harbors two carboxyl groups and an amino group, thus meeting the basic requirements to interact with excitatory amino acid receptors. We found that cinnabarinic acid acts as a partial agonist of type-4 metabotropic glutamate (mGlu4) receptors, with no activity at mGlu1, -2, -5, -6, -7, and -8 receptors either as agonist, antagonist or enhancer. We also tested the activity of cinnabarinic acid on native mGlu4 receptors by examining
(i) the inhibition of cAMP formation in cultured cerebellar granule cells and
(ii) protection against excitotoxic neuronal death in mixed cultures of cortical cells.
In both models, cinnabarininc acid behaved similarly to the prototypical mGlu4 receptor agonists, and its action was attenuated in cultures prepared from mGlu4 receptor knockout mice. Cinnabarinic acid did not activate truncated mGlu4 receptors lacking the N-terminal Venus-flytrap domain, as opposed to the mGlu4 receptor enhancer, N-phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide

(PHCCC). Mutagenesis and molecular modeling experiments showed that cinnabarinic acid acts as an orthosteric agonist interacting with residues of the glutamate binding pocket of mGlu4. We conclude that cinnabarinic acid is a novel endogenous selective orthosteric agonist of mGlu4 receptors endowed with neuroprotective activity. This function might be relevant under conditions in which the kynurenine pathway is activated in the CNS, as occurs during neuroinflammation.

P2.096

Spinal cord injury alters adult neurogenesis in the rat forebrain

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Following spinal cord injury (SCI), a rapid cellular response evolves at the injury site to form a dense scar tissue. However, despite this local tissue remodeling process, the cellular consequences of SCI in the brain remain unknown. We have investigated the impact of a SCI on adult brain neurogenesis niches by applying a BrdU pulse chase coupled to both neurogenesis, astrogliosis and microgliosis during the subchronic and chronic phases of injury. We show here that subchronic SCI downregulates neurogenesis by altering the formation of newly generated neurons in the adult forebrain regions: the subventricular zone of the lateral ventricule and the subgranular zone of the hippocampus; and activates microglia in the dorsal vagal complex of the hindbrain region. Neurogenesis remains downregulated in the hippocampus during the chronic phase. In the cervical spinal cord, subchronic SCI upregulates mainly astrogliosis and microgliosis, while neurogenesis is minor. We suggest that these brain cell dynamic modulations are probably due to inflammation and/or decrease of physical exercise; two factors that are known to affect adult brain neurogenesis. This new observation highlights a new dimension of SCI damage distal from the spinal cord and demonstrates brain vulnerability to SCI.

P2.097

Chronic imaging of the in vivo spinal cord using with multi-photon fluorescent techniques: a method for placing and maintaining spinal cord windows

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High-resolution imaging of the intact and pathological central nervous system (CNS) over time in live mammals is a powerful way to understand CNS function and how the CNS responds to injury. One way to examine the CNS in vivo is to acquire multi-photon fluorescent images through glass windows implanted over exposed sections of the CNS of fluorescent transgenic mice. Methods for the placement and maintenance of windows over various regions of the mouse brain are well established and their use is becoming increasingly more common. However, to date, multi-photon fluorescent imaging of the mouse spinal cord over time has required invasive surgery for each imaging session. Here we show preliminary results for a method to implant and maintain windows over the spinal cords of adult mice. Briefly, we exposed the T13 to L2 vertebrae of Thy1-CFP/Lys6-GFP or wild-type C57BL/6 mice. These vertebrae were joined together using dental cement and a dorsal laminectomy was performed at L1, followed by a dorsal 'pin-prick' spinal cord injury. A coverslip window was placed over the spinal cord and held in place using a combination of cyanoacrylate and dental cement. We found that this approach yielded windows that were stable and caused little discomfort to the mice. However, in most cases tissue began to develop between the window and spinal cord within 2-3 days. This tissue was highly vascular and contained Lys6 positive monocyte derived macrophages. This tissue progressively obstructed visibility of the spinal cord and reduced the multi-photon imaging depth

until the cord was not visible by 1-2 weeks post-surgery. To improve image quality we removed the window, surgically removed the tissue, and covered the spinal cord with a new window. In addition, the rate of tissue growth under the window was slowed by treatment with anti-inflammatory drugs. Together, these approaches have allowed us to acquire images for up to 8 imaging sessions over the period of 28 days. These image sets provide a detailed picture of axonal retraction/growth and cellular interactions with high spatial and temporal resolution. Collectively, our results show that windows implanted over the spinal cords of adult mice are a feasible and powerful method to examine in vivo cellular dynamics in space and time.

P2.098

Novel insights into the functional interaction between the Parkinson's disease-related proteins PINK1 and Parkin in cell models

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Most of the autosomal recessive forms of Parkinson's disease are caused by the mutation of two genes: PARK2, coding for the cytosolic E3 ubiquitin-protein ligase Parkin; and PARK6, encoding the mitochondrial seronine/threonine kinase PINK1. Genetic analyses in drosophila have linked Parkin and PINK1 to a common pathway involved in protection of mitochondrial function and integrity. Recent studies highlighted a role of these proteins in regulating mitochondrial fusion and fission and in inducing selective autophagy of damaged mitochondria. Our group recently revealed recruitment of Parkin to the mitochondrial surface where it appears to regulate the mitochondrial abundance of its novel substrate SCHAD (Short-chain-3-hydroxyacylCoA dehydrogenase), a matrix enzyme directed to mitochondria by an N-terminal tageting signal (MTS). We used biochemical approaches to explore the possibility that Parkin affects the mitochondrial import of PINK1, which is also addressed to mitochondria via an N-terminal MTS. In mammalian cells, PINK1 is detected as a full length precursor of 63 kDa ($PINK1_{63kDa}$) and a mature mitochondrial protein of 52kDa ($PINK1_{52kDa}$). In PC12 cells in which Parkin is expressed under the control of the *tet-off* gene regulatory system, we observed a significant increase of the $PINK1_{52kDa}$ / $PINK1_{63kDa}$ ratio in the presence of Parkin both in enriched mitochondrial fractions and in total lysates. This difference was abolished when mitochondrial protein import was blocked by dissipating the mitochondrial membrane potential ($D\Psi m$) essential for this process. To confirm these data, we used HeLa cells expressing the matrix protease Lon under control of the *tet-on* system to block mitochondrial import without impairing $D\Psi m$. In this model, the presence of Parkin did not change the $PINK1_{52kDa}$ / $PINK1_{63kDa}$ ratio. However, it significantly stabilized both the $PINK1_{63kDa}$ precursor and the mature mitochondrial $PINK1_{52kDa}$ isoform in import-competent but not in import-deficient cells. This stabilization was compromised by PD-causing Parkin variants. In conclusion, our results suggest that Parkin modulates the mitochondrial import of PINK1 and provide new insights into the reciprocal regulations of these proteins within the PINK1/Parkin pathway.

P2.099

Early somato-dendritic alterations in lumbar motoneurons of $SOD1^{G85R}$ juvenile mice

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We previously found altered electrical properties and over-branched dendrites in lumbar motoneurons from superoxide dismutase 1 (SOD1) mutant mice (an amyotrophic lateral sclerosis model) at second

postnatal week although hindlimb paralysis appear at 8 months of age. Here, we studied whether the abnormal branching occurred at birth and was related to altered synaptic supply of motor nuclei. Lumbar motoneurons in WT and SOD1 pups were intracellularly recorded, stained and further reconstructed in 3D as previously described [1, 2]. The reconstructed dendrites were morphometrically analyzed and used to build computer models of WT and SOD1 motoneurons to assess the functional consequences of the structural difference. Synaptophysin and GFAP expression was analyzed at two postnatal ages (P3 and P8) in the whole lumbar segment by western blot, and more specifically in the motoneuronal pools by immunohistochemistry. Immunolabeling of synaptophysin was significantly less intensive in pools of SOD1 motoneurons compared to WT ones at both ages. WT and SOD1 motoneurons had a comparable number of dendritic branches at P3-P4 but SOD1 dendrites had longer terminals. The number of branches did not differ between P3 and P9 in WT motoneurons, indicating that the dendrites only elongated at that period. On the contrary, SOD1 motoneurons over-branched precisely between P4 and P8. Glial staining in the ventral and lateral spinal cord was lower in SOD1 mice compared to WT ones at P8 suggesting replacement of some glial tissue by supernumerary dendrites. Simulation based on realistic morphology using passive multicompartmental model in NEURON software environment and Multiple Run Fitter tool allowed to study the average effectiveness of charge transfer along proliferated dendrites in SOD1 P8-P9 MNs. Our results suggest that the overbranching of dendrites in lumbar motoneurons of SOD1 mice might perfectly compensate for the lower number of presynaptic terminals reaching the motoneuronal pools. These morphological changes may represent the earliest compensatory mechanisms detected in SOD1 motoneurons.
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P2.100

Multi-Target-Directed Ligands as treatment of Alzheimer disease: novel compounds with both anti-acetylcholinesterase and serotoninergic 5-HT4 receptor agonist activities

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Alzheimer´s disease (AD), the most common form of dementia among the elderly, is a chronic, progressive and degenerative disorder of the brain with a loss of memory and cognition. Drugs acting on the acetylcholine-mediated system have been favored but, it is now necessary to improve the treatment with more etiological approaches. A new strategy based on the assumption that a single compound may be able to hit multiple targets is emerging, and seems particularly relevant for the treatment of neurodegenerative syndromes, which involve multiple pathogenic factors. This concept known as “Multi-Target-Directed Ligands” (MTDLs) can be used with a great potential benefit towards multiple targets implicated in the complex AD.
Herein, we targeted cholinergic and serotoninergic systems and conceived new ligands with both anti-acetylcholinesterase and serotoninergic 5-HT4 receptor agonist activity.
Here, we report the results of two different experiments.
a) ***In vivo*.** Using object recognition test (“episodic-like” memory test) in the mouse, we showed that co-administration of subactive doses of donepezil (0.3 mg/kg; ip), *a noncompetitive, reversible inhibitor of acetylcholinesterase* and RS 67333 (0.1 mg/kg), *a partial agonist of 5-HT4 receptor*, before the task extended the memory trace. This suggests a synergic action of these two drugs on acquisition/consolidation of memory processes and pharmacologically validates the concept of our “MDTL” strategy.
***b) In vitro*.** We aimed at giving to some of our 5-HT4 receptor ligand, previously synthesized, an additional AChE inhibitory activity. Among many synthesized compounds, MR31115 was selected, as a hit on the basis of its in vitro dual activity as AChEI (IC_{50} = 597 nM) and 5-HT4 receptor ligand (Ki = 45 nM). The pharmacological profile of MR31115 is currently under investigation in order to establish its agonist or antagonist activity.

P2.101

Complex regulation of p73 isoforms expression during neuronal apoptosis in response to DNA damage or alteration of the amyloid beta precursor polypeptide function

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Genetic ablations of *p73* have shown its implication in the development of the nervous system. However, the relative contribution of DNp73 and TAp73 isoforms in neuronal functions is still unclear. In this study, we have analyzed the expression of TAp73 and DNp73 isoforms during neuronal apoptosis induced by alteration of the amyloid-b precursor protein (APP) function or cisplatin. We observed a concomitant up-regulation of a TAp73 isoform and a down-regulation of a DNp73 isoform. The shift in favor of the pro-apoptotic isoform correlated with an induction of the p53/p73 target genes such as *Noxa*. At a functional level, we showed that TAp73 induced neuronal death and that DNp73 have a neuroprotective role towards APP alteration or cisplatin. We investigated the mechanisms of p73 expression and found that the TAp73 expression was regulated at the promoter level. In contrast, regulation of DNp73 protein levels were regulated by multiple proteases and phosphorylation at residue 86. Thus, this study indicates that tight transcriptional and post-translational steps of the p73 isoforms ratio play an important role in neuronal survival.

P2.102

Nasal OEC transplantation promotes respiratory recovery after cervical spinal cord contusion

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Cervical spinal cord injuries frequently induce, in addition to para and tetraplegia, respiratory insufficiency which constitutes a major complicating factor and increases dependency (assisted ventilation) and the probability of post-trauma death.
In order to counterbalance respiratory deleterious effects we tested the impact of engraftment of nasal olfactory ensheathing cells (OEC), considered as a promising therapeutic strategy for spinal cord repair. However, while the vast majority of fundamental studies were focused on the recovery of locomotor function, the efficiency of this cellular tool for repairing respiratory motor dysfunction, which affects more than half of paraplegic/tetraplegic patients, remains unknown.
EC transplantation was performed in conditions close to clinical situations, i.e. using a rat contusion model that mimics the mechanisms encountered after a C2 cervical contusion that induces a persistent hemi-diaphragmatic paralysis (Baussart et al., 2006). The respiratory rat model has been used since it has now been established that rodent constitutes an appropriate pre-clinical model for treating spinal cord injury, at least for respiratory dysfunction (Kastner and Gauthier, 2008).
We assessed the therapeutic efficiency of the delayed transplantation (2 weeks post-contusion) of nasal OECs within the injured spinal cord. Functional recovery was quantified with respiratory behavior tests, diaphragmatic electromyography and neuro-electrophysiological recording of the phrenic motoneurons while axogenesis was evaluated using immunohistochemistry.
We show that 3 months post-transplantation, nasal OECs improve

i) breathing movements,
ii) activities of the ipsilateral diaphragm and corresponding phrenic nerve, and
iii) axonal sprouting in the injury site.
We also demonstrate that this functional partial recovery is mediated by the restoration of ipsilateral supraspinal command. Our study brings further evidence that olfactory ensheathing cells could have clinical application especially in tetraplegic patients with impaired breathing movements.

P2.103

N-3 Polyunsaturated fatty acid supplementation improves hyperdopaminergic phenotypes in mice invalidated for dopamine transporter (DAT-KO)

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N-3 Polyunsaturated fatty acids (PUFA) are dietary essentials, and are critical to brain development and function. Increasing evidence suggests that Attention Deficit-Hyperactivity Disorder (ADHD) in children is associated with an imbalance in PUFA composition, with abnormal low levels of the main n-3 PUFA, DHA (22:6n-3).
Mice lacking the gene encoding the DAT have elevated dopaminergic tone. In addition, they exhibit novelty-driven hyperactivity and stereotypic activity that are paradoxically improved in response to psychostimulants. The goal of this study was to take the advantages of this mouse genetic model of ADHD to test the potential therapeutic effect of n-3 PUFA supplementation.
N-3 PUFA supplementation (fish oil: 2g/100g diet providing 7.1% DHA and 1.5% EPA) was given to the dams throughout lactation, and then directly to the pup diet from weaning up to behavioural assessment at adulthood. N-3 PUFA therapeutic effect was tested on two ADHD-related symptoms: novelty-driven hyperactivity and behavioural lateralization. Brain lipid composition and tissue content in monoamines were quantified in both striatum and frontal cortex.
Preliminary data showed that the N-3 PUFA supplementation significantly increased the DHA levels and decreased the arachidonic acid (AA, 20:4n-6) levels in phospholipid membranes. N-3 PUFA supplemented DAT-KO mice exhibited a significantly reduced horizontal and vertical hyperactivity at both 1 and 4 months of age. In addition, this calming effect was associated with a significant lower falling-in striatal dopamine levels in supplemented mutant mice, compared to DAT-KO mice raised under control diet.
This experimental design offers a unique opportunity to test the extent to which environmental factors (diet) may influence the ability of a system to compensate for a genetic deficit to maintain or restore normal function. The gene-environment interaction plays a key role in complex diseases, such as neurological and psychiatric disorders.

P2.104

Feasibility of high-density scalp somatosensory evoked potential recordings in macaque monkeys: a pilot study

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The goal of the present pilot study was to establish a simple and minimally invasive method to record somatosensory evoked potentials (SSEPs) from the whole scalp surface in anaesthetized adult macaque monkeys using a high-density electrode array.
Recordings were performed with a customised EEG cap containing 32 electrodes regularly distributed over the scalp while the monkey was anaesthetized (2.5% sevoflurane). Electrical stimulations were delivered separately either to the median nerve at the wrist or to the tibial nerve at the ankle (0.5Hz repetition rate (1 sweep every 2 seconds), intensity slightly above the visible motor threshold, total of 75 sweeps). The SSEP data were analysed both conventionally in terms of component amplitude and latency at selected scalp locations and topographically by cluster analysis of the voltage maps. This topographical analysis is a data-driven approach and reveals a series of scalp topographies reflecting the steps in information processing.
Although responses were somewhat variable in amplitude and latency across the different recording sessions, they were topographically very reproducible. The map topography of the responses obtained after either median or tibial nerve stimulations was in line with the somatotopic organisation of the sensorimotor cortex.
Our data show that SSEPs can be successfully and reproducibly recorded from a multichannel EEG cap in macaque monkeys. This minimally invasive method to record large-scale neuronal networks in real-time can be useful if repeated assessment of the cortical activity is desired, for example to study functional damage and recovery after a central nervous system lesion. In this case, topography of SSEPs will allow to assess the possible cortical reorganisation of neuronal networks and relate it to functional recovery. The tool we developed is very relevant in the context of promoting non-invasive approaches also in animal research.

P2.105

Hypersensitivity to environmental cues as a common basis for the maintenance of addiction and post traumatic stress disorder: a potential role of monaminergic uncoupling?

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Addiction and posttraumatic stress disorder (PTSD) are two pathologies characterized by their hypersensitivity to environmental cues leading to high emotional reviviscences which are responsible for the high susceptibility to relapse which is associated with these two pathologies. Recently, Tassin (2008) showed that decoupling between the noradrenergic and serotonergic systems evidenced by a behavioral sensitization, as well as by a marked norepinephrine and serotonin release within the prefrontal cortex, in response to an injection of psychostimulants, constitutes one of the first stages of addictive processes. We formulated the hypothesis that such increases in monoamine release in reaction to exposure to environmental cues may well serve as a physiological basis for the hyperreactivity to retrieval cues associated with addiction and PTSD. To support this hypothesis, it was decided to investigate uncoupling in rats exposed to an animal model of PTSD (the Single Prolonged Stress; Khan and Liberzon, 2004) consisting in three successive stress (restraint, forced swim, discomfort in dry ice). After different time intervals following PTSD (4, 15 and 30 days), rats were tested using either
(1) a battery of behavioral tests in order to assess the validity of our PTSD model or
(2) an amphetamine behavioral sensitization test in order to investigate uncoupling.
As it is well known that addiction and PTSD develops only in 17 to 20% of the exposed subjects, PTSD rats were either considered as a whole group or separated according to potential vulnerability traits such as reaction to novelty, anxiety and impulsivity quantified before PTSD. Consequences of the data obtained in this series of experiments will be discussed in relation with our hypothesis.

P2.106

Electrophysiological mapping of the mesencephalic locomotor region in a non human primate during bipedal locomotion

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Although pathophysiological mechanisms underlying Freezing Of Gait (FOG) in Parkinson´s Disease remain unknown, experimental studies established that the Pedunculopontine Nucleus (PPN) and the Mesencephalic Locomotor Region (MLR) could play a crucial role in FOG leading this area as a new target for DBS. Nevertheless, preliminary outcomes are very heterogeneous and precise target remains under debate. Accordingly, the aim of this study was to perform an electrophysiological mapping of the MLR in primate during bipedal Locomotion (Loc) using Micro Electrode Recording (MER) and Stimulation (MES)
Methods: Experiment was performed on 1 *Macaca fascicularis.*
MER and MES were performed during BaseLine (BL) and Loc. Acute MES were performed at the site where neurons in relation to locomotor behavior were recorded.
Stimulating parameters: pulse width (0,06-0,5ms); Freq. (10-25-50-130Hz); Current int. (0,1-1 mA)
Results: 93 neurons within the MLR were recorded. According to their response to Loc, we could defined 3 groups:
·Non responder neurons (low Firing Rate (FR))
·Neurons that responded to loc by changing the FR from tonic to a phasic pattern
·Tonic neurons with FR around 5 Hz that responded to Loc by decreasing or increasing the FR respectively around 2 or 10 Hz.
Low FR neurons were mainly located postero-laterally presumably in PPN pars cholinergic while phasic responders were found in the anterior-superior part of the MLR i.e. cuneiform nucleus/PPN dissipatus.
A total of 25 sites were tested by MES and different motor responses were induced :
·Muscular jerks (BL)
·Cyclic flexion of Contro-Lateral (CL) leg (BL)
·Bilateral movements of the legs into a startle-like position (BL)
·Blockage of the CL leg (BL and Loc)
·Increased flexing amplitude of CL leg (Loc)
Conclusion: We could record for the first time on a behaving primate, neurons within the MLR that heterogeneously responded to bipedal Loc. Indeed, this region encompasses an area that contains both cholinergic and non cholinergic neurons. This gives electrophysiological clues of the involvement of this area during Loc. Furthermore, using MES, we could induce locomotor-like movements depending on the stimulating parameters that will provide new insights for further chronic DBS studies of the MLR.

P2.107

The frontotemporal dementia-associated Charged Multivesicular protein 2B regulates the maturation of dendritic spines

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Frontotemporal dementia (FTD) arises from degeneration of neurones in the anterior cortical lobes, and has a genetic cause in 30% of cases. In the rare familial form FTD-3, disease is linked to a dominant mutation in CHMP2B (Charged Multivesicular body protein 2B), a subunit of the highly conserved ESCRT-III (Endosomal Sorting Complex Required for Transport-III). ESCRT-III forms membrane-associated filaments that are responsible for deformation and cleavage of membranes during endosomal trafficking and other cellular activities. It has been proposed that pathogenic CHMP2B mutants cause neurodegeneration by inducing endosomal and autophagy defects and ensuing accumulation of toxic material. In transfected hippocampal neurones in culture, we show that FTD-linked mutants of CHMP2B impair the morphological maturation of dendritic spines and lower the frequency and amplitude of miniEPSCs, without apparent changes in autophagy or survival. These effects may be due to dominant negative activity of the mutants, because CHMP2B is required for normal growth of spine heads, as indicated by depletion experiments. Subcellular fractionation and immunofluorescence microscopy indicate that CHMP2B and other ESCRT-III subunits are significantly enriched in dendritic spines. In heterologous cells, we find that CHMP2B polymerizes under the plasma membrane rather than at endosomes. Overexpressed CHMP2B specifically deforms the plasma membrane into rigid, 5-50 micron-long, 100-300 nm wide protrusions. Immuno- and cryo-electron microscopy reveal that these protrusions are generated by a helical polymer tightly attached to the inner side of the protruding plasma membrane. Furthermore, these CHMP2B structures appear to reorganize other ESCRT-III subunits and actin filaments. We propose that CHMP2B directs the assembly of a novel scaffold that directly remodels the dendritic membrane and contributes to the morphogenesis and /or stability of mushroom spines. Disease-linked mutants may perturb this assembly with detrimental consequences for long-term survival of specific neuronal populations.

P2.108

Electrophysiological biomarkers of intolerance of change in autism

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Background: In addition to difficulties in communication and social interaction, the presence of repetitive and restricted behaviours and interests forms part of the diagnosis of autism spectrum condition (ASC). Although of major importance in daily life, this last area has not received much research interest and the brain mechanisms underlying this fundamental feature of the autistic disorder are still not fully understood. The clinical observations of autistic patients show that they react in an unusual way to unattended sensory stimuli that appear in the environment. Moreover an abnormal orientation towards novel stimuli and a difficulty in selecting relevant information has been proposed in people with ASC. The resistance to change might thus reflect a basic disorder in the processing of unusual stimuli. This study aims to examine auditory change-detection and its neural basis in children with ASC and to test for electrophysiological patterns that are quantitatively related to intolerance of change.
Methods: Brain mechanisms involved in the automatic detection of auditory frequency change were studied through the Mismatch Negativity (MMN) and the P3a in 27 children with ASC aged 5-11 years matched with 27 healthy children. Behavioural assessment, including intolerance of change, was also performed using the Behaviour Summarized Evaluation sale (BSE-R).
Results/discussion: MMN in children with ASC showed significantly shorter latency than in controls suggesting acute brain reactivity to deviancy. Moreover, children with ASC had significantly larger P3a, indicating a greater tendency to switch attention toward irrelevant deviant events. Electro-clinical relationships showed that shortening of MMN and P3a latency was more marked in children who displayed greater difficulties to tolerate change. MMN and P3a abnormalities in ASC thus reflect an atypical processing of change that might underlie resistance to change in these children.

P2.109

TXNIP, which mediates insulin resistance in diabetes, is early over-expressed in the brain of the 5XFAD Alzheimer (AD) mice model and is induced by the amyloid beta peptide (Aβ) in vitro

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Alzheimer´s disease (AD) is the main cause of dementia among people age 65 and older, affecting more than 25 million people in the world currently. There is some evidence that nearly 80 percent of individuals with Alzheimer's disease also have cardiovascular disease at autopsy. The broader implications of such findings support the hypothesis that systemic vascular factors are risk factors for developing Alzheimer's disease. This risk encompasses different forms of cardiovascular disease, including coronary artery disease, carotid atherosclerosis, history of hypertension or high cholesterol, Type II diabetes and stroke or transient ischemic attacks. Several studies, including our own, have demonstrated that thioredoxin interacting protein (TXNIP) is one of the early response genes to hyperglycemic conditions and is highly induced by diabetes, where it mediates vascular and glial inflammation and neuronal dysfunction. TXNIP is necessary to mediate insulin resistance in diabetes. Recent studies indicate that insulin signaling is impaired in the AD brain, leading to neuronal and vascular dysfunction. Moreover, we demonstrated that TXNIP is required to induce the expression of IL-1b by glial cells during inflammation. Because of the role of TXNIP in inducing insulin resistance and inflammation, we investigated TXNIP expression in the brain of an AD mice model 5XFAD. The expression and function of TXNIP following amyloid beta peptide (Aβ) addition was analyzed *in vitro* in primary astrocytes and microglia, in brain endothelial cell line ERB4 and in differentiated SHSY5Y neuronal cells.
We found that TXNIP is early over-expressed (at 4 months) in the hippocampus of the AD mice. TXNIP over expression parallels enhanced inflammation in AD brain and the first appearance of cognitive decline. TXNIP is also early induced in vitro following Aβ addition in astrocytes, endothelial, and neuronal cells. Aβ addition induces intracellular re-distribution of TXNIP in microglial cells. Because of the role of TXNIP in insulin resistance and inflammation, our study open the way for the understanding the role of risk factors in AD progression. Indeed, TXNIP can be considered the link between diabetes and AD.

P2.110

Elastin (ELN) genetic polymorphisms as a risk factor for familial intracranial aneurysm

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Background: Intracranial aneurysm (IA) is characterized by an abnormal dilatation of the artery that is usually saccular. The rupture of an aneurysm is the main cause of subarachnoid hemorrhage (SAH). Genetic factors may play a role in the pathogenesis of IAs. Among the risk factors are highlighted

genetic polymorphisms of the elastin (ELN) - responsible for arterial elasticity - as well as smoking and drinking.
Objectives: Evaluate the influence of ELN polymorphism and frequency of smoking and alcohol consumption in patients with IA and their families.
Methods: 397 individuals distributed in 6 groups: G1 - 33 (IA familial history); G2- 92 (G1 family); G3- 43 (sporadic IA); G4- 172 (G3 family); G5- 20 (control individuals); G6- 37 (G5 family). DNA was extracted from whole blood, followed by amplification by Polymerase Chain Reaction (PCR). Post-PCR product was subjected to restriction enzyme MvaI and agarose gel electrophoresis. Statistical analysis considered the Fisher exact test or X^2, $P < 0.05$.
Results: The G allele is highlighted in G6 (0.72) compared with G2 (0.57, $P = 0.06$). The prevalence of genotype G / G G6 (54%) compared to G2 (33%, $P = 0.03$). Moreover, the model heterozygous mutant - / A (AA + AG) stood out in G2 (35%) compared to G6 (12%, $P = 0.03$). As for smoking, there is a higher frequency of smokers in G1 (79%) and G3 (61%) compared to G5 (29%, $P < 0.0001$ for both). The same was true for alcoholism (G1-40%, 36% and G3-G5-20%, $P < 0.05$).
Conclusions: The G allele differs ELN between G2 and G6, showing up as a possible protective factor for AI. Moreover, the prevalence of genotype - / A in G2 reveals its potential as a risk factor for AI. Furthermore, smoking and drinking may be associated with the disease.
Keywords: Intracranial aneurysm, genetic polymorphisms, elastin

P2.111

Early postnatal alterations of lumbar motoneurons in SOD1G93A low expressor line mutant mice

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease diagnosed at adulthood characterized by selective and progressive death of motoneurons (Mns). The mechanisms underlying the degeneration of motoneurons are still unknown and there is no curative therapy. Mutations of the Cu,Zn superoxide dismutase (SOD1) enzyme account for 20% of familial and 5% of sporadic ALS cases. Transgenic SOD1 mice are standard animal models of ALS that allow to study the presymptomatic period of this pathology. Changes in excitability of SOD1 Mns have been described in several laboratories in different transgenic lines and preparations. In the $SOD1^{G85R}$ transgenic mouse model, we found, together with excitability changes, early postnatal abnormalities in lumbar network activation and sensorimotor reflexes, and described dendritic overbranching of lumbar Mns in the second postnatal week. In the present study we used the $SOD1^{G93A\ low}$ heterozygous, low expressor line model, recorded in the in-vitro isolated spinal cord preparation in which synaptic environment and dendritic integrity are preserved, to describe the development of passive and active membrane properties and dendritic morphology of spinal L5 $SOD1^{G93A\ low}$ Mns over the critical period P6-P11 when the animals start to weight-bear. Resting membrane potential, input resistance and conductance of WT littermates and SOD1 Mns were similar. Differences on the spike potential amplitude and time course and rheobasic current were seen. When restricted to the P8-P11 period, these differences were enhanced, indicating a different maturation process during the second post natal week. In $SOD1^{G93A\ low}$ Mns, the close correlation between Rheobase and Rn was no longer observed. No differences were found in the frequency gain of repetitive discharge in response to injected currents. The morphology of $SOD1^{G93A\ low}$ Mns, compared to WT litter mates, was characterised by a more complex dendritic arborization resulting from a greater number of primary dendrites per neuron, and not by ramification of these dendrites as seen in $SOD1^{G85R}$ Mns. These results show a disturbed maturation of mutant Mns that differs from that described for $SOD1^{G85R}$ Mns and suggest differences in properties and membrane distribution of sodium channels.

P2.112

The rescuing effects of nicotine for midbrain dopamine neurons are unmasked by persistent depolarizing stimuli

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Epidemiological and experimental evidences indicate that the depolarizing alkaloid nicotine is protective for Parkinson's disease vulnerable dopamine neurons. The underlying mechanism of this effect remains, however, unclear. Using midbrain cultures, we established that depolarizing treatments that either block K^+ channels or activate Na^+ channels are necessary to unmask the rescuing effects of nicotine for dopamine neurons. In addition to providing partial protection *per se*, the adjuvant treatments allowed immediate sensitization of dopamine neurons to the effects of nicotine through a mechanism that required voltage-dependent calcium channel (VDCC) activation and moderate elevations in cytosolic calcium (Ca^{2+}_{cyt}). Using a pharmacological approach, we established that the neuroprotective action of nicotine (but not that of concurrent depolarizing treatments) required the activation of alpha 7-nicotinic acetylcholine receptors and subsequently that of T-type VDCCs. Corroborating this finding, nicotine provided no protective effects to dopamine neurons in midbrain cultures prepared from genetically engineered mice lacking the alpha 7-nicotinic receptor subtype. Signaling studies revealed that Ca^{2+}_{cyt} elevations evoked by nicotine and concomitant depolarizing treatments served to activate a survival pathway involving the calcium effector protein calmodulin and phosphatidylinositol 3-kinase. Collectively,our data support the idea that the protective action of nicotine is activity-dependent and gated by Ca^{2+}_{cyt} .

P2.113

Cell death and forced differentiation of glioblastoma stem cells induced by targeting Nkx2.2 and Ngn2 genes

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Glioblastomas (Gb) are high grade incurable brain tumours. Like many cancers, they contain a variable subpopulation of highly tumorigenic and radio-resistant cells, referred to as cancer stem cells. They typically grow without adhesion to form neurospheres, and they generate several cell types after differentiation. Transcription factors are key elements to regulate stem cells self-renewal / differentiation and the aim of our work was to identify transcription factors involved in these processes. For this purpose, we compared the expression of 7 neural transcription factors between different grades of gliomas and in glioblastomas cell cultures by Q-PCR. These analysis led us to focus on *NKX2.2* and *NGN2* genes whose functions were explored in glioblastoma stem cells culture. We found

that NKX2.2 is required for Gb cells proliferation and survival and NGN2 is able to induces differentiation into electrophysiologically active neurons accompanied with cell death. Nkx2.2 and Ngn2 are 2 new targets for manipulating Gb stem cells differentiation and survival.

P2.114

Genetic-background modulation of core and variable autistic-like symptoms in *Fmr1* knock-out mice

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Despite recent research on autism spectrum disorders (ASD), no animal models with specific construct validity are currently available. *Fmr1*-KO mice, a confirmed model of Fragile X syndrome (FXS), are a promising approach to study ASD. FXS is due to a mutation in the *FMR1* gene leading to a lack of Fragile X Mental Retardation Protein which plays a pivotal role in synaptic functioning. As FXS patients often display autistic symptoms and approximately 30% of them meet the full diagnostic criteria for ASD, these two syndromes may share some common underlying mechanisms.
The diagnosis of ASD is based on a triad of core symptoms including alteration of social interaction and communication, occurrence of repetitive behaviours, and secondary symptoms such as sensory hyper-reactivity, reduced prepulse inhibition (PPI) of the acoustic startle reflex, hyperactivity, sleep-pattern alterations, and epilepsies. Hence, a useful animal model should present behavioural features that resemble at least one of the ASD core symptoms and ideally some secondary ones.
Fmr1-KO mice present many characteristics of FXS, including macro-orchidism, hyperactivity, and cognitive deficits. The validity of the *Fmr1*-KO mouse as a model for ASD has not been convincingly demonstrated yet, although it has been widely employed in the last years. In addition, the *Fmr1*-KO is available on two genetic backgrounds (FVB and C57BL/6J), which may explain some of the conflicting results obtained with these mice up till now.
The aim of this study was to investigate whether *Fmr1*-KO mice are a suitable model for ASD. *Fmr1*-KO and their wild-type littermates on the two genetic backgrounds were examined on a battery of tests modelling the clinical symptoms of ASD including epilepsy. *Fmr1*-KO mice displayed autistic-like core symptoms of altered social interaction and occurrence of repetitive behaviours with additional hyperactivity. These alterations were modulated by the genetic background: it appears that C57BL/6J may be more suitable for further research on core autistic-like symptoms.
In conclusion, the *Fmr1*-mouse line does not recapitulate all of the core and secondary ASD symptoms, but still can be useful to elucidate the neurobiological mechanisms underlying specific ASD-like endophenotypes.

P2.115

Altered neuronal reactivity to cocaine within the reward circuit following subthalamic nucleus deep brain stimulation

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The subthalamic nucleus (STN) is a critical component of a complex network controlling motor associative and limbic functions. Indeed deep brain stimulation (DBS) of STN is the most common surgical therapy applied to Parkinsonian patients, and is very effective in alleviating tremor, rigidity, and hypokinesia. Interestingly it has also been shown that STN lesion or DBS decrease motivation for cocaine while increasing that for food (*Baunez et al., 2005, Rouaud et al., 2010*). However, the cellular substrates of these effects remain unknown, especially within the reward circuit.
The aim of our study was to characterize the cellular consequences of STN-DBS and to elucidate how STN-DBS interferes with acute cocaine effects in the brain. We especially focused on limbic structures (prefrontal cortex, nucleus accumbens, amygdala,..) in addition to some motor areas (dorsal striatum, globus pallidus, ..). Long Evans male rats were subjected to STN-DBS (130Hz, 60 µs, 50-150µA) or not for 30 min before acute ip cocaine injection (15mg/kg) and sacrificed 10 min following the injection. Their locomotor activity was recorded from the start of STN-DBS until the time of sacrifice. We have then analysed the expression of two immediate early genes (*arc* and *c-fos*) by in situ hybridization, and especially catFISH (cellular and temporal analysis of Fluorescent In Situ Hybridization).
Our preliminary data provide evidence that (1) STN-DBS did not significantly modify the expression of *arc* and *c-fos* mRNA in control conditions, except in the globus pallidus; (2) Cocaine acute injection induced *c-Fos* and *arc* gene expression in almost all areas; (3) STN-DBS potentiated cocaine effects on *arc* gene expression in the nucleus accumbens shell and dorsal striatum; but prevented *c-fos* expression in the prefrontal cortex, nucleus accumbens core and shell, dorsal striatum. Our data show that STN-DBS is able to modulate the acute cellular effects of cocaine in the brain, and especially in the limbic structures, but may have opposite responses on *c-fos* and *arc* expression.
Our findings are crucial to determine how STN-DBS exerts its cellular effects to modify behavioural response to cocaine, and to understand if specific neural circuits encode the motivation for food versus cocaine.

P2.116

Examination of amygdala physiology and related cognitive learning in constitutive Ilrapl1 mutant mice, a model of intellectual disabilities

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Constitutive absence of Il1rapl1 has been found to be associated with cognitive disorders in humans. Also, the Ilrapl1 protein associates with both pre and post synaptic partners, its disruption leading to both secretion deficits and defects in the organization of postsynaptic densities (Gambino et al., 2007; Pavlowsky et al., 2010). Here we tested the amygdala-related behavioral and physiological consequences of constitutive Il1rapl1 mutations. Specifically, we found that Il1rapl1 mutation leads to the absence of LTP at a major excitatory input expressing a PKA-dependent form of presynaptic plasticity. At a functional level, we identify the presynaptic deficit as being downstream of AC activation, thus excluding that it could be due to the post-synaptic deficits in dendritic spine shape and function observed in these animals. The poster will also present behavioral results obtained using a classical cued fear conditioning and extinction procedure. All together, our data identify Il1rapl1 expression as being important for proper presynaptic function, possibly determining cognitive abilities. Thus we propose that in addition to reported post-synaptic deficits, presynaptic defects may take an important part to the physiopathology of CDs.

P2.117

A new circuit model for morphine action on dopamine neurons

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Morphine is a potent analgesic with high addictive potential in specific contexts. Our data reveal that *in vivo*, morphine targets the GABAergic tail of the ventral tegmental area, also called rostromedial tegmental nucleus, disrupting the balance between inhibition and excitation on dopamine neurons. These results suggest that the excitatory context of dopamine neurons may tune the addictive potency of morphine.

P2.118

The motor, limbic and associative cortico-subthalamic pathways are not fully segregated in the rat

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Classically, the subthalamic nucleus (STN) is known for its role in the cortico-basal ganglia-thalamocortical motor circuit. This is evidenced by the improvement of motor disability in Parkinson's disease by STN high frequency stimulation. However, some patients suffer from cognitive and emotional changes. These side effects are most likely caused by current spread to the cognitive and limbic territories in the STN. It remains unclear whether these different circuits are segregated or integrated within the STN. To address this, we performed an electrophysiological study in the rat and investigated the responses of individual STN cells to the electrical stimulation of different cortical regions. We hypothesized that these circuits are integrated and that therefore one STN neuron would respond to stimulation of both motor and non-motor regions. After baseline recordings, successively the motor (MC), cingulate (CG), pre-limbic (PrL), infra-limbic (IL) and agranular insular (AI) cortices were stimulated. Peristimulus time histograms (PSTHs) were generated and responses were measured. The responses were calculated per cortical stimulation site, as were the number of overlapping responses to the different cortical areas. The STN neurons had an average spontaneous firing rate of 8.3 (SEM 0.45) Hz. Within the STN 79% of the neurons responded to stimulation of the MC, CG, PrL, IL and AI. Of these neurons, 37% showed a triphasic response, 10% had only an early excitation, while 37% had only a late excitation and 3% a combination of the early and late excitation, 13% showed only the STN specific long-lasting inhibition. Most neurons responded to only one cortical region, but 36% of the neurons showed an early response to both MC and AI, which originates from the hyperdirect pathway, even more neurons responded with a late excitation to stimulation of different cortical regions. These data suggest that in the rat the different circuits are not fully segregated but partially integrated. In addition, it seems that the hyperdirect pathway is organized in a more segregated way, than the indirect pathway.

P2.119

Lithium application interferes with hippocampal development but does not involve GSK3beta

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Most hippocampal dentate granule cells are born early postnatally. Their migration is controlled by Reelin, a protein secreted by Cajal-Retzius cells in the marginal zone of the dentate gyrus. An identified component of the Reelin signalling cascade is the enzyme glycogen synthase kinase (GSK)3 beta. Activity of GSK3beta is downregulated by Reelin or by application of pharmacological inhibitors, such as lithium chloride (LiCl). Lithium is also known as a drug for the treatment of neurological disorders thought to be causally related to developmental neuronal migration defects. In a first study, we investigated whether LiCl interferes with developmental processes known to be regulated by Reelin signalling. For this purpose we used hippocampal slice cultures. Different concentrations of LiCl were added to the incubation medium. With increasing concentrations of LiCl, we observed a loss of the proper arrangement of the dentate granule cell layer, suggesting that lithium interfered with the positioning of dentate granule cells. Next, we investigated whether LiCl treatment of the cultures affected Reelin content in the incubation medium. We found that Reelin content decreased with increasing LiCl concentrations. We also studied the effects of lithium on the morphology of Reelin-secreting Cajal-Retzius cells. Application of LiCl in the range of therapeutic concentrations induced neurite retraction of Cajal-Retzius cells in a dose-dependent manner. In order to compare the specificity of the LiCl-induced Reelin decrease, these experiments were repeated by replacing LiCl with sodium chloride (NaCl) or SB415286, a specific inhibitor of GSK3beta. No similar effects were observed neither with NaCl nor with SB415286, indicating that these effects were lithium-specific, but were not induced by GSK3beta inhibition.
In ongoing experiments, we now study whether or not similar migration defects as observed in vitro are also seen in vivo in adult animals. Mice aged one month were injected with 0.6 M LiCl or NaCl for 15 days by daily i.p. injections. 24h after the last treatment, the animals were perfused, the brains paraffin-embedded and sectioned, and the sections processed for cresyl violet staining.
(supported by the DFG: SFB 780 to M.F. and FO 223/6-1 to E.F.)

P2.120

Anorexia coexists with psychoactive and rewarding effects when mediated by serotonin 4 receptors in the nucleus accumbens

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Anorexia imbalances homeostasis and often triggers death, suggesting that a control system although unidentified, competes with signaling of the energy request. It may include the reward system because anorexia induced by serotonin 4 receptors (5-HTR_4), in the nucleus accumbens (NAc), uses a peptide connected to addiction (CART: cocaine- and amphetamine-regulated transcript). Whether common neuronal mechanisms bind inevitably anorexia to rewarding and psychoactive effects is, however,

unknown. Considering that the absence of 5-HTR_4 decreased anorexia induced by the drug ecstasy (MDMA), we tested whether a lesser anorexia comes along with a lesser psychoactive and rewarding effect. The psychoactive effect (hyperactivity) and stereotyped behavior induced by ecstasy were reduced in mice lacking 5-HTR_4 (KO) or treated with a 5-HTR_4 antagonist (RS39604). Hypolocomotion was associated with diminished 5-HT turnover index in the NAc. Injecting RS39604, in the NAc, reduced MDMA-induced hyperactivity. Infusing RS39604, in the NAc, suppressed the rewarding effect of MDMA. Considering that anorexia induced by MDMA but not, by the non-rewarding fenfluramine, is maintained in an addiction-related model: the 5-HTR_{1B} KO mice, we thought that the absence of 5-HTR_{1B} induced an over-expression of 5-HTR_4 and CART in the NAc. The response was positive. In the absence of 5-HTR_{1B}, inactivating 5-HTR_4 suppressed anorexia induced by MDMA. A mimicked over-expression of 5-HTR_4 using viral transfer in the NAc increased CART and satiety. Findings indicate that an impaired activity of 5-HTR_4, in the NAc, underlies the coexistence of anorexia and hyperactivity, which may likely challenge homeostasis because of the involvement of these receptors in reward.

P2.121

Subchronic deep brain stimulation of centromedian-parafascicular complex alleviates movement disorders linked with Parkinson's disease and its treatment by L-DOPA but not dystonia in experimental rodent models

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The thalamic centromedian-parafascicular (CM/Pf) complex, mainly represented by Pf in rodents, is emerging as a key regulator of basal ganglia function. It has been proposed as a target for the neurosurgical treatment of movement disorders. In this context, we recorded the activity of Pf neurons and investigated the effects of its high frequency stimulation (HFS; 130 Hz; 6 days) in a rat model of Parkinson's disease and L-DOPA-induced dyskinesias. Lesioned rats present decreased cytochrome oxidase subunit I (COI) gene expression in the Pf, suggesting reduced neuronal metabolic activity. In vivo electrophysiological recordings of Pf neurons show shorter bursts with a reduced number of spikes in the burst but an increased intraburst frequency. Lesioned rats receiving chronic dyskinesiogenic L-DOPA treatment present in “OFF” L-DOPA (12 hours washout) a tendency towards normalization of thesechanges, whereas in “ON” L-DOPA they show an exacerbation of the intraburst frequency. These data reveal marked changes in the discharge pattern of Pf neurons in parkinsonian state and under L-DOPA, supporting the involvement of CM/Pf in movement disorders. HFSof Pf, while normalizing COI in Pf, has a moderate anti-akinetic action but totally reverses lateralized neglect, suggesting potent action on sensorimotor integration. Moreover, unlike HFS of the subthalamic nucleus (STN), Pf-HFS does not induce per se dyskinesias and significantly alleviates L-DOPA-induced forelimb dyskinesia. In situ hybridization analysis of the gene expression of markers of neuronal activity showed that Pf-HFS has a more widespread action than STN-HFS on the basal ganglia network and pointed to the preferential relationships between Pf and entopeduncular nucleus(EP) as a substrate for the differentimpact of HFS of the Pf versus STN on motor dysfunction. Since EP is a target for the neurosurgical treatment of dystonia, we next examined the impact of Pf stimulation (6 days) on the dt^{sz} hamster model of dystonia. Dyskinetic scores were not significantly alleviatedby Pf stimulation at 25, 60 or 130Hz, suggesting no short term efficiency of this target for the management of dystonia.
This work was supported by FRC, France Parkinson, CNRS, Université de la Méditerranée. LJ has anMENRTgrant.

P2.122

Characterization of heterotopic cells in the hippocampal CA3 region of doublecortin knockout mice

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Lissencephaly “smooth brain” consists of a set of rare brain disorders characterized by the lack of normal cortical convolutions and resulting in severe intellectual disability and epilepsy. In type 1 lissencephaly patients the neocortex consists of 4 disorganized layers of neurons, is very thick, and the hippocampus is also disorganized. 70-80 % of cases are due to mutations in either the LIS1 or doublecortin (DCX) genes. *Dcx* knockout (KO) mice show no major isocortical abnormalities, but have discrete hippocampal and interneuronal defects. They are also hyperactive, exhibit spontaneous convulsive seizures and *in vitro* the dysplastic CA3 region possesses a lower threshold for epileptiform events (Nosten-Bertrand et al., 2008). These mice therefore provide a useful model to further study how hippocampal lamination defects underlie the genesis of epileptiform activities. Towards this aim we are better characterizing abnormally positioned, or 'heterotopic' hippocampal neurons in *Dcx* KO mice. An abnormal double layer of neurons is apparent in the CA3 region, instead of a single layer present in wild type (WT) littermates. Mislocalized neurons may have aberrant connections and function abnormally, contributing to epilepsy and behavioral deficits. In this study, we have been characterizing the two *Dcx* KO CA3 layers by molecular and cellular studies:
1) by separating the two pyramidal cell layers in the CA3 region at P0 using laser capture microdissection and analyzing gene expression in each layer compared to WT by transcriptome studies;
2) by analyzing the evolution of the two cell layers during development using immunohistochemistry; and
3) by cataloguing defects observed at the subcellular level using electron microscopy studies.
These combined data are contributing to our understanding of the development of hyperexcitability in the Dcx KO hippocampus.

P2.123

Oligophrenin1 loss of function in mice impairs presynaptic long term plasticity and cognitive learning through correlative PKA over-activity

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Loss of Oligophrenin-1(OPHN1) function has been shown to be responsible for a syndromic form of mental retardation. A mouse model for the ophn1 deficiency have been recently described, these mice largely reproduce the phenotype observed in patients with abnormalities in behavior and dilated

cerebral ventricles. Here, we show that in some specific brain regions, including the hippocampus and the cerebral cortex, the absence of OPHN1 was associated with a several-fold increase in PKA activity, a master signaling molecule controlling presynaptic release probability. Accordingly, *ophn1* mutation leads to the absence of LTP at these two major excitatory inputs expressing PKA-dependent forms of presynaptic plasticity. Interestingly, some aspects of contextual and cued fear conditioning performed on *ophn1* mutant mice showed that both hippocampal- and amygdala-related learning abilities were affected. At the molecular level, we identify the presynaptic deficit as being downstream of the Adenylate cyclase activation, thus excluding that it could be due to the post-synaptic deficits in dendritic spines shape reported in this model. Thus, our data identify Oligophrenin1 expression as an important compound for proper presynaptic function.Thus we propose that in addition to reported post-synaptic deficits, presynaptic defects may take an important part to the pathophysiology of cognitive disorders.

P2.124

Chronic insomnia model after *in utero* exposure to antidepressant treatment in mice

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Serotonin selective re-uptake inhibitors (SSRI) are of increasing use in pregnant women, but their long-term effects on the child are largely unknown. Such putative effects on sleep regulation were examined in the adult progeny of mice treated with SSRI during gestation.
Methods: C57/BL/6 gestating females were treated from the first until the last day of gestation with fluoxetine (18 mg/kg/day in drinking water). Control animals drank plain water. Polygraphic sleep recordings were made at 8-16 weeks of age in the progeny exposed to prenatal fluoxetine treatment (FLX) or water (Control). Vigilance states (wakefulness, slow wave sleep and paradoxical sleep) were analyzed in basal conditions, and after a restraint stress challenge of 90 minutes duration.
Results: In basal conditions, both male and female FLX mice exhibited an increase in the duration of wakefulness (+16% and +10% of that in the control groups, respectively), and a concomitant decrease in slow wave sleep (-16.8% and -11%). Paradoxical sleep (PS) duration was not modified.
After 90 minutes of restraint stress, control mice displayed an increase of PS amounts appearing 2 hours after the end of the stress period and lasting for 6 hours (+54% and +135% of no stress conditions for males and females, respectively). In the FLX groups, females exhibited the same sleep modifications as controls. FLX male mice presented a smaller and non significant increase of PS amounts (+22%), but this increase was delayed so as to begin 4 hours after the end of stress period and to last for about 8 hours thereafter.
In conclusion, *in utero* exposure to fluoxetine in mice promotes persistent disturbances in sleep organization that evoke those of chronic insomnia and seem to be gender-dependent, since only the male progeny is strongly affected. These modifications are associated to a delayed response of sleep to stress. These results are consistent with previous data published by Armitage and Hoffmann (2001) that reported stronger sleep disturbances in depressed men than women.
Reference: Armitage R and Hoffmann RF (2001) Sleep EEG, depression and gender. Sleep Med Rev. 5:237-246.

P2.125

Assessing the role of vitamin D in Alzheimer's disease

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Vitamin D, the steroid hormone of sunlight, is increasingly recognised as a neurosteroid with potent neuro-immunomodulatory and neuroprotective effects within the nervous system. In patients with Alzheimer's disease (AD), i) a vitamin D deficiency, ii) decreased hippocampal mRNA levels of the vitamin D receptor (VDR) and iii) VDR polymorphisms have been reported1,2,3. It has also been shown that developmental vitamin D deficiency is associated with abnormal development and functioning of the central nervous system4,5. This deficiency alters the expression of a number of genes and proteins and we found that nearly half of them were associated with Alzheimer's disease. The aim of this study is to assess the effects of vitamin D supplementation in an Alzheimer's mouse model (5xFAD). Analysis will be based on i) quantification of histological markers of AD, amyloid peptide accumulation and neuroinflammation, after 10 weeks of vitamin D supplementation, ii) pangenomic transcriptome study of the genes and metabolic pathways affected by vitamin D supplementation and iii) in vitro studies designed to assess the effect of vitamin D on survival, cell cycle, neurite outgrowth and inflammation in primary neurone and astrocyte cultures. Preliminary results, based on the analysis of DNA microarray data, suggest that vitamin D regulates the expression of a number of genes involved in inflammation, synaptic transmission and mitochondrial functioning, pathways which are often affected in AD pathology.

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P2.126

Inhibition of the constitutive activity of serotonin 4 receptors in the nucleus accumbens produced binge eating

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The G-protein-coupled serotonin 4 receptors (5-HTR_4) may undergo agonist-independent isomerization from an inactive (R) to an active (R*) state (e.g. constitutive activity: accumulation of R*). Inverse agonists enhance R state; agonists enrich R* form while antagonists equilibrate R/R*, in culture cells. We found previously that activating 5-HTR_4, in the nucleus accumbens (NAc), a part of the reward system, decreases feeding via the cAMP/PKA/CART (cocaine- and amphetamine-regulated transcript). Infusing a competitive antagonist of 5-HTR_4 provoked hyperphagia in fed, but not in food-deprived mice and, did not change CART level. Here, we tested the effects of a 5-HTR_4 inverse agonist and found that it decreased the levels of cAMP and CART in the NAc and, produced “binge eating” in fed mice. The inverse agonist did also markedly increase feeding in food-deprived mice, which was not associated with decreased level in CART, but with an increased level in the orexigenic neuropeptide Y mRNA in the NAc. “Binge eating” following the inverse agonist treatment is not observed when combined with the competitive antagonist. Findings provide evidence that oscillation from R* to R state of 5-HTR_4 is respectively implicated in the control of CART and NPY in the NAc, related to an oscillation from an excessive inhibition/activation of feeding. This is therefore conceivable that an abnormal R/R* equilibrium underlies the coexistence of anorexia/bulimia, which remains to be further elucidated because it growingly induces death in adolescents in industrialized countries.

P2.127

Is blood-brain barrier disruption related to chronic epilepsy?

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In a previous study, we studied blood-brain barrier (BBB) disruption in the lithium-pilocarpine model of chronic epilepsy. We measured the expression of angiogenic factors and their receptors and checked tight junction integrity by staining zonula occludens 1 (ZO-1) protein. We showed that angiogenesis was associated with a permanent BBB disruption, which is known to contribute to epileptogenesis.
To assess that chronic epilepsy is related to BBB disruption, we investigated vascular changes in the chemical kindling model, which induces a progressive hyper-excitability without spontaneous seizures. This model was obtained by repetitive injections (every 48h) of sub-convulsive dose of pentylenetetrazol (PTZ, i.p.). The progressive aggravation of seizures was measured by EEG recording and behavioural observation according to Racine scale. Rats were sacrificed at different time-points, and vascularisation, tight junction integrity and angiogenic factors were evaluated.
At first, we showed that seizures started synchronously in different structures (hippocampus, thalamus and cortex). At score1 we observed:
i) a peak of VEGF expression,
ii) a significant neovascularisation,
iii) a decrease of ZO-1 staining along microvessels.
At later scores, we noticed:
i) a return of VEGF expression back to basal level,
ii) no more increase in vessel density,
iii) a regular staining of ZO-1.
Therefore, we showed that angiogenic processes and BBB disruption are transient in this model. These surprising results strengthen the hypothesis of a correlation between BBB permeability and spontaneous seizures.

P2.128

Montpellier vectorology facility: lentiviral vector production

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Re-engineered viruses are a widely used tool for nucleic acid delivery, transgene expression, and gene therapy. Among the various available viral vectors, lentiviral vectors allow for long term, stable *in vitro* and *in vivo* gene transfer. They can transduce both dividing and quiescent cells in a stable manner. Their tropism can be modified by changing their envelope glycoprotein. Based in Montpellier, the Vectorology facility provides a service of lentiviral vector production to the scientific community.
Web site: http://ifr3.igf.cnrs.fr/plateforme/index.php?page=vectorologie
The service includes:
1- Purification and concentration of viral particles produced after co-transfection of the gene transfer plasmid (supplied by the customer), the envelope plasmid and the packaging plasmid into HEK-293T cells.
2- Titration of the lentiviral particles. The p24 capsid protein concentration is measured by ELISA to assess the total amount of particles produced. When the transfer vector bears a reporter gene (eGFP, DsRed, etc.), we perform FACS analysis to determine the infection titer.
3- Finally, a safety test is performed to check the replication-defectiveness of the viral particles.
We also provide support for customers to define the best experimental strategies for their research programs (pseudotypes, use of specific promoters, choice of the best vector, etc.).

A research and development program has also been set up in order to optimize the current vectors and investigate the use of other viral systems for gene transfer.
The Vectorology facility is open to academic and private partners.

P2.129

Psychosocial impact of Parkinsonian dysarthria: relevance of a new self-evaluation scale

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The psychosocial impact of motor speech disorders, as for example dysarthria, is well-documented in the literature, including the speaker's point of view. However, dedicated tools able to objectify this concept are still missing. In order to overcome this deficit, Walshe *et al.* (2009, Int J Lang Comm Disord, 44, 693-715) developed the Dysarthria Impact Profile (DIP), an auto-questionnaire that could have the potential to fill this need.
Our objective was to adapt a French version of the DIP and to assess its relevance for the description of the psychosocial impact of dysarthria in Parkinson´s Disease (PD). The scale was administered to 7 PD patients (mean age = 74.4 ± 8.8 years), presenting with varying degree dysarthria, and 7 age-matched control subjects (mean age = 73.0 ± 4.4 years). The psychometric properties of the DIP were estimated. For statistical comparisons, other self-evaluation questionnaires were also administered to the PD population: a global and PD-specific quality of life questionnaire (Parkinson's Disease Questionnaire - PDQ-39; Peto *et al*., 1995, Qual Life Res, 4, 241-248) and the Voice Handicap Index (VHI; Jacobson *et al.*, 1997, Am J Speech Pathol, 6, 66-70). A motor evaluation, using the part III of the Unified Parkinson's Disease Rating Scale (UPDRS), was also carried out.
The DIP was able to significantly differentiate the two control and PD populations: DIP total score for PD patients was significantly lower than for controls (Mann Whitney U Test, $p< 0,025$). The DIP showed good internal consistency (Pearson r test = 0.96) but variable intra-rater fidelity according to the different dimensions explored by the DIP. Data from PD patients revealed a strong correlation with those of the VHI (Pearson r test = 0,85), "gold standard" questionnaire for vocal deficits.
Our data demonstrated and statistically objectified that disorders of oral communication in PD patients have a debilitating impact on daily living activities. The scale still ought to be refined and its psychometric properties confirmed on a larger population. Once validated, the DIP may represent a useful tool for clinicians and researchers when assessing the psychosocial impact of dysarthria, in addition to the standard evaluation.

P2.130

Protein chip technology for the rapid detection of botulinum neurotoxin activity

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Botulinum neurotoxins (BoNT's) are potent poisons but also precious therapeutic agents for the treatment of a wide range of affections. BoNT/A, B and E produce proteolytic cleavage of the SNAREs (VAMP2 / synaptobrevin and SNAP-25) that ensure synaptic vesicle fusion and acetylcholine release from motoneurone terminals. Our aim is to develop rapid and sensitive in vitro assays for the endoprotease activity of botulinum neurotoxins in environmental or human samples, to replace the in vivo mouse assay, currently in routine use. Using synaptic vesicles as substrate, endoproteolytic

cleavage of VAMP2 was detected by surface plasmon resonance in 10min with 2pM native BoNT/B. Contamination of liquid food products with low picomolar amounts of BoNT/B was revealed within 3h. BoNT/B activity was detected in 5 h in all sera from patients with type B botulism, but not in healthy controls or patients with other neurological diseases. We have now extended this protein chip assay to all BoNT serotypes affecting humans. This novel method has thus potential applications in food safety, serological diagnosis of human botulism or in monitoring the quality of pharmaceutical preparations.

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P2.131

Early alteration of hippocampal somatostatin systems in THY-Tau22 mice

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The vulnerability of telencephalic somatostatin (SRIF) interneurons in Alzheimer's disease (AD) is associated to functional impairments in learning and memory systems. Recent data also suggest that Tau phosphorylation impacts on telencephalic SRIF interneurons. In the present study, SRIF peptide expression and receptors distribution were analyzed in a relevant experimental model for AD displaying progressive development of the Tau pathology, the THY-Tau22 strain. Mice were sacrificed at three different time points : 4 months of age when hippocampal Tau pathology is starting and associated with slight cognitive impairments, at mi-course (7 months) and at 11-15 months when Tau pathology is maximal and cognitive alterations are frank. By qRT-PCR, SRIF mRNA levels in the hippocampus were consistently lower at all time points in transgenic mice as compared to wild type controls (THY-Tau22 : 1059±114, 1042±115, 652±36 vs 1167±106, 1222±111, 768±58 controls : , $x10^{-7}$ mRNA copy number, n= 5 in each group $p< 0.05$, ANOVA). Cortistatin mRNA levels were not affected. Among the five sst receptor subtypes, only sst4 receptors were decreased in THY-Tau22 hippocampus as measured (i) at the mRNA level by qRT-PCR (THY-Tau22 : 202±19, 157±36, 84±5 vs controls : 253±28, 221±11, 120±11 $x10^{-7}$ mRNA copy number , n= 5 in each group $p< 0.001$, ANOVA) and (ii) at the protein level by binding experiments with $^{125}I\ Tyr_{25}\ SRIF_{28}$ autoradiography in the CA1, using the selective sst4 agonist L-803087 (THY-Tau22 : 6804±441, 5085±305, 3517±525 vs controls : 6892±615, 6625±439, 5346±364 nC1/mg protein, n= 3-4 in each group $p< 0.01$, ANOVA). These results indicate that SRIF interneurons and sst4 receptors, their main target in the mouse CA1, are affected early during the progression of Tau pathology and that this decrease is consistently observed overtime in THY-Tau22.

This work is supported by ANR-2010-MALZ-003-01

P2.132

Impact of early life stress on alcohol consumption and on the short- and long-term responses to alcohol in adolescent female rats

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We examined the interaction between early life stress and vulnerability to alcohol in female rats exposed to prenatal restraint stress (PRS rats). First we studied the impact of PRS on ethanol preference during adolescence. PRS slightly increased ethanol preference *per se*, but abolished the effect of social isolation on ethanol preference. We then studied the impact of PRS on short- and long-term responses to ethanol focusing on behavioral and neurochemical parameters related to depression/anxiety. PRS or unstressed adolescent female rats received 10% ethanol in the drinking water for 4 weeks from PND30 to PND60. At PND60, the immobility time in the forced-swim test did not differ between PRS and unstressed rats receiving water alone. Ethanol consumption had no effect in unstressed rats, but significantly reduced the immobility time in PRS rats. In contrast, a marked increase in the immobility time was seen after 5 weeks of ethanol withdrawal only in unstressed rats. Hippocampal levels of neuropeptide Y (NPY) and mGlu1a metabotropic glutamate receptors were increased at the end of ethanol treatment only in unstressed rats. Ethanol treatment had no effect on levels of corticotropin-releasing hormone (CRH) in the hippocampus, striatum, and prefrontal cortex of both groups of rats. After ethanol withdrawal, hippocampal levels of mGlu1 receptors were higher in unstressed rats, but *lower* in PRS rats, whereas NPY and CRH levels were similar in the two groups of rats. These data indicate that early life stress has a strong impact on the vulnerability and responsiveness to ethanol consumption during adolescence.

P2.133
Immunotherapy for stroke

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Background: Tissue-type plasminogen activator (tPA) is the only available emergency treatment for acute ischemic stroke. tPA (endogenous and exogenous) has two faces in acute ischemic stroke: beneficial fibrinolysis in the vascular bed, but damaging effects on the neurovascular unit and the brain parenchyma. All together, the limitations lead to only 10% of stroke patients being treated with tPA, among which only 40% actually display neurological improvement. To improve this profile, we aimed at developing a novel treatment strategy for stroke, relying on antibodies targeting the pro-neurotoxic effects of tPA.
Methods and findings: We have previously shown that tPA can worsen ischemic brain damages in rodents, thanks to its ability to interact with the amino terminal domain of the NR1 (ATD-NR1) subunit of glutamatergic NMDA receptors. Accordingly, we first used recombinant protein engineering and immunoglobulin production technologies in order to obtain antibodies that specifically recognize the ATD-NR1 in mice and humans. In vitro, these antibodies prevented the potentiating effect of tPA on NMDA glutamatergic receptors, without altering basal neurotransmission. Then, based on a dedicated model of murine thrombo-embolic stroke coupled to MRI, NIRF, confocal microscopy, behavior assessments, we demonstrate the efficiency of immunotherapy in a complete pre-clinical screen: after a single administration alone or with late tPA-induced thrombolysis, antibodies dramatically reduce ischemic brain injuries, improve long-term neurological outcome and, in parallel, attenuate critical ischemic events including blood-brain barrier leakage and activation of matrix metalloproteinases -3 and -9, and the Platelet-Derived Growth Factor-CC pathway.
Conclusions: Our immunotherapy strategy is thus able to limit ischemic histological and neurological damages in mice, and extends the therapeutic window of tPA-driven thrombolysis. Thus, the prospect

of this immunotherapy is an extension of the range of treatable patients, whether used as a monotherapy or, in combinations, to extend the therapeutic window for thrombolysis.

P2.134

Olesoxime promotes oligodendrocyte maturation and remyelination in mouse models of demyelination

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Multiple sclerosis is a neurodegenerative disease characterized by episodes of immune attack of oligodendrocytes leading to demyelination and progressive functional deficit. One therapeutic strategy could consist in stimulating the spontaneous regenerative process observed in some patients. Myelin regeneration requires endogenous oligodendrocyte progenitors migration and activation of the myelination program at the lesion site. In this study we have tested the ability of olesoxime, a neuroprotective agent, to promote remyelination in the rodent adult central nervous system *in vivo*. Here, we show that olesoxime promotes myelination in naïve animals not only during development but also in the adult brain. Using rat primary cultures and mouse forebrain slices we demonstrate that this compound directly promotes OPC maturation and myelin formation without affecting their proliferation or migration. Furthermore, using both a focal (LPC) and a widespread (cuprizone) mouse model of demyelination, we show that olesoxime favors OPC maturation and remyelination. This effect allows to reduce the lesion load assessed by T2-weighted MRI in the LPC model, and to enhance motor skill recovery in the cuprizone model.
Our observations further prove that oligodendrocyte maturation and myelin synthesis can be beneficial for myelin repair and functional recovery. It provides the proof of concept that olesoxime is a potential candidate for the treatment of demyelinating diseases.

P2.135

Chronic Agomelatine treatment corrects the abnormalities of the sleep/wake cycle and the circadian rhythm of motor activity induced by prenatal restraint stress in adult rats

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The rat model of prenatal restraint stress (PRS) is particularly valuable for the study of the mechanisms involved in the pathophysiology of anxiety and depression since adult PRS rats show endocrine and behavioral abnormalities that are corrected by antidepressant medication. PRS induce alterations of circadian rhythms as a phase advance of both circadian corticosterone secretion and locomotor activity. Moreover, PRS rats present a prolongation of corticosterone response to stress

which is correlated to an increase REM sleep duration and fragmentation. Here we have considered the influence of two weeks manipulation on the sleep structure of control and PRS animal. For that, animal were surgically implanted with chronic electrode for tow EEG and one nuchal EMG. After two weeks of recovery and habituation to the sleep recording procedure, PRS and control rats were submitted for two weeks to a daily manipulation including intragastric saline gavage (chronic stress, CS) or were left undisturbed. PRS by itself reduced non-REM sleep duration and increased REM sleep duration and fragmentation. Two weeks of CS amplified the effect of PRS on non-REM and REM sleep. Moreover, the two weeks of CS induced an increase of wake duration in PRS animal and a decrease of REM sleep duration in control animal. Finally, the CS increased the number of wake, non-REM and REM sleep episodes in PRS animal indicating an increased sleep fragmentation. In conclusion, PRS animal show sleep alterations that are amplified by CS. These observations reinforce the concept of a strong linkage between sleep quality and stressing events and enforce to consider the circadian homeostasis in the mediation of the consequences of chronic stress on neuroplasticity in PRS animal.

P2.136

GABAergic hypothesis in major depressive disorders demonstrated by paired-pulse TMS

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Neurophysiological hypotheses of major depressive disorders (MDD) are generally based on serotonin, norepinephrine or dopamine impairment. However, the neuropathophysiology is still not fully understood and recent publications highlight the involvement of GABA in depressive disorders (Levinson et al, 2010; Croakin et al, 2010). Transcranial magnetic stimulation (TMS) is a good noninvasive tool allowing the observation of GABAergic and glutamatergic intracortical pathways. Studies using TMS applied over the motor cortex have shown modifications in cortical excitability particularly in intracortical inhibition (ICI) processes (for review, Croakin et al, 2010).
Our study was aiming to observe cortical excitability in MDD patients compared to healthy subjects. Fourteen medicated inpatients with MDD (DSM-IV diagnosis) were included (8 right-handed males and 6 females, 2 of whom left-handed; the mean age was 51±15 years). The control group included 9 subjects without any psychiatric disorder history or medication (mean age 34 ±11 years).
Results were analyzed with Wilcoxon non-parametric statistical test. We found a decrease in patients' baseline compared to healthy subjects. Intracortical inhibition (ICI) showed a group effect for each interstimuli interval (2 and 4 ms) and each hemisphere, with a significant decrease in patients *vs.* controls. No group effect was observed for cortical silence period (CSP) measures.
These results clearly show an ICI deficit in MDD, which may be related to a $GABA_A$-mediated dysfunction (Kujirai et al, 1993). On the other hand, there is no group effect on CSP which is known to be dependent on $GABA_B$ receptors activation (Siebner et al, 1998). Overall, our results suggest a deficit in $GABA_A$ functioning, whereas $GABA_B$ would be normal or with a tendency to be more activated. This different dysfunction of GABA receptors may be related to a more particular dysregulation of $GABA_A$/$GABA_B$ interaction, $GABA_B$ having a regulator function over neurotransmission; or to a more specific chloride channel dysfunction linked to the $GABA_A$ receptor. It is important to note that medication is a limitation to our results and interpretations. Nevertheless, the GABAergic hypothesis in depression must be seriously considered.

P2.137

A polysialic acid mimetic peptide promotes functional recovery in rodent models of spinal cord injury

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Patients with spinal cord injury (SCI) usually suffer from permanent and often devastating disabilities. Recovery after SCI is impaired by a non-permissive environment and by the intrinsic poor ability of neurons to support process outgrowth. Numerous studies have demonstrated the therapeutic value of the linear homopolymer of alpha 2,8-linked sialic acid (PolySialic Acid, PSA), on the regrowth of injured axons and on the prevention of the excitotoxic cell death under pathological conditions.
The Rougon laboratory has previously developed a new innovative gain of function approach using a cyclic PSA-NCAM mimetic peptide (PR21) to modulate PSA functions (Torregrossa et al., 2004).
In the present non clinical study we investigated the therapeutic potential of PR21. Following transection or contusion SCI in rodents, PR21 improved locomotor performance. In the transection model, delivery of PR21 directly at the lesion site decreased the time of return to continence. At the cellular level, we observed that PR21 was able to increase serotoninergic axonal density at and caudal to the lesion site, and to act on astrocytes by decreasing reactive gliosis.
In an *in vitro* model, PR21 demonstrated a protective effect against glutamate cytotoxicity.
Finally, in a preliminary chronic toxicological study, PR21 intravenously delivered to rats at one hundred times the therapeutic dose used in the contusion rat model, did not induce mortality or behavioral adverse effects.
Our data supports the notion that PSA-NCAM is an important factor to consider when treating spinal cord injury and points to PR21 as being a promising drug candidate.

P2.138

Neuroadaptation induced by early life stress in the nigrostriatal motor circuit: prenatal restraint stress as a model of compensatory parkinsonism

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Early life stress triggers a pathological epigenetic programming that leads to persistent changes in synaptic plasticity and behavior. We used adult rats subjected to prenatal restraint stress (PRS rats) to examine the impact of early life stress on the function of the basal ganglia motor circuit in the adult life. Male adult PRS rats (5-7 months of age) showed a marked reduction in depolarization-evoked 3H-dopamine release in superfused striatal synaptosomes, with no changes in 3H-noradrenaline, glutamate and GABA release in the striatum, and 3H-dopamine release in the hippocampus and prefrontal cortex. The reduction in 3H-dopamine release was associated with an increase in striatal levels of dopamine, DOPAC, and homovanillic acid (which was more prominent in the right striatum), and profound changes in the striatal levels of noradrenaline and serotonin. These changes have been interpreted as compensatory mechanisms aimed at rescuing the striatal motor programming in spite of the reduction in dopamine release. It is quite likely that adaptive changes might also develop in striatal projection neurons of PRS rats. Accordingly, PRS rats showed a robust reduction in haloperidol-induced catalepsy, and an increase in apomorphine-induced stereotypes. We found that these

animals had a greater expression of KCC2, a membrane transporter that pumps chloride ions out of neurons, thereby allowing chloride influx in response to GABA-receptor activation. The increase expression of KCC2 might reinforce the GABA-mediated inhibition of striatal projection neurons of the indirect pathway, thereby limiting the hyperactivity of the pathway resulting from the reduction in dopamine release. We are currently examining the expression of receptors that regulate the activity of striatal projection neurons, such as mGlu4 and mGlu5 metabotropic glutamate receptors, A2A adenosine receptors, and CB1 cannabinoid receptors. We conclude that early life stress has a strong impact on the function of the striatal motor circuit, and that PRS rats might represent a model for the study of neuroadaptive changes occurring in response to a defect in nigro-striatal dopaminergic transmission.

P2.139

In vivo measurement and *in silico* interpretation of changes in the shape of hippocampal spikes during the epileptogenesis process

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Acquired epilepsies begin with an initial insult leading to the recurrence of spontaneous seizures. Drug-resistance affects 30% of patients. Temporal lobe epilepsy (TLE) represents the most resistant form of epilepsy since 90% stay refractory to any medication.
The epileptogenesis process is still largely unknown, mainly because it often involves an initial seizure-free period where no clinical monitoring is made. Nevertheless, during this latent period, some electrophysiological markers such as hippocampal epileptic spikes (HES) are observed in local field potentials few days after the initial lesion in various animal models of TLE. Although some recent studies have suggested that changes occur in the frequency of these HES, it is still unclear whether the shape of these events evolves over the latent period and how this evolution, if any, can be related to underlying pathophysiological mechanisms.
A general objective in our group is to develop prevention procedures for refractory forms of epilepsy. A natural approach would be to act during the epileptogenesis process. Here, this specific study aims to characterize and to understand, in terms of possible alterations of underlying neuronal networks, the evolution of HES during epileptogenesis. This study was performed in an *in vivo* mouse model of mesial TLE. This model consists in a micro-injection of kaïnic acid into the hippocampus which induces both structural (sclerosis) and functional (abnormal intracerebral electroencephalographic signals or iEEG) changes. We have developed an automatic method to detect HES in iEEG, and to measure their amplitude and duration. In addition, a detailed computational model of the hippocampus (CA1), was used to physiologically interpret observed modifications in terms of excitability-related parameters.
Over the latent period, results showed a significant increase of the amplitude and duration of HES. In the computational model, these changes could be explained by
i) an increase of AMPAergic currents,
ii) a decrease of GABAergic currents and
iii) an increase of the $GABA_A$ reversal potential.
Considering these electrophysiological changes and, hence, identifying some pathophysiological causes, could lead to modulate or perhaps to stop the epileptogenesis process.

P2.140

Mitogen- and stress-activated protein kinase 1-induced neuroprotection in Huntington's disease: role of chromatin remodeling at the PGC-1-alpha promoter

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Huntington´s disease (HD) is a neurodegenerative disorder due to abnormal polyglutamine expansion in huntingtin protein (Exp-Htt). This expansion causes protein aggregation, leading to neuronal dysfunction and death. Transcription dysregulation due to Exp-Htt participate in neuronal death in HD. We have previously shown that mitogen- and stress-activated kinase (MSK-1), a nuclear protein kinase involved in chromatin remodeling through histone H3 phosphorylation, is deficient in the striatum of HD patients and model mice. Restoring MSK-1 expression in cultured striatal cells prevented neuronal dysfunction and death induced by Exp-Htt. To study the neuroprotective role of MSK-1 *in vivo*, we used infections with a lentiviral vector allowing overexpression of Exp-Htt within the striatum of rats, alone or along with MSK-1. Overexpression of Exp-Htt alone induced a down regulation of MSK-1 within the infected area while co-infection with both Exp-Htt and MSK-1 induced an overexpression of MSK-1 despite the overexpression of Exp-Htt. Of interest, MSK-1 overexpression attenuated Exp-Htt-induced down regulation of DARPP-32 expression 4 and 10 weeks after infection and enhanced NeuN staining after 10 weeks. MSK-1 induced constitutive hyperphosphorylation of H3 and CREB, indicating that MSK-1 has spontaneous catalytic activity. MSK-1 overexpression also upregulated PGC-1alpha, a transcriptional co-activator involved in mitochondrial biogenesis. Chromatin immunoprecipitation indicated that transcriptional regulation of PGC-1alpha is directly linked to increased binding of MSK-1, along with H3 and CREB phosphorylation of the PGC-1alpha promoter. MSK-1 knock-out mice showed spontaneous a ventricular enlargement, a neuropathologic feature of HD, and a higher susceptibility to systemic administration of the mitochondrial neurotoxin 3-NP. These results indicate that MSK-1 a key upstream regulator of anti-apoptotic genes and neuroprotection in HD. They highlight a possible gene therapy using MSK-1 in early phases of HD treatment.

P2.141

Kinematic patterns of modified grasp (tenodesis) in C6 quadriplegic patients

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Despite sensorimotor disability of the four limbs, C6 quadriplegic patients can achieve functional grasp. Grasping kinematic analysis of tetraplegic patients has been poorly reported in the literature. This study investigated the kinematic parameters in pointing and tenodesis grasping in these patients while performing tasks of daily life.
Four complete C6 quadriplegic patients and four healthy subjects were included.
Each subject performed three different tasks: i) pointing to two targets (aligned with the midsagittal plane and shifted 30°in the right space) with the forefinger, ii) reaching and grasping a 7 cm diameter apple; iii) reaching and grasping a vertical floppy disk.
Movements were recorded with an optoelectronic system at a sampling rate of 50Hz. The kinematic parameters computed were: Movement Time (MT), Peak Velocity (PV), wrist extension and pointing accuracy.
In both pointing and grasping tasks, patients showed a longer MT associated with a weaker PV compared to control subjects. Pointing errors were slightly more pronounced in the sagittal plan.

In the grasping tasks, the main difference was observed for the wrist angle. This angle was more negative in quadriplegic patients during transport phase indicating a more pronounced wrist flexion compared to control subjects, whereas it was more positive during grasp phase indicating a more important wrist extension. Moreover, in the patients' group, the wrist extension was enhanced in the floppy disk task compared to the apple task.
From this kinematic analysis, two distinct phases of the quadriplegic subjects' wrist angular profile could be described. A passive wrist flexion during the transport component of the movement leading to a maximal finger aperture in order to completely wrap the object to grasp and an active wrist extension during the grasping component resulting in an effective closure of the hand, known as “tenodesis effect”. In the floppy disk task, the wrist extension was more enhanced leading to an increase of the tension in the Flexor Pollicis Longus with a more effective lateral pinch.
Analysing modified grasp Kinematic patterns in quadriplegic patients might improve the understanding of the upper limb motor control following spinal cord injury.

P2.142

Prenatal stress elicits induces long-lasting changes in methylation of schizophrenia-related genes in the mouse prefrontal cortex

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The postmortem brain of schizophrenia is characterized by an overexpression of DNA-methyl-transferase (Dnmt1 and 3a) enzymes in telencephalic GABAergic neurons. This up-regulation may result in DNA hyper-methylation in the promoter region of specific genes related to schizophrenia, which include the genes encoding for reelin, glutamic acid decarboxilase 67 (GAD67), brain-derived neurotrophic factor (BDNF), and group-II metabotropic glutamate (mGlu2, -3) receptors. Here, we examined the impact of early life stress on methylation of schizophrenia-related genes using as a model mice exposed to prenatal restraint stress (“PRS mice”). In unstressed mice, levels of Dnmt1 and 3a mRNA were very high soon after birth, and then progressively decreased in the first 2 months of postnatal life. Exposure to PRS (30 min twice daily during the last ten days of pregnancy) markedly increased the levels of Dnmt1 and 3a mRNA in the prefrontal cortex of the adult offspring (+175% and +350%, respectively). Adult PRS mice also showed an increase in DNA methylation of reelin, GAD67, and BDNF IX gene promoters in the frontal cortex, associated with reductions of GAD67, BDNF IX, reelin, mGluR2 and mGluR3 mRNA levels. PRS showed a schizophrenia-like phenotype, characterized by changes in the exploratory activity, a defect in pre-pulse inhibition, and a decrease in social interaction. Some of these defect were corrected by valproate or by the mGlu2/3 receptor agonist, LY379268. These data suggest that PRS in mice is a valuable model for the study of epigenetic changes associated with schizophrenia and for the identification of novel therapeutic targets.

P2.143

Isoflurane anaesthesia precipitates brainstem Tauopathy and airway dysfunction in young Tau-P301L mice

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Introduction: The common volatile anaesthetic isoflurane is suspected to hasten tauopathy and post-operative cognitive decline in Alzheimer Disease (AD) (Baranov et al., 2009). Tau-P301L mice, a mouse model of tauopathy, develop early cognitive deficits and late motor dysfunction with brain tauopathy, as well as airway dysfunction with brainstem tauopathy (7-8 months) and they die prematurely at 8-12 months (Terwel et al., 2005; Dutschmann et al., 2010).
Aim and methods: Isoflurane anaesthesia precipitated brain tauopathy in young Tau-P301L mice (Planel et al., 2009). Here, we examined the effects of isoflurane on hyperphosphorylation of Tau protein within the brainstem using immunohistochemistry and on airway function and dysfunction using double chamber plethysmography in young wild-type and transgenic Tau-P301L mice (age 4 months) prior to and after isoflurane anaesthesia (1.3% isoflurane/ 30% O_2, 4 hours).
Results: At age 4 months, Tau-P301L mice showed normal airway function and phosphorylation of Tau prior to anaesthesia. However one week after isoflurane anaesthesia, the same mice showed significant airway dysfunction, i.e. reduced airflow despite a marked increase in the respiratory chest movements. In these mice, Tau protein was highly hyperphosphorylated in the Kölliker-Fuse, a pontine respiratory-related nucleus controlling upper airways. Interestingly, the deleterious effects of isoflurane on airway function of Tau-P301L mice were totally prevented by artificial ventilation during anaesthesia with air but not with a hypoxic (10% O_2) or hypercapnic (7% CO_2) mix of gas. In addition, artificial ventilation also significantly reduced hyperphosphorylation of Tau in the Kölliker-Fuse. Pre-treatment of Tau-P301L mice with memantine (20 mg/kg), a N-methyl-D-aspartate receptor antagonist already used to treat AD, also totally prevented the isoflurane-induced airway dysfunction.
Conclusion: Isoflurane anaesthesia prematurely induces airway dysfunction in spontaneously breathing, but not artificially ventilated or memantine treated, young Tau-P301L mice. These mouse experimental data could have implications for patients suspected to suffer a primary or secondary tauopathy but requiring volatile anaesthesia for surgery.

P2.144

D-amphetamine induces an increased dopamine release in the core part of the nucleus accumbens in adult rats following a neonatal functional blockade of the prefrontal cortex

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Schizophrenia (SZ) is a complex neuropsychiatric disorder thought to result from a defective connectivity between several integrative regions stemming from developmental failures (Weinberger & Lipska, 1995; Lewis & Levitt, 2002). Various anomalies reminiscent of early brain development disturbances have been observed in the patients' left prefrontal cortex (PFC) (Akbarian et al., 1993; Kalus et al., 2000). Data obtained over the last 30 years support the hypothesis of a dopaminergic (DA) dysfunctioning in SZ (Harrison, 1999; Carlsson et al., 2001). Psychostimulants, such as D-amphetamine, can exacerbate symptoms in SZ patients (Sato et al., 1992; Lahti et al., 1995; 2001). Recent cerebral imaging studies showed that D-amphetamine produces a more important increase of the striatal DA release in SZ patients (Laruelle et al., 1996; Abi-Dargham et al., 1998). In the context of animal modeling of the pathophysiology of SZ, the present study was designed to investigate the effects of D-amphetamine on DA responses in the nucleus accumbens in adult rats, following a neonatal inactivation of the left PFC (infralimbic/prelimbic region). Transient functional blockade of the left PFC was carried out by local TTX microinjection in 8-day-old rats, i.e a critical time of the neurodevelopmental period (Clancy et al., 2001). DA variations were recorded in the left core part of the nucleus accumbens using in vivo voltammetry in freely moving adult rats (11 weeks). Control animals received an i.p. injection of NaCl (0.9%); D-amphetamine was injected i.p. at 0.75mg/kg or 1.5mg/kg doses. The results were the following: 1) A clear dose-effect was observed for the two conditions (PBS and TTX microinjection at postnatal day 8 (PND8) ; 2) With the highest D-amphetamine dose (1.5mg/kg), DA increase in the core was more elevated in TTX microinjected animals than in PBS microinjected animals. These data suggest that rats microinjected with TTX in the left PFC at PND8 display a more important reactivity to D-amphetamine than controls. Taken together, our findings may contribute to a better understanding of the pathophysiology of SZ.
Acknowledgements: The authors thank S. Eybrard for her technical help.

Support : INSERM & Région Alsace, Fondation de France (FM), EDF(AL).

P2.145

Spadin, a sortilin-derived peptide, a new concept in the antidepressant drug design

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Current antiepressant treatments require several weeks of administration before a therapeutic effect can be observed but remain inadequate for many individuals. Improving the treatment and the prevention of depression is challenging. The two pore-domain potassium channel, TREK-1 has been identified as a new target in depression and it has been hypothesized that TREK-1 antagonists might be effective antidepressants. We validated the antidepressant effects of spadin a peptide derived from the maturation of the N-terminus part of the neurotensin receptor 3 (NTSR3 /Sortilin) that specifically blocks TREK-1 channel.
Spadin efficacy was studied through five different animal behavioral models, namely the Porsolt forced swim, the tail suspension, the conditioned suppression of motility, the learned helplessness and the novelty-suppressed feeding test.
Spadin blocks the TREK-1 activity in COS-7 transfected cells and CA3 hippocampal neurons. These effects are absent in TREK-$1^{-/-}$ mice. Spadin does not affect the activity of four other K2P channels. Spadin increases the efficacy of serotoninergic neurotransmission. Similarly to that observed in TREK-$1^{-/-}$ mice, spadin induces a resistance to depression in the five behavioral models. Spadin appears to be specific for the depression because it had no effect in three anxiety animal tests: the elevated plus maze, the stair case and the white-dark box. More importantly, a spadin intravenous 4-day treatment not only induced a strong antidepressant effect but also enhanced hippocampal phosphorylation of CREB protein and neurogenesis, considered to be key markers of antidepressant action after chronic treatment with selective serotonin reuptake inhibitors. Additionally, Spadin does not affect other functions of TREK-1, for example pain sensitivity or epileptogenic susceptibility is not increased by a spadin treatment.
All our data together with the Alpha Screen dosing method, indicated that Spadin could be used as an antidepressant molecule but also as a biomarker for depression disease. Spadin can be considered as a putative endogenous antidepressant of new generation with a rapid onset of action.

P2.146

Adaptive immunity in Parkinson´s disease: the regulatory T cell response

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Parkinson Disease (PD) is a movement disorder characterized by a massive and progressive loss of dopaminergic neurons (DNs) in the substantia nigra *pars compacta* (SN*pc*). While cell autonomous mechanisms of neuronal cell death are not fully understood yet, it is becoming increasingly accepted that non-cell autonomous mechanisms develop as well and are instrumental in neuronal cell demise in PD. Thus, innate immune responses mostly mediated by microglial cells are though to be toxic for DN and involved in progressive neurodegeneration. Aside microglia-driven neuroinflammation, we have recently documented the presence of cytotoxic and helper T cell infiltrates within the SNpc both in

human post-mortem specimen and in a mouse model of PD. Importantly, we could demonstrate that this adaptive immune response contributes to neuronal cell death as well. Our goal is now to develop therapeutic strategies aimed at dampening these deleterious immune responses. One promising way to achieve this goal is the manipulation of a specific T cell population, i.e. the CD4+/CD25+ regulatory T cells (Tregs). These T cells are essential for the control of immune responses and have the potential to modulate neuroinflammation through the secretion of anti-inflammatory cytokines such as Il-10. To give support to such approach we studied the Treg responses to PD-like injury in the MPTP mouse model. Our preliminary data show that we can follow Foxp3+ Tregs in the central nervous system.
Keywords: Parkinson disease, Neuroinflammation, Regulatory T cells, adaptive immunity.

P2.147

Epigenetic programming induced by prenatal stress leads to a reduced expression of synaptic-vesicle associated proteins and a selective impairment of glutamate release in the adult hippocampus

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The rat model of prenatal restraint stress (PRS) is particularly valuable for the study of epigenetic mechanisms regulating resilience to stress in the adult life and for the identification of novel drug targets in the treatment of stress-related disorders. Mothers were exposed to restraint stress from day 11 of pregnancy until delivery. PRS reduced maternal care in the first week of postnatal life and led to an increase in anxiety-like behavior and a reduced number of proliferating neuroprogenitors in the hippocampal dentate gyrus of the adult offspring. To facilitate the identification of novel molecular processes involved in the epigenetic program induced by PRS, we performed a proteomic analysis in the adult hippocampus. We found that PRS induced changes in the expression profile of a number of proteins, involved in the regulation of synaptic vesicles, signal transduction, cytoskeleton dynamics, protein synthesis, and energetic metabolism. Immunoblot analysis showed reductions in hippocampal levels of synapsins, synaptophisin, and synaptobrevin, three proteins that regulate intracellular trafficking and membrane fusion of synaptic vesicles. Interestingly, these changes were associated with a substantial reduction in depolarization-evoked glutamate release from hippocampal synaptosomes, with no changes in GABA release. We also found substantial changes in the expression of proteins that had already been related to stress, such as LASP-1, fascin, and prohibitin. These observations indicate that PRS leads to enduring changes in glutamate release in the hippocampus and suggest that an impairment of glutamatergic transmission is a component of the epigenetic program induced by early life stress.

P2.149

Is the Substantia nigra part of sensory structures?

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How the activities of the substantia nigra (SN) promote movement ?

1- We recorded Substantia nigra pars reticulata (SNR) neurons (n=132) in freely moving cats performing a simple reaction time (RT) task.
At the conditioned stimulus (CS), the cat lifts a paw for a food pellet, if fast enough (RT< 400 ms). Early responses (20 to 80 ms) to the CS were more often activation (A; n=49) than inactivation (I; n=18). CS response amplitudes of successful or failed trials (RT>400 ms) were alike. They did not correlate to RTs values.
2- Seconds after reward, the steady activity of most A neurons increased, but decreased for some I neurons.
Successive CS responses of A neurons were correlated positively for 10 trials. For some I neurons negative correlations lasted from the 6^{th} to the 10^{th} trials. Rewards impinged on SNR activities on 12s to 120s ranges.
3- Blockade with 1ml lidocaine (0,3ml/min) induced after 4-5 minutes and for 20 minutes:
a. Slow trial rate, down from 5 to 3/min.
b. RT shift from 300-400 up to 400-500 ms.
c. Failure to act on CS. Late RTs rate surged from 5 to 20 %.
A cat under SNR block or a Parkinson's disease (PD) affected patient presented similar RT distributions. So the effects of PD treatments on RTs allowed a pharmacological analysis of the SN network.
4- Age-matched healthy volunteers and PD patients were tested on the simple RT task.
RT values stepped-down after about 11 trials for healthy people (n=16; about 45 % SD) and 13 trials for l-dopa treated patients (n=7; about 25 % SD). The step-down, transferred from the first hand to perform to the second, was a motor learning process. Untreated patients (n=6) did not improve. Under anticholinergic drugs (n=12), the RTs increased along the session. Under mixed cures (n=12) motor learning was seldom restored. These effects were highly significant (p< 0.0001). Anticholinergic drugs and l-dopa were antagonists.
Suggestions: The SNR neuron had features expected for a sensory input to a movement program.
The SNR neurons are part of a Turing machine-like learning process.
The SN, rather than the striatum, could be the primary site of action of l-dopa.

P2.150

Sensory-related anticipatory activity in motor cortical neurons predicts their implication in movement preparation

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The prior knowledge about when a movement should be initiated strongly influences its preparation, reflected in the behavioral performance (reaction time) and the neuronal activity. Here we show that the expectation of visual information, also in epochs void of motor preparation, modulates the activity of motor cortical neurons.
We trained two monkeys in a reaching task involving two consecutive delays of equal duration. Each trial started by a time cue (TC), informative about the duration of the two delays (both either 1 or 2sec). At the end of the first delay (D1), one of six peripheral targets was flashed for 55ms (SC, spatial cue), and then masked by the onset of the remaining targets (distracters). At the end of the second delay (D2), the GO signal requested a movement to the cued target. Thus, during D1 no information about the movement was available, but the extremely short presentation of SC forced the animal to attend to its expected moment of presentation.
We recorded the activity of 744 and 470 neurons in the motor cortex of two monkeys during task execution. We classified the neurons according to their activity during D1. A large proportion of the neurons (49 and 38% for monkey T and M, respectively) significantly changed their activity before the onset of SC, i.e. in the absence of motor preparation. Among these, as many neurons increased (D1up) as decreased (D1down) their activity, in a gradual way. The D1up neurons responded phasically to SC, but were less active during movement. The D1down neurons changed their activity more gradually after SC, but were more active during the movement execution phase.
In conclusion, we find two populations of motor cortical neurons with a specific non-motor anticipatory behavior related to the visual spatial information. Furthermore, their subsequent activity after the onset

of the spatial cue suggests that they participate to two complementary networks involved in different stages of visuomotor behavior: the D1up neurons rapidly process the sensory information, whereas the D1down neurons take over for movement preparation and execution.
Funding: ANR (ANR-NEUR-05-045-1); AXA Research Fund.

P2.151

Respiratory-related odor sampling: deciphering the effects of frequency and flow rate variation on olfactory bulb activity

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Olfactory sampling behaviour (namely sniffing) seems to be a part of the olfactory percept. First, olfactory bulb (OB) activity is known to be highly related with respiration. Second, olfacto-motor activity or sniffing is highly variable (Youngentob, 1987). Nevertheless, impact of sniffing variations on OB activity, and particularly at Mitral/Tufted(M/T) cells and local field potential (LFP) levels, was poorly studied. In a first study (Courtiol et al., 2011), we showed that nasal flow rate had a strong effect on M/T cell activity, LFP gamma and beta oscillations, and on the synchronization between spikes and LFP oscillations. In the present study, our aim was to understand the effect of sniff frequency variation alone and combined to flow rate variation on OB activity. For this purpose, we used an anesthetized model of double-canulation tracheotomized rats allowing a fine control of nasal airflow dynamics. Both LFP and M/T cell responses to odors were recorded. Our results highlight a tradeoff effect between frequency and flow rate on OB odor response.
This work was supported by a grant from "Agence Nationale de la Recherche" (#ANR-07-NEURO-030).

P2.152

Specific and regionally restricted correlations between monoamine tissue content in cerebral structures involved in cognition

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Cognitive information processing involves the integration of numerous cortical and subcortical regions whose relations are modulated by the dopamine (DA), noradrenaline (NA) and serotonin (5-HT) monoaminergic systems. The widespread nature of these monoaminergic networks, innervating the encephalon and arising from cell bodies confined to the brainstem, is one of the main difficulties in analyzing their functions and interactions. We have examined the functional relationships between brain areas within a particular monoamine system network and between distinct monoaminergic systems in various brain areas involved in decision-making, through a global approach by correlating the monoamine tissue content.
Bilateral punches of twenty brain regions were taken on a cryostat from frozen Sprague-Dawley rat brains (n=35). NA, DA and 5-HT tissue contents, measured using a sensitive HPLC/electrochemistry system, were expressed in ng/mg of tissue. Significant correlations were searched for between the monoamine content of brain regions.
As expected, NA and 5-HT were present in all brain regions, ranging from 0.1 (anterior insula, AI) to 0.38 ng/mg of tissue (hippocampus) and 0.07 (posterior cingular cortex) to 0.94 ng/mg (posterior

insula, PI). DA tissue content, less homogeneous, was higher in dorsomedial striatum (8.6 ng/mg) compared to extrastriatal tissues (< 0.5 ng/mg). Significant correlations between tissue content for each monoamine followed specific regional patterns. For instance, infralimbic cortex and amygdala monoamine contents were correlated (negatively in central nucleus for DA; positively in basolateral nucleus for NA); prelimbic cortex and nucleus accumbens shell (negatively for DA; positively for NA); cingulate cortex and ventrolateral striatum (negatively for DA and 5-HT). The cortical concentration of the three monoamines did not correlate with each other. PI and AI displayed a higher number of correlations with other brain regions, notably via the 5-HT system, that did not overlap.
In conclusion, this work provides a useful database of the monoamine tissue content in brain regions that reveals intriguing anatomical correlations. We suggest that these connections highlight anatomo-functional circuitries that display large inter-individual differences.

P2.153

Neuronal encoding of temporal features of an overtrained motor sequence in the primate striatum

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The striatum is involved in the acquisition and retention of motor skills. It is well established that temporal structure of a series of sensori-motor events is an important determinant of sequence learning, but the contribution of striatal neurons in the processing of these temporal aspects remains unclear. To address this issue, we recorded the activity of phasically active neurons (PANs), which correspond to striatal projection neurons, mainly in the posterior putamen of three monkeys performing reaching movements to targets in a fixed repeating sequence. Behavioral performance and PAN activity were compared in a cued condition in which the timing of each target-stimulus was externally signaled, as opposed to an uncued condition in which no timing signal was available. We also examined the effect of a shortening of the interval between successive targets (4 to 2 s) in the uncued condition. Comparison of task performance between conditions showed a reduction in movement latency when target-stimuli became more predictable in time. PANs displayed phasic or sustained activations at different periods during the task performance. Among recorded PANs 50% (42/84) and 31% (19/62) showed changes in the temporal characteristics of their patterns of activation after removing the external cue and after shortening the inter-target interval, respectively. The modification of the temporal structure of the sequence of movements causes a reorganization of PAN activations during the task performance. These results suggest that neurons in the so-called “sensorimotor striatum” may take part in adapting behavior to changing temporal structure of sequential stimuli and corresponding actions, thus contributing to the role of this striatal region in the flexible adjustment of overtrained motor sequences.

P2.154

Subthalamic nucleus high-frequency stimulation generates a concomitant synaptic excitation-inhibition in Substantia nigra *pars reticulata*

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Deep brain stimulation of the subthalamic nucleus (STN-DBS) has emerged as an efficient treatment for Parkinson's disease. In addition, DBS is viewed as a promising tool to treat various neurological and neuropsychiatric disorders. However, mechanisms underlying beneficial effects remain debated. Accordingly, we performed double patch-clamp recordings of neighboring GABAergic SNr neurons in

sagittal rat brain slices to analyse the cellular effects of STN stimulation on SNr neurons by recording simultaneously synaptic currents and firing activity. We showed that high frequency of the STN (STN-HFS) caused an increase of the spontaneous firing activity in half of SNr neurons while the remaining ones displayed a decrease. At the synaptic level, STN stimulation triggered inward current in 58% of whole-cell recorded neurons and outward current in the remaining ones. By a pharmacological approach, we showed that STN-HFS evoked effects were mediated in all neurons by a balance between AMPA/NMDA receptors and GABAA receptors whose ratio promotes either a net excitation or a net inhibition. Extracellular in vivo injections of phaseolus, an anterograde tracer, revealed that GABAergic pallido-nigral fibers travel through the STN whereas striato-nigral fibers travel bellow it. Therefore, electrical stimulation of the STN does not only recruit glutamatergic axons from the STN, but also GABAergic passing fibers from the globus pallidus. For the first time, we showed that STN-HFS induces concomitant excitatory/inhibitory synaptic currents in SNr neurons byrecruitment of STN efferences and passing fibers allowing to a tight control on basal gangliaoutflow.

P2.155

Power fluctuations in beta and gamma frequencies in rat globus pallidus: association with specific phases of slow oscillations and differential modulation by dopamine D1 and D2 receptors

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Modulation of oscillatory activity through basal ganglia-cortical loops in specific frequency bands is thought to reflect specific functional states of neural networks. A specific negative correlation between beta and gamma sub-bands has been demonstrated in human basal ganglia, and may be key for normal basal ganglia function. However these studies were limited to Parkinson's disease patients. To confirm that this interaction is a feature of normal basal ganglia we recorded local field potential (LFP) from electrodes in globus pallidus (GP) of intact rats. We found significant negative correlation between specific frequencies within gamma (≈60 Hz) and beta (≈14 Hz) bands. Further, we show that fluctuations in power at these frequencies are differentially nested within slow (≈3 Hz) oscillations in the delta band, showing maximum power at distinct and different phases of delta. These results suggest a hierarchical organization of LFP frequencies in the rat GP, in which a low frequency signal in the basal ganglia can predict the timing and interaction of power fluctuations across higher frequencies. Finally, we found that dopamine D1 & D2 receptor antagonists differentially affected power in gamma and beta bands and also had different effects on correlation between them and the nesting within delta, indicating an important role for endogenous dopamine acting on direct and indirect pathway neurons in the maintenance of the hierarchical organization of frequency bands. Disruption of this hierarchical organization and subsequent disordered beta-gamma balance in basal ganglia disorders such as Parkinson's disease may be important in the pathogenesis of their symptoms.

P2.156

Noradrenergic control of the subthalamic nucleus and motor behavior

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The subthalamic nucleus (STN) is a basal ganglia component playing a key role in motor control. Although the modulation of STN neuronal activity by dopamine is extensively studied, the modulation by noradrenaline is still unknown. The present study aimed to investigate
1) the effects of local injection of noradrenergic agents on the firing rate and patterns of STN neurons and
2) the impact of noradrenergic agents injection into the STN on locomotor activity in intact and 6-hydroxydopamine lesioned rats.
Using selective agonists and antagonists of a1 and a2 adrenergic receptors (AR), we show that STN neurons have functional a1- and a2-AR controlling STN firing. We further demonstrate that local injection of clonidine, an α2-AR agonist, induced a switch from tonic to bursty pattern without changing the firing rate. This bursty activity was reversed by the local injection of idazoxan, an a2-AR antagonist. Furthermore, clonidine injection into the STN reduced locomotor activity in sham and 6-OHDA-lesioned rats. In contrast, local injection of phenylephrine, an α1-AR agonist increased the firing rate of STN neurons without changing the firing pattern. In parallel, phenylephrine did not induce any change in locomotor activity. This is the first evidence showing that α1-AR activation is able to modulate the firing rate of STN neurons and that the activation of α2-AR modulates the firing pattern of these neurons. Furthermore, our data show a direct link between the bursty pattern of STN neurons and hypokinesia.

P2.157

Impact of cerebral inflammation on cognition: cortical LPS increases taste aversion learning

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Cerebral inflammation that is observed in many cerebral physiopathological situations is known to alter neuronal activity and to affect cognitive processes. More specifically, hippocampal inflammation impairs several hippocampal-based learning in rodents. However, it is still unclear whether similar effect can be obtained by cortical inflammation and what are the mechanisms by which inflammation modulates neuronal activity and cognitive processes.
For this purpose, we use conditioned taste aversion (CTA) as cortical-dependent learning. Food learning, especially CTA, is essential for the well-being of individuals. The insular cortex (IC) is crucial for CTA acquisition and retrieval since IC inactivation greatly impairs CTA. Precisely, the aim of our study is to investigate the impact of cortical inflammation restricted to the IC on CTA memory formation and retrieval.
CTA acquisition consists in associating a novel sweet taste with a gastric malaise. During the test (assessed 3 days later), the sweet taste is given to rats and liquid consumption is measured as an index of aversion. To induce cortical inflammation, lipopolysaccharide (LPS) is infused bilaterally in the IC.
When LPS is infused in IC 1.5 hours before CTA acquisition, i.e. taste-malaise pairing, liquid consumption of LPS-injected rats is lower during the test compared to controls. LPS infusion in IC 1.5 hours before the retrieval test has no effect, demonstrating a specific effect of LPS on acquisition. To evaluate whether intracortical LPS has aversive properties by itself (which could explain the enhanced CTA acquisition), LPS was infused 1.5 hours before novel sweet taste presentation (without malaise induction). Three days later, control and LPS-treated rats show similar increase of sweet taste consumption, leading to the conclusion that the LPS is not acting as an aversive agent. Finally, we know that the NMDA-NR2B subunit phosphorylation in IC neurons is required for the acquisition of CTA. Knowing that inflammation is able to impact the glutamatergic transmission as well, we postulate that the enhanced CTA acquisition observed after LPS infusion may be due to an increase of NMDA-NR2B subunit phosphorylation. Western blot experiments are currently performed to test this hypothesis.

P2.158
Acid-Sensing Ion Channel 3 (ASIC3) in postoperative pain

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Iatrogenic pain consecutive to a large number of surgical procedures has become a growing health concern. The etiology and pathophysiology of postoperative pain are still poorly understood, but hydrogen ions appear to be important in this process. We have investigated the role of peripheral Acid-Sensing Ion Channels (ASICs), which form depolarizing channels activated by extracellular protons, in a rat model of postoperative pain (i.e., hindpaw skin/muscle incision). We report high levels of ASIC-type currents (~77%) in sensory neurons innervating the hindpaw muscles, with a prevalence of ASIC3-like currents. The ASIC3 protein is largely expressed in lumbar DRG neurons innervating the plantar muscle, and its mRNA and protein levels are increased by plantar incision 24 H after surgery. Pharmacological inhibition of ASIC3 channels with the specific toxin APETx2 or in vivo knockdown of ASIC3 subunit by siRNA led to a significant reduction of postoperative spontaneous, thermal and postural pain behaviors (spontaneous flinching, heat hyperalgesia
and weight bearing). Together, these data demonstrate a significant role for peripheral ASIC3-containing channels in postoperative pain.

P2.159
Chronic pain is not necessarily correlated to surexepression of spinal glial activation markers

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Nowadays, glia are thought to contribute to chronic pain. We therefore investigated whether spinal glial activation correlates with the apparition of pain symptoms in two different models of chronic pain in rats: a bone cancer pain model induced by injecting MRMT-1 rat mammary gland carcinoma cells into the right tibia and a peripheral neuropathic pain model obtained by right sciatic nerve ligation. In both models, but not in sham-cancer and sham-neuropathic, mechanical allodynia and hyperalgesia of the affected limb developed, after 21 (cancer) or 10 (ligation) days post surgery. There was also an increased number of Fos positive nuclei in the superficial (I-II) and deep (V-VI) ipsilateral laminae of dorsal horn in cancer rats with non-painful palpation of the injected paw compared to unstimulated cancer rats and stimulated/unstimulated sham-cancer rats. Surface occupied by GFAP (Glial fibrillary acidic protein, marker of reactive astrocytes) immunoreactivity (IR) in ipsilateral laminae I-II and V was similar in cancer and sham-cancer rats and increased in neuropathic compared to sham-neuropathic rats. In the same way, qRT-PCR analysis of GFAP transcripts within ipsilateral dorsal horn showed no difference between cancer and sham-cancer rats and an increase in neuropathic compared to sham-neuropathic rats. In lamimae I-II, we found a similar number of astrocytes with an IR for S100beta protein (specific astrocytic calcium binding protein) in sham-cancer and cancer rats and an increased number in neuropathic compared to sham-neuropathic rats. Finally, we found no change in OX-42 (antibody recognizing the complement receptor 3 expressed by activated microglia) and iba1 (specific microglial calcium binding protein) IR in laminae I-II and V of sham-cancer and cancer rats while we

found an increase in OX-42 and iba1 IR in ipsilateral laminae I-II and V compared to controlateral ones in both neuropathic and sham-neuropathic rats.
Altogether, our findings demonstrate that chronic pain induced by spinal nerve ligation is correlated with spinal astrocytic and microglial activation and this is not necessarily true for chronic pain induced by bone cancer, suggesting that glia play different roles in the development and maintenance of chronic pain in these two models.

P2.160

Comparison between the consequences of trauma-induced hearing loss and aging-induced hearing loss on the spectro-temporal receptive fields in the guinea pig auditory cortex

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Aging is the most prominent cause of hearing loss and it produce severe degradations in speech understanding. While many studies have described age-related alterations at the peripheral (cochlear) level, very few studies have tried to determine the relative contribution of central vs. peripheral changes to the age-related hearing deficits. The present study describes functional age-related alterations obtained from the same animals at the cortical and at the peripheral level.
Multiunit activity and local field potentials were recorded in the auditory cortex of three groups of urethane anesthetized guinea pig: Aged (>3years, n=6), young (3-4months) and young adults (8 months old, n=6) exhibiting a trauma-induced hearing loss. From the frequency tuning curves, we quantified the cortical acoustic thresholds, the response strength, the latency and duration of the response and the breadth of tuning. These parameters were also quantified from auditory brainstem responses (ABR) collected on the same animals. The results show that both aging and acoustic trauma reduced the response strength of ABR and of cortical evoked responses. The response latencies of the ABR were increased but those of the cortical evoked responses were even more increased. By comparing the ABR threshold and the cortical LFP thresholds we observed that in addition to peripheral hearing loss, aging also induced a “cortical hearing loss”. Aging also increased the duration of neural responses and reduced the receptive field bandwidth, effects which were not found in traumatized animals. Altogether, these results emphasize that presbycusis is a physiological situation resulting from interactions between peripheral hearing loss and biological aging of the central nervous system.
Supported by ANR grant “Hearing Loss” and by FRC.

P2.161

Tonotopic organization of spontaneous activity in auditory cortex revealed with voltage-sensitive dye imaging

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Ongoing brain activity patterns interact with incoming stimuli to affect sensory processing, and abnormal spontaneous activity might form the basis of the phantom percepts experienced during tinnitus, a condition which often follows noise trauma. However, the spatiotemporal structure of spontaneous activity across auditory cortex, and the mechanisms underlying its genesis, remain unknown. To measure the patterns of spontaneous activity across defined tonotopic fields of auditory cortex, we measured population neural activity with voltage-sensitive dye imaging (VSDI; using the dye RH-1691) in the anesthetized guinea pig while presenting complex time-varying acoustic stimuli or

during silence. Using a dense array of partially-overlapping 50 ms tone pips (8 frequencies per octave spanning six octaves), we obtained spectro-temporal receptive fields (STRFs) for individual imaging pixels and used them to define the tonotopic organization of the primary (A1 and DC) and belt auditory cortical fields. Spontaneous activity during long epochs of silence was subsequently measured, and consisted of a continuum of events known to occur during slow-wave sleep or during certain anesthesia regimes. These ranged from prolonged (several hundred millisecond) “upstates” of increased fluorescence to shorter semi-regular “spindle” oscillations, both of which occurred non-uniformly across the cortical surface. By grouping all pixels in areas A1 or DC according to sound frequency preference (obtained from STRFs), we show that these spontaneous events occur in patterns that reflect tonotopic organization within and across cortical areas. More specifically, during both upstate and spindle events, activity correlation between pixels in A1 or DC decreases as their frequency-preference diverges; but additionally, pixels in A1 are also highly correlated with (even far-away) pixels in DC sharing similar frequency preference. The tonotopic patterns observed for cortical spontaneous activity constrain mechanistic hypotheses regarding their genesis, and could be susceptible to perturbation following noise trauma.

P2.162

Thalamus driven cortical brain state change in the awake mouse

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Cortical brain states profoundly regulate perception, learning and cognition, but little is known about the mechanisms controlling different brain states. Here, we investigate state changes occurring during wakefulness in the mouse barrel cortex, a brain region known to process tactile information relating to the mystacial whiskers. During inactive periods of quiet wakefulness, neurons in the barrel cortex exhibit slow, large-amplitude membrane potential oscillations. During active behaviors, the slow synchronous membrane potential dynamics are suppressed in the barrel cortex and are replaced by higher frequency smaller amplitude membrane potential fluctuations that maintain the neurons at a more depolarized level. This cortical state change is centrally generated since it persists after deprivation of the sensory inputs from the whiskers. Recording local field potentials across different cortical areas and the hippocampus together with nucal muscular activity showed that state changes occurred as a global phenomenon in relation to motor activity. We used a combination of juxtacellular, local field potential and whole-cell recordings with pharmacological inactivation and optogenetic stimulation in the awake mouse to investigate the role of the thalamus in controlling state changes. We found that active wakefulness is accompanied by a marked increase in firing rate of thalamocortical neurons. Optogenetic stimulation of thalamic neurons during quiet wakefulness reproduces the cortical activation observed during active wakefulness. On the contrary, pharmacological inactivation of the thalamus increases the cortical slow activity during quiet wakefulness and blocks the main component of the cortical activation during active wakefulness, i.e. high frequency membrane potential fluctuations and depolarization. However a state change remains and occurs as a prolonged silent (Down) state in cortical neurons during active wakefulness. The cortical state change was abolished when thalamic inactivation was accompanied by the blockade of muscarinic receptors at the cortical level. Our results demonstrate that the thalamus plays a key role in regulating cortical brain states during wakefulness, with an additional contribution of neuromodulators.

P2.163

Spontaneous eye movements during visual mental imagery

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Background: There is strong evidence that eye movements reflect the positions of objects during the verbal description of a previously seen picture. However, in most studies, the participants performed imagery of new visual materials that they had studied shortly before; suggesting that the oculomotor pattern observed during visual perception was stored in short-term memory and reenacted during mental imagery. However, does the relationship between imagery and perception persist over time? It is still not known whether a spatial correlation between eye position and verbal recall is observed while exploring mental images stored in long term memory and built from various types of learning (visual or contextual). To this aim we recorded eye movements during mental imagery of the map of France.
Methods: In the first experiment we asked nine naïve subjects to imagine the map of France and to name as many towns as possible in 2 minutes, while keeping their eyes open. In the second experiment, the participants were asked to give a maximum of French towns whose names began with a given letter (A, B...etc) and to answer simples questions related to France towns. In both experiments, we recorded the participants' eye movements using a head mounted SMI eye tracker. For each subject we compared the gaze location, at the time of pronouncing each town, with the real location of this same town according to the Global Positioning System (GPS).
Results: For the first experiment, the X and Y coordinates of the gaze, at the time of pronouncing the names of towns, correlated highly with the longitude and latitude of these same towns respectively, in seven subjects. Only the X coordinate of gaze correlated with the longitude of towns in one subject and no correlations were found in another subject. For the second experiment, no correlations were found between the towns GPS coordinates and the gaze coordinates at the time of pronouncing the town names.
Conclusion: These results confirm that eye movements during imagery are not random but reflect the spatial structure of the imagined scene, though this scene is retrieved from long term memory. These findings contribute to evidence that the eyes are connected with the cognitive processes that occur during imagery.

P2.164

Dynamic uptake of lipid nanocapsules in cochlear cells after in vitro and in vivo administration

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Hearing impairment affects approximately 10% of the population and increases in frequency four-fold with aging. Hearing deficits are often caused by loss of sensory hair cells and primary auditory neurons due to a variety of factors that generate oxidative stress (e.g. noise, ototoxic drugs, and aging). Unfortunately, there are currently no effective treatment of hearing deficits. A first step would thus be the design of an effective delivery system able to bring therapeutic agents into cochlear cells. The aim of this study was to evaluate the ability of nanoparticles to deliver pay loaded siRNA or drugs into cochlear cells. For this purpose, we first manufactured lipid core nanocapsules (LCNs) labelled with Nile red or FITC to visualize their penetration into the cochlear cells. Then, we loaded LCNs with a siRNA against p27, a specific marker of non-sensory epithelial cell, to evaluate their efficiency in delivering therapeutic agents to cochlear cells.
Two complementary types of *in vitro* rodent models were used in this study. The organ of Corti explant allowed investigating LCNs distribution in the sensory epithelium. The cochlear slice allowed investigating LCNs distribution in other cochlear structures such as the scalae and the stria vascularis. In addition, the adult mouse *in vivo* model was used to determine whether LCNs can penetrate the cochlear membranous partitions and be incorporated into different cell types within the cochlea.
Our results showed that the incubation of cochlear explants with 1/10 to 1/10000 dilutions of LCNs did not induce any toxic effects to cochlear cells. When cochlear explants were incubated with LCNs incorporating Nile red or FITC, the corresponding fluorescence was seen in almost all cochlear cells. The cochlear explants treated 48 hours with LCNs loaded with a siRNA against p27 showed a significant decrease of p27 expression. In addition, our *in vivo* results showed that small LCNs (20nm)

can reach the cochlear cells through the intact round window membrane. These results are very promising in term of clinical use of LCNS as vehicles to bring into cochlear cells drugs for curing hearing deficits.

P2.165

Ozone inhalation activates stress responsive regions of the central nervous system

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Ozone (O_3), a major component of air pollution, has considerable impact on public health. Besides the well described respiratory tract inflammation and dysfunction, there is accumulating evidence indicating that central nervous system (CNS) functions are affected by O_3 exposure. However, the mechanisms through which O_3 exerts toxic effects on the CNS remain poorly understood, and the possible involvement of a neural pathway has never been examined. This work aimed at precisely characterizing CNS neuronal activation after O_3 inhalation using Fos staining in adult rat. Our experimental conditions, namely 0.5 - 2 ppm O_3 exposure during 1.5 - 120 hours, caused a sustained time- and dose-dependent lung inflammation evidenced by a bronchoalveolar increase in leukocytes number. O_3 inhalation also induced a sustained time- and dose-dependent neuronal activation in the dorso-lateral regions of the NTS overlapping terminal fields of lung afferents carried by the vagus nerves. In contrast, we did not detect any neuronal activation in the thoracic spinal cord where terminates lung primary afferents carried by spinal nerves. Furthermore, we highlighted a neuronal activation in interconnected central structures such as the caudal ventrolateral medulla, the parabrachial nucleus, the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the paraventricular hypothalamic nucleus. Overall, our results demonstrated that O_3 challenge evoked a lung inflammation that induced, through the vagus nerves, the activation of NTS neurons and promoted a pattern of neuronal activation that appeared similar to the one involved in the systemic stress response.

P2.166

Effects of partial removal of intracortical inhibitions on spectrotemporal receptive fields and on response to conspecific and heterospecific vocalizations in the guinea pig auditory cortex

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Over the last decade, several laboratories have investigated the neural code which operates in auditory cortex (ACx) for discriminating natural stimuli. It was shown in several species that ACx neurons display temporal patterns of discharges in response to conpecific vocalizations which differ at presentation of artificial versions (e.g. time-reversed) of these vocalizations. One intriguing question is whether these temporal patterns are generated by the cortical network (i.e., by the interplay between excitations and inhibitions converging on a given cortical cell) or by subcortical areas and then passively transmitted to ACx. To answer this question, we recorded neuronal activity in ACx of urethane anesthetized guinea pigs before, during and after a partial pharmacological blockage of intracortical inhibitions. Multiunit activity and local field potentials were collected by a 16 electrodes array positioned in the tonotopic field A1 and tuning curves were quantified by presenting gamma tones from 0.1 to 36kHz at 75 to 35dB. Then, the responses to conpecific and hererospecific vocalizations were tested. This protocol was repeated after a 4min application of $GABA_A$ (Gabazine: GBZ) or of $GABA_B$ antagonist (Saclofen, CGP 55485) applied at 10µM on the cortical surface, a concentration which never induced seizure activity. A 4min application of GBZ significantly (paired t-

test, p< .05) increased the response strength, the tuning width and the response duration obtained with gamma tones. Under these conditions, GBZ also increased the responses to vocalizations and did not disorganize temporal spike patterns: in fact, the across-trials temporal reliability of the response was even enhanced during GBZ application. In contrast, Saclofen and CGP applications did not induce significant changes in the tuning curves and did not affect the responses to vocalizations. These data suggest that fast intracortical inhibitions mediated by $GABA_A$ receptors modulate both the tuning curves parameters and the responses to natural sounds. They also suggest that temporal spike patterns detected at presentation of communication sounds might not be generated at the cortical level.
Supported by ANR grant “Hearing Loss” and by FRC.

P2.167

Erythropoietin in blood and brain blunt the hypercapnic ventilatory response in mice

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Changes in arterial Pco_2, Po_2 and pH are the strongest stimuli sensed by peripheral as well as central chemoreceptors to adjust lung ventilation to the metabolic demand. There are evidence showing that the carotid bodies, the main peripheral oxygen sensors, as well as some central chemosensitive structures respond to changes in both Pco_2 and Po_2. Recently it was demonstrated that erythropoietin (Epo), the main regulator of red blood cell production, contributes significantly to increase the hypoxic ventilatory response. This effect has been attributed to the presence of Epo receptors both in carotid bodies and in key brainstem structures involved in the control and integration of the respiratory signal. Therefore, we hypothesized that Epo plays a dual role in chemoreception by acting on peripheral and central structures, although its contribution to the hypercapnic ventilatory response has never been explored. In this study, we make benefit of two transgenic mouse lines constitutively expressing high levels of human Epo in brain only (Tg21) or in both brain and plasma (Tg6), the later leading to excessive erythrocytosis. The basal and hypercapnic ventilatory parameters in transgenic mice and corresponding control animals were measured by plethysmography and *in vivo* electrophysiology techniques. Moreover, chemical (Aranesp and phenylhydrazine) and chirurgical (spleenectomy) approaches have been applied to assess the role of excessive erythrocytosis and plasma level of Epo. Our results clearly show that excessive erythrocytosis and increased level of plasma Epo contribute to blunt the hypercapnic ventilatory response. In addition, data from Tg21 mice demonstrate that augmented level of cerebral Epo also decreases the ventilatory response to CO_2. These findings may be relevant to better understand respiratory disorders occurring at high or low altitude.

P2.168

Tianeptine preferentially recruits GABAergic neurons within the corticolimbic circuit and prevents the stress-induced sensitization of startle

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Exposure to traumatic stress can precipitate bouts of depression and anxiety, while modulation of GABAergic interneurons within corticolimbic regions of the brain might provide therapeutic benefits for these disorders (Kalueff and Nutt, 2007; McNally, 2007). Because previous work indicates that the antidepressant drug tianeptine has stress-protective effects (e.g., Rocher, et al 2004), we studied the impact of immediate post-stress tianeptine treatment (10 mg/kg) on neuronal activation and on subsequent stress-induced behavior. Elevated platform stress (EPS) exposure increased Fos immunoreactivity within numerous corticolimbic regions in the rat brain, and in contrast to the antidepressant drugs imipramine or venlafaxine, post-stress tianeptine treatment caused a decrease in Fos-immunoreactive neurons within the central nucleus of the amygdala (CEA). Tianeptine also increased the percentage of neurons in the prelimbic cortex and basolateral amygdala that were double-labeled for Fos and the GABA-synthesizing enzyme glutamic acid decarboxylase 67 (GAD67). These results indicate that tianeptine preferentially recruits GABA-releasing neurons within corticolimbic regions. Tianeptine given after EPS exposure also prevented a lasting sensitization in the magnitude of the rat's startle reflex. Tianeptine is recognized as a modulator of glutamatergic synaptic transmission (McEwen, et al 2010), yet its mechanism of action is not well delineated. Our results suggest the drug might regulate glutamatergic processes by affecting the activation of GABAergic neurons. Additionally, because startle sensitization is a model for post-traumatic stress disorder, tianeptine might be effective for reducing the sequela of behavioral symptoms that emerges after a traumatic event by modulating inhibitory neurons within the corticolimbic network.

P2.169

Activation of C-fibers by low frequency sinusoidal electric currents in humans

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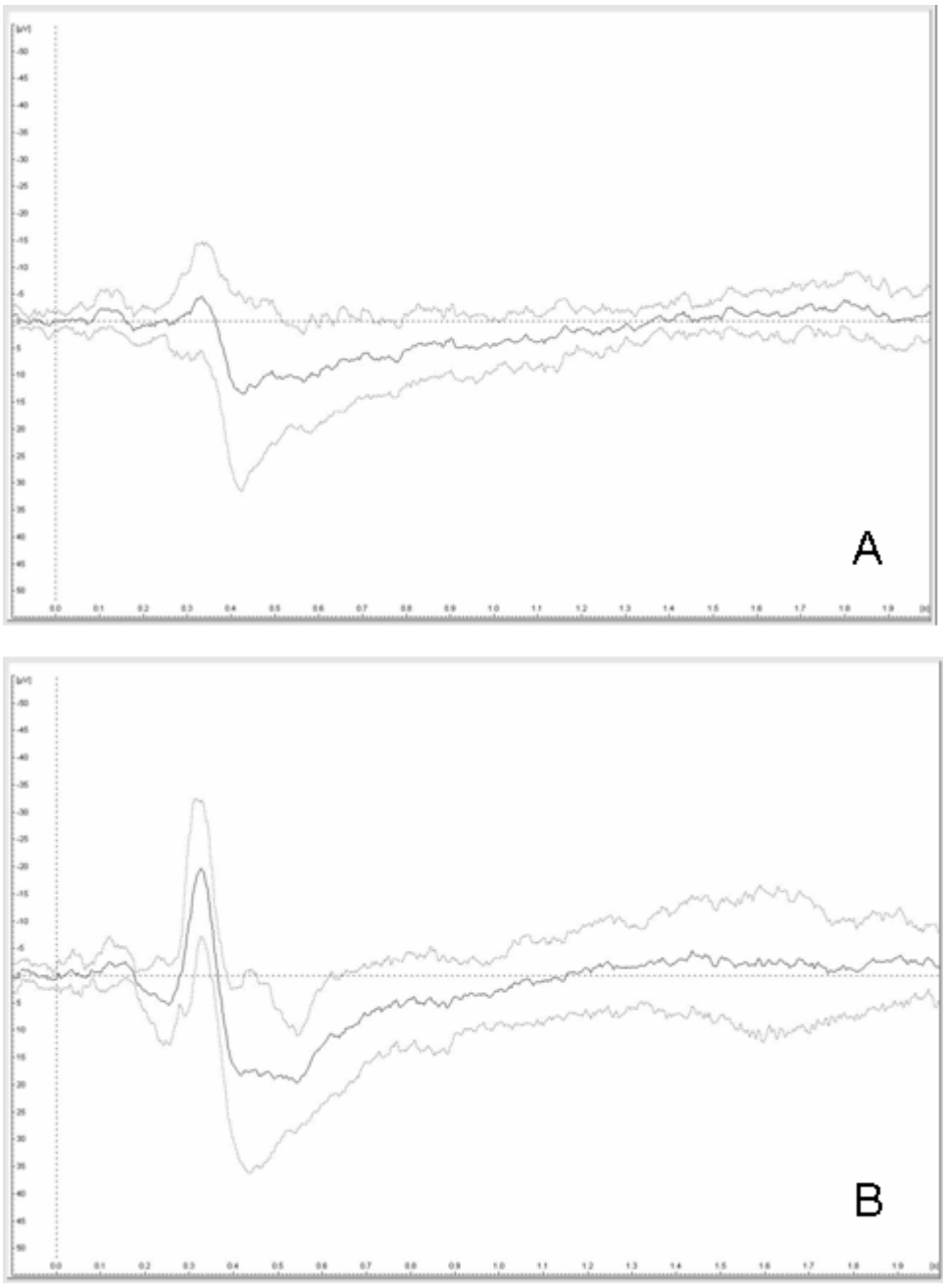
Laser and contact heat stimulation have proved efficient for assessing the activity of thermal and pain-related Ad and C-fibers. Although transcutaneous electrical nerve stimulation can directly activate Ad- or C-fibers, concurrent activation of Aß-fibers is unavoidable when high intensity electrical stimuli are applied. However, it has been suggested that various frequencies of electrical sine wave stimuli may be used to evaluate the selective function of Aß-, Ad- and C-fibers. Specifically, unmyelinated C-fibers are to be activated by a 5-Hz stimulus, thinly myelinated Ad-fibers by 250-Hz stimuli, and large myelinated Aß-fibers by 2000-Hz stimuli. Because Aß-, Ad- and C-fibers have different conduction velocities, the selective activation of Ad- and C-fibers can be assessed based on evoked potential latencies. The vertex N2/P2 component is in the 160-390 ms range when Aß- and/or Ad-fibers are stimulated and N2/P2 component in the 750-1200 ms range when only C-fibers are activated. We tested whether a 5-Hz sinusoidal electric stimulus could selectively activate C fibers and consequently generate ultra-late N2/P2 components in the 750-1200 ms range. We found that a 5-Hz sine wave stimulus, whether of high or low intensity, elicited N2/P2 complexes only in the late latencies (160-390 ms), suggesting that Ad-fibers were concomitantly activated along with C-fibers. These findings are in agreement with those of previous simulation studies suggesting that activation of fibers of diameter less than 2.5 µm (i.e., C-fibers) at the 5-Hz frequency requires accompanying activity from Aß- and Ad- fibers.



[Grand-average of electrical evoked potentials]

P2.170

Identification of ventral intraparietal area (VIP) with multiple sensory stimulations: a functional magnetic resonance imaging (fMRI) study in awake monkeys

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The ventral intraparietal area (VIP) of the macaque monkey is shown to be the site of visual, tactile, vestibular and auditory multisensory convergence and integration, and is mostly involved in processing stimuli moving with respect to the subject. As a result, this cortical area is thought to play a key role in the perception of relative self-motion with respect to the environment and in the representation of close extra-personal space.
Here, we used functional magnetic resonance imaging (fMRI) in behaving monkeys to precisely map area VIP. Three different sensory modalities (visual, auditory and tactile) were tested in blocks in two monkeys while they maintained active fixation. In relation with the response properties of VIP neurons, we specifically used stimuli evoking a movement percept in the near extra-personal space. The activity evoked by these stimuli was contrasted with either the activity evoked by their corresponding scrambled or static stimuli (visual and auditory modalities controls), or with the activity evoked by the active fixation of a blank screen in the absence of any sensory stimulation (tactile modality control). Within the intraparietal sulcus, we identified two regions co-activated by moving visual stimulations and tactile stimulation of the face, and one very small region co-activated by visual and auditory stimulations. No region was co-activated by the three modalities. All of these bimodal regions are distributed along the fundus of the intraparietal sulcus. The most anterior bimodal region is visuo-tactile and overlays the classical description of the anterior intraparietal area AIP. The intermediate bimodal region is also visuo-tactile and matches the classical description of VIP. The most caudal bimodal region is visuo-auditory and is in the direct vicinity of this latter visuo-tactile region. It is unclear whether this caudal region represents a functional subdivision of VIP or an independent intraparietal area to be described. However, it is clear from our study that, in contrast to what has been described in humans, monkey VIP cannot be described on the basis of a polymodal conjunction of visuo-auditory-tactile responses. The functional significance of this result remains to be explored.

P2.171

Multiple desensitization mechanisms of mechanotransducer channels shape firing of mechanosensory neurons

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How desensitization of mechanotransducer currents regulates afferent signal generation in mammalian sensory neurons is essentially unknown. Here, we dissected desensitization mechanisms of mechanotransducer channels in rat sensory neurons that mediate the sense of touch and pain. We identified four types of mechanotransducer currents that distribute differentially in cutaneous nociceptors and mechanoreceptors and that differ in desensitization rates. Desensitization of mechanotransducer channels in mechanoreceptors was fast and mediated by channel inactivation and adaptation, which reduces the mechanical force sensed by the transduction channel. Both processes were promoted by negative voltage. These properties of mechanotransducer channels suited them to encode the dynamic parameters of the stimulus. In contrast, inactivation and adaptation of mechanotransducer channels in nociceptors had slow time courses and were suited to encode duration of the stimulus. Thus, desensitization properties of mechanotransducer currents relate to their functions as sensors of phasic and tonic stimuli and enable sensory neurons to achieve efficient stimulus representation

P2.172

Mapping the networks of barrel cortex involved in associative learning

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Neuronal circuits in primary sensory cortices exhibit plastic changes during altered sensory experience. We are interested in the function of cortical circuits in associative learning in which animals learn to anticipate an aversive event, here a tail shock, when a particular neutral sensory

stimulus occurs, here a whisker deflection. Such fear conditioning transforms sensory maps by a learning-specific expansion of the neuronal representation of that particular whisker in the whisker cortical map. Our aim is to identify neuronal circuits that underlie this learning-dependent transformation in the somatosensory cortex and to study how this plasticity contributes to the control of behavior. To this aim, we combine behavioral studies and electrophysiological recordings in vivo. Here, C57Bl6 mice are trained in a differential conditioning paradigm in which the A, B and C whiskers are individually deflected. Mice are head-restrained and their whiskers are deflected with piezoelectrics along the caudo-rostral axis. After the habituation sessions, conditioning are daily trials of 20 min over three days. Only C whisker-stimuli are co-terminated with an electrical tail shock (200 µA, 250 ms). Learning is evaluated after the last conditioning day in the same apparatus by measuring the mice cardiac responses (CR), evoked by whisker deflection. Successfully trained mice (CR^+) exhibit a specific bradycardia at the deflection of the C whiskers, while CR^- mice do not present any cardiac changes. A third group is pseudo-conditioned mice that are trained as CR^+ mice, but for which whisker deflections and shocks are not contingently paired.
Neuronal responses evoked by whisker deflections are recorded in barrel cortex. In one hand, epidural field potentials are monitored with planar multielectrode arrays affixed onto the surface of cortex and survey the transformation of the whisker map. On the other hand, local field potentials are recorded in every cortical layer simultaneously with multi-shank 32-channel electrodes. Electrophysiological recordings are performed in trained, awake, mice during the retention test to reveal the modifications of neuronal activity paired with the expression of fear cardiac response.

P2.173

Visuo-tactile extinction-like phenomena in neurologically healthy participants: physiological limits to multisensory perception

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Sensory extinction is a relatively common neurological syndrome in brain-lesioned patients, which manifests itself by a failure to report a contralesional stimulus only when it is presented concurrently with an ipsilesional stimulation [double simultaneous stimulation (DSS)], while perception of single stimulations (SS) is preserved. Extinction might occur within a sensory modality (e.g. visual, tactile…), but also across modalities (e.g. visuotactile), revealing interactions between information from different senses. Here, we sought to determine whether visuotactile extinction-like phenomena exist in healthy participants. To do so, we had participants perform a task similar to the clinical assessment of extinction, in which they had to respond “left”, “right”, “both” or “none” to tactile and/or visual stimuli delivered to either hand alone or to both hands. Tactile stimuli consisted of electrotactile stimulations applied on the distal phalanx of each index finger, and visual stimuli were red LEDs located near the hand (~5cm). The intensity of the stimuli was determined independently on each side so that the participant would detect about 90-100% of stimulations. Each hand was placed palm-up on the table top directly in front of the shoulder, and participants maintained fixation on a central cross in front of them at all time. They responded by releasing a pedal with the foot (or feet) corresponding to the side(s) where they had perceived a stimulation. Participants´ performance on DSS was significantly lower than on SS, and was also inferior to the joint probability of detecting either single stimulus alone, thus mimicking clinical extinction. These results reveal that extinction-like phenomena between visual and tactile stimuli exist in neurologically healthy participants, thus suggesting that clinical extinction might be an exacerbated manifestation of a physiological limit to the sensory capacities of the normal brain, the spatial bias observed in patients being a mere consequence of the lesion lateralization. This normal model of cross-modal extinction will also be a useful tool to study multisensory interactions in the intact brain.

P2.174

BDNF/TrkB a major signaling pathway in the central control of food intake and swallowing

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The neurotrophin BDNF and its high affinity receptor TrkB have been shown to contribute to the central control of food intake, acting as an anorexigenic signalling pathway. We focused on the caudal brainstem which plays an important role in food intake regulation since it contains the whole neuronal circuitry responsible for meal size regulation under the control of oro-gastro-intestinal stimuli, as well as central pattern generators for the rhythmic motor activities involved in feeding behavior, such as chewing, licking and swallowing. We performed a series of experiment in order to
1) to evaluate whether BDNF can modulate food intake directly inside the DVC,
2) to explore the link between the melanocortinergic signaling and the BDNF/TrkB signaling within the DVC and
3) to test the hypothesis that BDNF may also modulate swallowing in the DVC.
We first showed that local administration of exogenous BDNF to the dorsal vagal complex (DVC) triggers anorexia, whereas endogenous BDNF content in the DVC is modulated by nutritional status, leptin and cholecystokinin (CCK). We also showed that icv delivery of MC3/4R agonist and antagonist within the 4th ventricle of adult rats respectively increased and decreased the BDNF protein content within the DVC, whereas exogenous BDNF was able to block the orexigenic effect induced by 4th-icv administration of an MC4R antagonist. By using an analogue sensitive kinase allele murine model ($TrkB^{F616A}$ mice), we also showed that the pharmacological blockade of TrkB kinase activity abolished the anorexigenic effect of a selective MC4R agonist and of CCK. Therefore BDNF appears to be a downstream of melanocortinergic signalling pathway within the DVC. We also showed that BDNF protein content within the DVC is decreased by stimulation of the superior laryngeal nerve (SLN) that triggers swallowing, whereas BDNF microinjections in the DVC inhibits swallowing triggered by SLN stimulation. This inhibition is reversed when BDNF is co-injected with biccuculine, suggesting that GABAergic transmission is a downstream effector through which BDNF inhibits swallowing. These results suggest that BDNF can be considered as a common integrator for both positive and negative feedback controlling food intake behavior within the DVC.

P2.175

Short-term effects of unilateral lesion of the primary motor cortex (M1) on ipsilesional hand dexterity in adult macaque monkeys

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Although the arrangement of the corticospinal projection in primates is consistent with a more prominent role of the ipsilateral motor cortex on proximal muscles, rather than on distal muscles involved in manual dexterity, the role played by the primary motor cortex on the control of manual dexterity for the ipsilateral hand remains a matter a debate, either in the normal function or after a lesion. We therefore tested the impact of permanent unilateral motor cortex lesion on the manual dexterity of the ipsilateral hand in 11 macaque monkeys, within a time window of 60 days post-lesion. For comparison, unilateral reversible pharmacological inactivation of the motor cortex was produced in an additional monkey. Manual dexterity was assessed quantitatively based on three motor parameters

derived from two reach and grasp manual tasks. In contrast to the expected dramatic, complete deficit of manual dexterity of the contralesional hand that persists for several weeks, the impact on the manual dexterity of the ipsilesional hand was generally moderate (but statistically significant) and, when present, lasted less than 20 days. Out of the 11 monkeys, only 3 showed a deficit of the ipsilesional hand for 2 of the 3 motor parameters, and 4 animals had a deficit for only one motor parameter. Four monkeys did not show any deficit. The reversible inactivation experiment yielded results consistent with the permanent lesion data. In conclusion, the primary motor cortex exerts a modest role on ipsilateral manual dexterity, most likely in the form of indirect postural control.

P2.176

Müller glial cell proliferation and generation of new photoreceptors during Retinal degeneration in Spinocerebellar ataxia 7 mouse model

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Spinocerebellar ataxia 7 (SCA7) is a polyglutamine (polyQ) expansion disease characterized by cerebellar and retinal degeneration. The SCA7 R7E mice display a progressive retinal dysfunction that is due to the loss of rod differentiation phenotype. After one year of disease duration, surviving rods have lost their segments and show no degenerative sign. Interestingly, at early disease stages, a transient apoptotic wave associated with cell proliferation is observed in the outer nuclear layer (ONL). In this study, we analysed the origin, identity and fate of the proliferating cells to understand their role in the SCA7 retinopathy.
SCA7 and WT littermate mice were injected with the bromodeoxyuridine (BrdU) at different post-natal days (PN) ranging from PN12 to PN42 to label proliferating cells. Other mice received a single dose of BrdU at PN14 and subsequently analysed from PN14 to PN42 to trace the proliferating cells over time. Mitotic cells were labeled by immunofluorescence with phospho-histone 3 (PH3) antibody, while identity and fate of proliferating cells were studied using retinal cell specific markers and TUNEL staining.
While few BrdU+ cells are detected in the WT retina, they are abundant in SCA7 retina from PN 12 to PN 42, with a peak of BrdU+ cell production at PN 17. Most BrdU+ cells are located in the inner nuclear layer (INL) at PN 12. However, they are exclusively located in the ONL from PN 21. By tracing the proliferating cells, we show that BrdU + cells migrate from the INL to the ONL and express rod specific markers. These BrdU+/rod+ cells persist for up to 3 weeks in SCA7 retina, whereas the number of BrdU+/ rod - cells decline over time.
Our results suggest that in response to polyQ toxicity in SCA7 retina, Muller glial proliferate, migrate to the ONL where they might transdifferentiate into persistent rod photoreceptors, while trandifferentiation of Müller glia to photoreceptors was shown to occur in mammalian retina upon acute injury, our study proposes that this transdifferentiation phenomenon might also be an inherent feature of some retinal degenerative diseases.

P2.177

Raclopride or high-frequency stimulation of the subthalamic nucleus stops cocaine-induced motor stereotypy and restores associated alterations in prefronto-basal ganglia circuits

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Stereotyped movements are key symptoms of various neuropsychiatric and neurologic disorders such as Tourette's syndrome, obsessive compulsive disorder and punding as observed in humans addicted to cocaine and in Parkinson disease patients over treated by L-Dopa. In rodents, a challenge injection (after sensitization and withdrawal) of psychomotor stimulants induces motor stereotypies.
We recently showed that motor stereotypy is linked to functional alterations in the prefrontal but not sensorimotor cortico-basal ganglia circuits. Indeed, during strong cocaine-induced motor stereotypy: 1- information transmission through all trans-striatal and trans-subthalamic pathways of the prefrontal basal ganglia circuits is reduced 2- dopamine (DA) and acetylcholine (ACh) releases are respectively increased and decreased in the prefrontal territory of the dorsal striatum 3- restoration of striatal cholinergic transmission leads to the arrest of motor stereotypy.
Neuroleptic and high-frequency stimulation of the subthalamic nucleus (HFS STN) are currently used to treat motor stereotypies. Thus, here we investigated the effect of these two therapeutical approaches on cocaine-induced motor stereotypies and on functional alterations described in the prefrontal cortico-basal ganglia circuits. We showed that an intraperitoneal injection of the neuroleptic, raclopride (low dose; 0,4mg/kg), as well as HFS STN (100Hz, 8V, 60µs pulse width) rapidly leads to the arrest of cocaine-induced motor stereotypies. Furthermore, both approaches normalize *in vivo* NMDA-evoked ACh release and HFS STN normalizes NMDA-evoked DA release in the prefrontal territory of the dorsal striatum. Finally, raclopride restores the transfer of cortical information to the substantia nigra pars reticulata (SNR), the output structure of the basal ganglia, via direct and indirect trans-striatal and hyperdirect trans-subthalamic pathways, in the prefrontal basal ganglia circuits. HFS STN strongly alters (blockade in 59% of the recorded SNR cells) neuronal activity in the SNR.
Therefore, we propose that both raclopride and HFS STN stop cocaine-induced motor stereotypies by partially (HFS STN) normalizing functional alterations in the prefrontal basal ganglia circuits.

P2.178

Distinct role of the GABA transporters GAT-1 and GAT-3 in the regulation of extracellular concentrations of GABA in the rat hippocampus

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Tonic inhibition in the brain is mediated by extrasynaptic GABA(A) receptors and controls neuronal and network excitability. Disturbances in tonic inhibition play a role in epilepsy and psychiatric disease. The magnitude of tonic inhibition depends on the concentration of extracellular GABA (e[GABA]). We have shown that hippocampal e[GABA] is responsive to stressful challenges (1). However, the regulation of e[GABA] by the different GABA transporters (GAT) under *in vivo* conditions is largely unknown.
To investigate the role of GAT-1 and GAT-3, the two main GABA transporters in the rat brain, we performed *in vivo* microdialysis in the hippocampus of freely behaving Sprague-Dawley rats. GAT-specific inhibitors were locally infused by reverse dialysis and GABA concentrations were measured by HPLC. All procedures were conforming the Animals (Scientific Procedures) Act 1986.
Infusion of the GAT-1 inhibitor NNC-711 caused a dose-dependent increase in e[GABA] (EC_{50}: 1.20±0.30 µM, Emax: 1001±92%). In contrast, the GAT-3 inhibitor SNAP-5114 exerted effects only under conditions of elevated e[GABA]. Thus, 100 µM SNAP-5114 was without effect on e[GABA] when infused on its own, but augmented the effects of 1 µM NNC-711 (Emax: 716±30% versus 566±32% for NNC-711 alone, $P< 0.01$). Co-infusion of SNAP-5114 also potentiated the effect of 50 mM KCl on e[GABA] (Emax: 1733±268% versus 976±165% for KCl alone, $P< 0.05$). Interestingly, when synaptic transmission was blocked by infusion of tetrodotoxin, NNC-711, but not SNAP-5114, was still able to significantly increase e[GABA]. Furthermore, the vast capacity of GAT-1 is demonstrated by the ≈70-fold increase in e[GABA] during co-infusion of KCl and NNC-711.
We hypothesise that GAT-1 and GAT-3 are differentially involved in the control of e[GABA] under particular (patho)physiological circumstances. We are currently investigating whether the specific neuroanatomical localisation of hippocampal GAT-1 and GAT-3 (i.e. synaptic versus extrasynaptic and neuronal versus glial) could explain their differential role in the regulation of e[GABA].
Sponsor: The Wellcome Trust (083163/B/07/Z).
(1) De Groote, L, Linthorst, ACE (2007) Neurosci 148:794-805

P2.179

Context- and direction-selective frequency modulations of motor cortical LFP beta oscillations

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The local field potential (LFP) is a neuronal population measure, mainly reflecting the local synaptic activity around the electrode tip. Beta oscillations are often observed in motor cortical LFPs, but their functional role remains unclear. Studies of beta power modulations have related beta oscillations to a 'resting' motor cortex, maintenance of posture, attention, sensorimotor binding and motor planning. However, frequency modulations were largely overlooked. We here describe context- and direction-selective LFP beta frequency modulations in motor cortex. Two macaque monkeys were trained in a center-out reaching task with two consecutive delays. The first delay demanded attention in time in expectation of the briefly presented visual cue and the second delay involved visuomotor integration and movement preparation in one out of six possible directions. The frequency in both a low and a high beta band (around 20 and 30Hz) was systematically 2-4Hz lower during anticipation of the visual spatial cue in the first delay, compared to during visuomotor integration and movement preparation in the second delay. Furthermore, the beta frequency was directionally selective during preparation, with an average difference of about 3Hz between preferred and non-preferred directions, permitting movement direction decoding with the same accuracy as for beta power. The decoding performance was improved by combining power and frequency compared to decoding either feature in isolation. This suggests that beta frequency provides an additional informative signal that can be exploited in brain-machine-interfaces. We conclude that multiple beta bands co-exist in motor cortex, operating around specific main frequencies, but with context-related frequency modulations within each band. Frequency modulations are clearly as systematic and behaviorally meaningful as power modulations, meriting further attention. We suggest that the context-related analyses of peak frequency values may aid the quest for understanding the possible functional role of brain oscillations.
Funding: ANR (ANR-NEUR-05-045-1) and AXA Research Fund (to BEK).

P2.180

Functional segmentation of inhibition in cerebellar cortex

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The cerebellar cortex is subdivided into a reproducible array of parasagittal modules, each with characteristic afferent and efferent projections and pattern of gene expression. This banding can be revealed by Purkinje specific molecular marker Zebrin-II, a member of the large zebrin family. Zebrin-II is expressed by subsets of Purkinje cells (PCs), that form an array of parasagittal bands, alternating between Purkinje cells expressing zebrin-II (zebrin positive, ZII+) or not (zebrin negative, ZII-), symmetrically to the midline. Relationship between stripes organization and their function is still obscure. Here we investigate whether the zebrin-II molecular segmentation can delimitate functional segmentation of inhibitory interneurons in cerebellar cortex, using electrophysiological recordings on acute slices from transgenic mice expressing GFP in Zebrin-II positive bands (EAAT4-GFP).

We first characterized spontaneous inhibition received by PCs in both ZII+ and ZII- bands. If no major difference were observed in the frequency and amplitude of inhibitory events received by PCs, we found that somatic and dendritic inhibitory events can be discriminated by their kinetics. Then, by recording neighboring Purkinje cells in ZII+ and ZII- bands, we observed a robust difference in the characteristics of action potential driven IPSCs received by these two cells. This feature was also identified at the ZII+/- borders. Thus we propose that zebrin-II expression refine the transversal organization of propagated inhibition in the cerebellar cortex.

P2.181

Age-related white matter shrinkage in the posterior cingulum is related to atrophy in the hippocampus subiculum

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Although aging is characterized by shrinkage of both grey and white matter (WM), the interest of studying WM changes is quite recent and underlying mechanisms need to be further investigated. We previously highlighted a specific age effect on the volume of the hippocampus subiculum; here we hypothesize this could be paralleled by the shrinkage of specific WM tracts as a result of impaired subicular efferences.
61 healthy subjects aged 19 to 84 years underwent MRI examination on a 3-T scanner. T1-weighted data were handled with the VBM5 toolbox and high-resolution proton-density images were used to manually delineate three hippocampal subparts: Subiculum, CA1 and Other, as previously described (*La Joie et al., 2010*). The effects of age were then assessed (i) on WM density maps and (ii) on the volumes (adjusted for total intracranial volume) of hippocampal subfields. Correlations between these two measures were then performed.
Age-related WM density decreases were found, mainly in the anterior corpus callosum (CC), prefrontal lobe, posterior CC/cingulum bundle and fornix. Concerning hippocampal subfields, strong decrease with age appeared in the subiculum ($r=-0.64$; $p< 10-5$), a slighter effect was found for CA1 ($r=-0.36$; $p=0.004$), whereas the other subfields were spared ($r=-0.02$; ns). Subicular volume positively correlated to WM density in the posterior CC, the cingulum bundle and the fornix. This association was still significant when controlling for age. No significant correlation was found for CA1 nor the other subfields.
This study showed that with aging, WM undergoes extensive changes encompassing frontal but also limbic areas. Furthermore, WM density in limbic areas is correlated to the shrinkage of the subiculum, the major hippocampal output sending fibres through the fornix and to the retrosplenial cortex. It is likely that age-related alterations in the subiculum and in these WM tracts are related, and together participate to the impairment of a common brain network.

P2.182

Inverse agonism and agonism at 5-HT_{2C} receptors influence the electrophysiological response of substantia nigra pars reticulata neurons to stimulation of the medial prefrontal cortex

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Precise integration and processing of cortico-subcortical information is essential for appropriate control of motor behavior, mood and cognition. Serotonin2C (5-HT_{2C}) receptors are thought to regulate the

strength and characteristics of cortical input to basal ganglia, but underlying mechanisms remain unclear. The present study investigated the effects of the 5-HT_{2C} inverse agonist, S32006 (Millan et al. 2010, IJNP doi : 10.1017/SI461145710001045), compared to the 5-HT_{2C} agonist WAY163909 (Dunlop et al. 2005, JPET 313 : 862-869) on processing of medial prefrontal cortex (mPFC)-derived information by the associative/limbic territories of the basal ganglia using an extracellular single-cell recording approach. The influence of the 5-HT_{2C} agents on communication between the mPFC and basal ganglia was studied by analysing the response of substantia nigra pars reticulata (SNr) neurons to electrical stimulation (200, 300 and 500 µA; 0.3 ms; 0.3 Hz) of the mPFC in urethane-anesthetized rats, 30 and 45 minutes after their intraperitoneal injection. Electrical stimulation of the mPFC elicited an excitatory-inhibitory-excitatory (triphasic) response in a small population of SNr neurons corresponding to the sequential activation of the cortico-subthalamo-nigral, the cortico-striatonigral and the cortico-pallido-subthalamo-nigral pathways. At a dose of 10 mg/kg, S32006 enhanced the duration of the late excitatory response evoked by mPFC stimulation and marginally decreased the duration of the inhibitory response. On the other hand, WAY163909 (1 or 3 mg/kg) did not affect the latency or duration of the different phases but decreased the magnitude of the inhibitory response (at 3 mg/kg) and facilitated the appearance of the late excitatory response for SNr neurons that did not respond at lower intensities of cortical stimulation. Neither S32006 nor WAY163909 significantly affected the basal firing rate of SNr neurons under the influence of the mPFC. These results suggest that phasic and tonic controls exerted by 5-HT_{2C} receptors operate via distinct mechanisms on the excitability of the associative/limbic territories of basal ganglia.

P2.184

Evidence that gap junctional coupling contributes to catecholamine release in the adrenal medullary tissue: an *in vivo* study

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The regulation of catecholamine secretion is under a dual control involving an incoming initial cholinergic command from the splanchnic nerve and a local modulation through gap junctions. We previously reported that gap junctions ensure propagation of electrical and ensuing Ca^{2+} signals between chromaffin cells and increase catecholamine release in response to stimulation of a single cell. Whereas converging arguments strongly support that gap junctional coupling between chromaffin cells contributes to catecholamine release in acute slice preparation, its effective contribution to excitation-secretion coupling *in vivo* is still unknown. To address this, we developed a new approach to monitor simultaneously the electrical activity of chromaffin cells and catecholamine secretion *in vivo* in anaesthetized mice.
Extracellular recordings show that chromaffin cells exhibit intermittent bursts superposed on a continuous basal electrical activity. This activity is nerve-derived since it is suppressed by local application of TTX on the splanchnic nerve. Plasma catecholamines were assayed from blood samples collected directly from the adrenal vein. Basal adrenaline (Adr) and noradrenaline (Nadr) concentrations were 30 ± 6 nM and 17 ± 2 nM, respectively (n = 11). Splanchnic nerve stimulation at low (0.1 Hz, 5 min) or high (4 Hz, 2 min) frequency led to a significant increase of both Adr (x5 and x18, respectively) and Nadr (x1.5 and x3.5, respectively). The gap junction blocker carbenoxolone (CBX, 120mg/kg, *i.p.*) efficiently reduced Lucifer yellow (LY) diffusion within the medulla and reduced the release of NAdr evoked by a high, but not a low, frequency stimulation, when compared to the inactive analogue glycyrrhizic acid. Adr release was not affected. In mice submitted to a chronic stress (5d cold exposure), the expression of connexin36 and the diffusion of LY were increased. CBX not only decreased the nerve-evoked release of NAdr to a higher extend as compared to unstressed mice but also decreased the release of Adr.
These data demonstrate that
i) gap junctions are involved in excitation-secretion coupling in the adrenal medulla *in vivo* and
ii) gap junctional coupling dominantly contributes to catecholamine release in stressed mouse.

P2.185

Vitamin D_3 promotes functional and electrophysiological recovery in rat models of spinal cord injury

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After a spinal cord trauma, i) inflammation, ii) neurodegeneration, iii) glial scar and iv) limited regeneration are observed at the lesion site. The ultimate goal is to find a molecule, possibly FDA-approved, which is able to prevent these deleterious events. In previous works, we have shown that vitamin D exhibited i) immuno-modulatory, ii) neuro-protective, iii) anti-mitotic and iv) neuro-regenerative properties. In a proof of principle study, based on a rat model of peripheral nerve lesion, we demonstrated that vitamin D significantly i) increased axogenesis and axon diameter and ii) improved the responses of sensory neurons to metabolites such as KCl and lactic acid (Chabas et al 2008). Then, we extended our work to the central nervous system. In a first study, using a rat model of spinal cord compression at the T10 thoracic level, we delivered vitamin D_3 (cholecalciferol) at the dose of 50 IU/kg/day or 200 IU/kg/day. Three months later, ventilatory, motor and sensitive responses of the regenerated axons were electrophysiologically recorded. When compared to control animals, vitamin D-treated rats displayed a significant i) improvement of ventilatory frequency and ii) reduction of H reflex indicating functional improvements. In addition, when compared to the vehicle group, the UV 50 and UV 200 groups exhibit a significantly higher rate of axon crossing the lesion site ($P < 0.05$). In a second study, we used a rat model of cervical hemisection (C2) with a higher dose of vitamin D_3 (500 IU/kg/day) delivered weekly, during 12 weeks. Preliminary data indicate that vitamin D_3 improves locomotor recovery of treated animals. Furthermore, histological and electrophysiological experiments are in progress. Overall, the two sets of studies suggest a potential therapeutic benefit of vitamin D_3 after a medullar trauma.

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P2.186

Ghrelin/obestatin interaction on food intake and hypothalamic or brain stem neuronal activity

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Ghrelin and obestatin are two gut peptides originating from the same precursor, preproghrelin. While ghrelin stimulates growth hormone (GH) secretion and food intake, obestatin antagonizes these effects (Zizzari et al 2007). In Humans, preproghrelin gene polymorphisms have been associated with pathologies linked to an unbalanced energy homeostasis (*ie* anorexia nervosa and obesity). We hypothesized that one of those polymorphisms located in the obestatin sequence (Q to L substitution in position 90 of preproghrelin) may impact on the function of obestatin. In the present study, we tested the potency of native and Q90L obestatin to modulate ghrelin-induced food intake and neuronal c-Fos activity. Food intake was assessed in C57BL/6 mice and cFos activity was measured in NPY-

Tau-SapphireGFP mice expressing the GFP fused to the protein Tau (Pinto et al. 2004) or NPY-Renilla-GFP mice expressing humanized Renilla GFP under the transcriptional control of the mouse NPY promoter driving expression of a bright and stable fluorescent signal (Van Den Pol et al. 2009). Mice received saline, ghrelin alone (30 nmol ip) or ghrelin combined to native or Q90L obestatin (30 nmol each) in the early light phase. Ghrelin-induced food intake was equally antagonized by native and Q90L obestatin. In those conditions, Q90L was more potent than native obestatin to antagonize ghrelin-induced c-Fos activation within the arcuate nucleus of the hypothalamus and the nucleus tractus solitarius of the brainstem. Co-localisation within arcuate NPY neurons depended on the mouse model: in NPY-Tau-Sapphire GFP mice, ghrelin alone or combined to native or Q90L obestatin induced c-Fos expression in 6-8% of neurons independently of the treatment. Using Renilla-GFP mice, cFos was activated in up to 30% of arcuate NPY neurons with ghrelin treatment and this tended to be reduced by co-administration of either native or Q90L obestatin. These data support the hypothesis that native and/or Q90L obestatin antagonize ghrelin-induced food intake partly by acting through NPY neurons in the arcuate nucleus of the hypothalamus.

P2.187

Contribution of P2X4 to transcriptional remodeling in activated microglia

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Microglia are the main resident immunological cells the CNS, which exhibit distinct phenotypes depending on their local environment. In the healthy brain, microglial cells are in a surveillance state whereas upon rupture of CNS homeostasis, microglia enter activated states characterized by functional remodeling, accounting for the acquisition of immune phenotypes. Purinergic receptors are known to play important roles in microglia activation, and P2X4R have been suggested as potential therapeutic target to limit microglia-mediated inflammatory responses associated with brain diseases. To increase our understanding of microglial activation, here we have performed transcriptional profiling of cortical resting and activated microglia isolated from mice brain cortex in normal and inflammatory conditions (i.e. after icv administration of LPS and IFNg). We have also compared the transcriptional remodeling of microglia upon activation in wild-type and P2X4-deficient mice.
Our results show that quiescent microglia from of wild-type and P2X4 KO mice presented only very moderate differences in terms gene expression signatures. However, activated microglial cells from wild-type and P2X4R KO mice presented specific transcriptional profiles, which only partially overlapped. Our data suggest that P2X4R play a significant role in the regulation of pro-inflammatory functions of microglia.

P2.188

Evaluation of pollution on the neurosecretory cells of cerebroid ganglia and sexual cycle during an annual cycle of *Perna perna* (Mollusca, Bivalvia)

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Laboratory investigations on three mussel populations living along the Atlantic coast of Morocco were carried out i) to identify the different sexual stages throughout a year and specify spawning periods, ii)

to determine numerical variations of neurosecretory cells (NSCs) positive to the luteinizing hormone-releasing hormone (LHRH) in the cerebroid ganglia of these bivalves, and iii) to assess the degree of pollution in each mussel bed via the study of several biomarkers: acetylcholinesterase (AChE), catalase (CAT), glutathione S-transferase (GST) and malondialdehyde (MDA). Mussel samples were collected from an unpolluted site, a bed polluted with domestic wastewater (Hay al-Fath), and another contaminated with industrial wastewater (Mohammedia). Three spawning periods were noted in the unpolluted site instead of two periods in the polluted beds. Compared to the unpolluted site, the gametogenetic waves in mussels were longer at Hay al-Fath and shorter at Mohammedia. The quantitative development of NSCs in the cerebroid ganglia was correlated with the different stages of the sexual cycle. During gamete maturation, the number of NSCs increased whereas it decreased during spawning and/or sexual rest. In the site contaminated with domestic wastewater, the number of NSCs was significantly lower than those found in the bivalves from the other two sites. In all sites, the four biomarkers showed a seasonal cycle correlated to mussel sex. In polluted sites, high CAT and GST activities were generally found while AChE activity was low, particularly at Hay al-Fath. High levels of MDA were noted even in the unpolluted site, but with variations according to the site studied. In conclusion, pollution reduced the number of resting periods and changed length of gametogenetic waves in the sexual cycle of *P. perna*. In addition, it reduced the number of LHRH-positive NSCs in the cerebroid ganglia of mussels living in the site contaminated with domestic wastewater.

P2.189

Seasonal expression of gene clock proteins in the jerboa (*Jaculus orientalis*): modulation by 5-Methoxytryptophol and melatonin

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The jerboa is a seasonal breeder form the high continental shelves of Middle Atlas Mountains in Morocco. In this species, the maximal expression of the daily rhythm of pineal 5-Methoxytryptophol (5-ML) occurs in late summer-early autumn period, when animals are in sexual quiescence. In the jerboa 5ML modulates reproductive function since daily injections during the period of sexual activity in early spring (long photoperiod) induces gonadal quiescence. In addition, the 5-ML control of sexual function depends on the season. The site of action of 5-ML in the brain of the jerboa remains unknown since 5ML receptors have not yet been determined. To determine the target structures of 5ML and to explore the seasonal dependent control of reproductive functions of this pineal hormone, we assayed the expression of c-Fos and clock gene proteins. Sixty four adult female jerboas were captured in the field in early spring and transferred to the animal facility where they were maintained in natural photoperiod conditions. Animals were fed a diet of grain wheat and sunflower seeds ad libitum supplemented by lettuce leaves every 3 days.
During each season, jerboas (n=16) were divided in 3 groups and subcutaneously injected 2h after sunrise with 5ML (n= 6), MEL (n=6) or controls (n=4). 90 min later, animals were sacrificed, the brains fixed and sections cut in 25µm coronal sections for c-Fos, BMAL1, PER1 and PER2 immunocytochemistry. The results show that 5ML induces c-Fos in the SCN in autumn, when the 5ML rhythm is at maximal amplitude. In the paraventricular thalamic nucleus (PVT), c-Fos is induced in the spring- summer period during which the amplitude of 5ML rhythm is reduced. In addition, 5ML modulates BMAL1 content in the SCN and PVT during summer and PER2 in autumn. In the jerboa, 5ML acts specifically upon the SCN and PVT in a seasonal dependent manner. Thus, reproductive function in this species may be seasonally modulated by a 5ML control on BMAL1/PER2 clock gene proteins in addition to action control by melatonin.
This work is supported by *Volubilis Program* 2009 (no. 20565ZM), Rhône-Alpes MIRA/CMIRA (2009/2010), EUCLOCK (EU-FP6 project no. 018741 and an Accueil Pro fellowship from Region Rhône-Alpes to N Lakhdar Ghazal.

P2.190

Interactions between A30P and A53T α-synuclein mutations and rotenone in a transgenic rat of Parkinson's disease

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Parkinson's disease (PD) is characterized by a progressive and massive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) which leads to several clinical motor symptoms such as akinesia, rigidity and resting tremor. The molecular pathways leading to this pathological picture remains unclear, but it is believed that most sporadic cases are steming from a combination of environmental exposures and individual genetic susceptibility.
Exposures to pesticide and metals are contributing to risk factors. Rotenone, a commonly used pesticide provides certain advantage in modelling the pathogenesis of PD. Rotenone is a specific inhibitor of complex I (NADH- dehydrogenase). It crosses blood brain barrier and all membranes easily. Indeed rotenone administration affects many of the pathogenic pathways including oxidative stress, Lewy pathology and proteasomal dysfunction.
We decided to develop a transgenic Sprague Dawley (SPD) rat expressing human double mutated alpha-synuclein (hα-syn, A30P and A53T) under the control of rat tyrosine hydroxylase promoter. Alpha-synuclein gene is one of the genes involved in both familial and sporadic PD.
The aim of this study is to assess if the mutated hα-syn in our rat increase the effect of rotenone in dopaminergic neurons of the SN. First, we established the most appropriated dose of rotenone. This dose had to be relatively low to produce selective degeneration in weaken DA neurons by the presence of mutated hα-syn.
Wild type SPD rats were administered rotenone (2.75; 2.25; 1.5; 1.25 and 1.00 mg/Kg) by daily intraperitoneal injection. We performed behavioural tests for 3 weeks to evaluate motor function and molecular/cellular analyses of the striatum and substantia nigra after 4 weeks of rotenone administration. The inflammatory response of the brain parenchyma will also be investigated.
Our results indicate that the lower dose (1.00 mg/Kg/D) was the most appropriate as we got the best rat survival and several animals developed akinesia, rigidity, catalepsy and defects in motor coordination.
In regards of these results, we are currently testing our transgenic rat expressing the human double mutated alpha-synuclein at this lower dose to establish if these mutations potentiate neurotoxical effects of rotenone.

P2.191

Lymphocyte infiltration in the mouse hippocampus after kainate-induced status epilepticus

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Accumulating evidence indicates that infiltrating lymphocytes influence neuronal survival in many brain pathological conditions characterized by an inflammatory reaction. In the case of temporal lobe epilepsy, inflammation and blood-brain barrier (BBB) leakage play important roles in the pathogenesis of the disease. Yet, infiltration of blood cells into the parenchyma of epileptic brains remains controversial. We have previously characterize the activation of microglial cells in the hippocampus of C57Bl/6 mice following a status epilepticus (SE) induced by systemic kainate injections. The present study aimed at analyzing the BBB integrity and the infiltration of blood cells in this model.
Leakage through the BBB was evaluated using i.v. injections of Evans Blue at different time points during and after SE. We observed a major rupture of the barrier in the CA3 region of the hippocampus

during SE. At 24h post SE and at later points, however, BBB leakage was minimal and restricted to small areas of the hippocampus. We then used flow cytometry to analyze leukocyte infiltration in the hippocampus. We observed a monocyte infiltration which started at 24h post SE, peaked at 48h and returned almost to control values after 7 days. Infiltration of lymphocytes first appeared in the hippocampus at 48h post SE and peaked around 7 days. This infiltrate was dominated by CD8+ T cells but also included CD4+ and NK+ T cells. No B cells were detected in hippocampus at 1-7 days after SE. In parallel, we performed an immunohistochemical analysis on fixed brains to study the localization of infiltrating lymphocytes. We first observed CD3+ T cells at 48h post SE and these cells were closely associated with blood vessels. At 7 days post SE, the number of CD3+ T cells increased and no obvious association with blood vessels was observed, suggesting that T lymphocytes have left the peri-vascular space to enter the brain parenchyma. CD3+ cells were observed in all sub-regions of the hippocampus.
These results indicate that T lymphocytes infiltrate the hippocampus of mice after SE and that this infiltration is delayed compared to microglia activation and monocyte infiltration. The roles of these infiltrating lymphocytes in epileptogenesis remain to be determined.

P2.192

Mesenchymal stem cells: immunosuppressive strategy for the long term survival of intracerebral xenotransplant

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Cell transplantation is a promising therapeutic strategy for neurodegenerative disorders. Interesting results were obtained after the transplantation of human fetal neuroblasts into the brain of Parkinsonian´s patients but tissue availability and ethical concerns limit this approach. Other cellular sources have therefore to be found. Fetal pig neuroblasts are interesting candidates as cellular source, but intracerebral xenotransplants are systematically rejected, even under systemic immunosuppression. To optimize the immunosuppressive strategies for intracerebral transplantation, we are currently working on a local control of the host immune responses. In this perspective, the immunosuppressive properties of mesenchymal stem cells (MSC) offer great perspectives. Indeed, we studied the advantages of co-grafting syngenic MSCs with porcine neuroblasts (pNB) into the rat striatum. Two groups of animals (n=6 per group) were transplanted either with pNB alone or with both MSC and pNB. At day 63, no porcine neuron was detected in the striatum that received only pNB while 4 out of six rats transplanted with both pNB and MSC exhibited healthy porcine neurons. Interestingly, 50% of the rats co-transplanted with both cell types displayed healthy grafts with pNF70+ and TH+ neurons at 120 days post-transplantation. Q-RT-PCR analyses revealed a general dwindling of pro-and anti-inflammatory cytokines in the striatum that received the co-transplants. To evaluate the impact of pNB and MSC co-transplants on motor recovery, both cell types were implanted into the striatum of a rat model of Parkinson's disease (6-OH-dopamine lesion) without any additional immunosuppressor. Behavioral analyses performed at day 90 and 105 post-transplantation, clearly showed motor recovery in the rats transplanted with both MSC and pNB. No recovery was observed in the rats transplanted with pNB. Taken together, the present data indicate that the immunosuppressive properties of MSC have a great interest to promote long term survival of xenogeneic neurons in the brain.

P2.193

Traumatic brain injury alters the growth hormone axis in mice

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Traumatic brain injury (TBI) is a major cause of long lasting hypopituitarism in humans. The growth hormone (GH) axis is especially vulnerable, as about 25% of the survivors are GH-deficient as long as 3-years after TBI (Schneider et al., Lancet 2007, 368: 1461). The underlying mechanisms are largely unknown.To study this phenomenon experimentally, mice were anesthetized, underwent unilateral craniotomy above the right parietal cortex, and a controlled cortical impact was delivered with a pneumatic device. Bleeding was controlled, scalp was sutured, and mice received ketoprofen (ip 0.5mg/g) before recovery. A GHRH challenge (100ng) was performed in anesthetized animals, 7 days after TBI. Despite similar basal GH levels in control (4.8±0.7ng/ml, n=21) and TBI mice (3.7±0.7ng/ml, n=39), the GHRH stimulation was larger in control (64.6±8.8ng/ml; $p < 0.001$ vs basal) than in TBI mice (32.1±6.1ng/ml; $p < 0.001$ vs basal; $p < 0.005$ vs. control). Surprisingly, activated microglia, reactive astrocytes, and increased IgG levels were found at the ventromedial hypothalamus (5 control; 5 sham, 7 TBI) where the growth hormone releasing hormone (GHRH) and somatostatin (SRIF) neurons send their nerve terminals. However, no such changes could be observed in the vicinity of GHRH and SRIF cell bodies. Next, the long lasting changes of the GH axis were studied 30 days after TBI. These mice had impaired basal GH levels (0.3ng/ml, n=17; $p < 0.001$ vs control) that were enhanced by GHRH (24ng/ml, $p < 0.005$ vs basal), although this was lower than control ($p < 0.01$). Nevertheless, mRNA levels for pituitary GH and GHRH receptor were not changed in TBI mice. Importantly, the cellular alterations (reactive astrocytes, increased IgG levels) of the ventromedial hypothalamus had persisted in these mice (5 control; 5 sham; 7 TBI). In contrast, the spontaneous electrical activity and the spontaneous synpatic inputs of GHRH-GFP neurons were identical in brain slices from sham and TBI mice (n>10). The resting properties and the action parameters of these neurons were also not changed (n>10). Altogether, these data establish that the GH axis is profoundly and durably altered after TBI in mice, and that changes at the median eminence level of the GH axis might be involved in the sustained GH deficit.

P2.194

Transcortin contributes to sex differences of hypothalamic-pituitary-adrenal axis and despair-like behavior in mice

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Women and men present different risks in the occurrence and symptomatology of several psychiatric disorders such as depression. While social and cultural factors certainly contribute to these differences, biological sex differences seem also to play an important role. A biological system known to present sex differences is the hypothalamo-pituitary-adrenal (HPA) axis, the major neuroendocrine system involved in adaptive stress responses. Alteration of HPA axis activity (resulting in hyper or hypoactivity) due to environmental and/or genetic factors is involved in the development of depression. In our previous study, an original model of mice deficient in transcortin or CBG (circulating glycoprotein responsible for glucocorticoid hormones bioavailability) was presented. On adult male mice, we observed an increase of despair-like behavior in Cbg k.o.mice associated with glucocorticoid hyposignaling in response to stress. Because of the higher susceptibility to develop depression in women and the lack of preclinical studies on HPA hypoactivity animal models, female Cbg k.o.mice were investigated in basal and stressful conditions.
Similarly to male, plasma corticosterone levels across the nycthemeral cycle were unchanged in Cbg+/- mice whereas in Cbg-/- mice it was clearly reduced, especially at the circadian peak compared to wild-type mice. After stress, plasma total and free corticosterone (biologically active form) levels

were significantly decreased in Cbg-/- mice and were moderately reduced in Cbg+/- compared to wild-type. Estimation of urine corticosterone concentration through circadian cycle showed a higher corticosterone production in female compared to male mice that was dependent of the presence of CBG: similar levels were observed between Cbg-/- female and male mice of all genotypes.
Despite these HPA axis alterations and contrarily to males, Cbg-/- female mice showed no significant increase of despair-like behaviors as measured by forced swim and tail suspension tests. However, after ovariectomy differences between genotypes appeared showing that estrogens removal led to reduced despair-like behavior in wild type mice.
Thus, we demonstrated that CBG, induced by estrogen, contributes to sex differences of the HPA axis and to despair-like behavior.

P2.195

Effect of somatostatin receptor subtype 2 (Sst2) overexpression in human lactotroph and gonadotroph adenomas cells

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The most frequent pituitary adenomas develop from lactotroph and gonadotroph cells. Prolactinomas express dopamine (DA) receptor 2 (D2DR). In most cases, D2DR agonists control prolactin (PRL) secretion and cell proliferation. Nonfunctioning pituitary adenomas (NFPA) are mostly derived from gonadotroph pituitary cells. Transphenoidal neurosurgery is the first line treatment for NFPA, but it is often incomplete. No specific medical treatment is available. However, gonadotroph adenomas express low levels of D2DR and somatostatin (SRIF) receptor 2 (Sst2), while SRIF and DA analogs have low efficacy in controlling tumoral growth. Sst2 agonist, octreotide, is efficient in controlling 60% of growth hormone (GH) secreting adenomas. In cell culture, a chimeric SRIF-DA analog (dopastatin) is more efficient in controlling GH than Sst2 or D2DR agonists alone or combined. However, in lactotroph and gonadotroph adenomas, dopastatin showed only an efficacy similar to that of D2DR agonist. Sst2 overexpression in GH octreotide resistant adenomas reversed octreotide sensitivity.
The aim was to overexpress sst2 in 10 NFPA and 7 prolactinomas cell culture by mean of an adenoviral vector (AdSst2), with parallel eGFP adenoviral control infection (Adgfp). Validation of Sst2 transfer by real-time RT-PCR. Effect of Sst2 at various viral charges on cell viability. Comparative effect on cell proliferation, cell viability, and hormonal secretion of Sst2 agonist, D2DR agonist and dopastatin in AdSst2 and AdGFP cells.
Results:Sst2 overexpression was efficient in both NFPA and prolactinomas. D2DR mRNA was coexpressed in all cases. In NFPA, Sst2 gene transfer induced a dose dependant apoptotic mortality through a caspase mechanism. *At low viral charge*, AdSst2 cells became sensitive to sst2 agonist, either in term of PRL secretion (30 ± 5 vs. 7 ± 5 %) or cell viability (40 ± 10 vs. 15 ± 5 % in Ad GFP). Dopastatin was more effective than either Sst2 or D2DR agonist in Ad-Sst2 NFPA (65 vs. 50 vs 40 % , respectively) while it was only slightly more effective in PRL suppression in Ad-Sst2 prolactinomas.
In **conclusion**, in 2 different pituitary adenomas, coexpressing Sst2 and native D2DR, dopastatin showed differential effect: additive Sst2-D2DR in NFPA, dominant D2DR effect in prolactinomas.

P2.196

Transcortin deficient mice are insensitive to chronic stress

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Sustained or chronic stress can have numerous pathologic effects in vulnerable individuals, in particular on brain function. Part of these effects is mediated by glucocorticoid hormones, the end products of the hypothalamo-pituitary-adrenal (HPA) axis. In a previous study, we reported on the increased despair-like behaviour associated with glucocorticoid hypo-signalling after acute stress in a mouse model of transcortin deficiency called Cbg k.o. Transcortin or CBG (corticosteroid binding globulin) is a circulating glycoprotein that plays a crucial role in glucocorticoid hormones bioavailability in plasma and target tissues. This study was designed to analyze the effects of chronic stress on transcortin deficient mice.
Two paradigms were used to induce a chronic stress on the animals. The first one consisted of a four week treatment in which a stress was applied to the animals twice a day in a random fashion (cage tilt, wet bedding, air puff, etc.). The second was the social defeat paradigm in which mice are confronted for ten days, 5 min per day with an unknown resident mouse of bigger size.
Anxiety-related behavior was not found altered after any of the two chronic stress regimens in both controls and Cbg k.o as judged by open field or elevated plus maze tests. However, depressive-like behavior was increased after both chronic stress paradigms in controls mice as measured in a forced swim test. In Cbg k.o., this trait is elevated before chronic stress and does not increase further after any of the two chronic stress regimens.
Thus, these experiments show that transcortin deficient mice show an endogenous despair-like behavior that is not aggravated by chronic stress while the same paradigm increases this depressive-like trait in control mice. The role of glucocorticoids in this insensitivity to chronic stress of the CBG k.o. mice is under study.

P2.197

Anxiety level assessed in the open field test is regulated by endogenous or exogenous melatonin in female rats: contribution of the pinealectomy

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The pineal gland is the major site of secretion of melatonin (Mel). In all studied species, Mel is released in a circadian rhythm. The results reported in our previous studies have shown that melatonin plays a role in behavior and modulates anxiety levels in rats. The purpose of this study is to confirm these results by studying the impact of pinealectomy in the presence or absence of exogenous melatonin on the level of anxiety assessed in the Open Field Test (OFT).

The pinealectomised rats (PX) or sham operated (SH) were submitted to PL (16L/8D) and received chronic injections of Mel (4mg/kg) or NaCl (9‰) during 8 weeks.

After the treatmen,t the anxiety level was evaluated using the test of OFT. In this test, the time spent in the central area (TCA) and the number of returns to the center (NRC) reflected the anxiolytic effects, while the total number of squares visited (TN) by rats reflected the overall locomotor activity. The results, analyzed by ANOVA followed by the test "t" showed that:

1. SH/Mel groups exhibited a significantly higher TCA and NRC than those obtained in Px/Mel groups ($p < 0.001$), whereas locomotor activity was not affected by Mel treatment. The exogenous Mel produced then a highly signinificant anxiolytic effect.

2. TCA and NRC of SH group were significantly higher when compared to the TCA and NRC PX group ($p < 0.05$). The pinealectomy induced an anxiogenic effect and the endogenous Mel caused a significant anxiolytic effect.

The obtained results clearly showed that endogenous or exogenous Mel produced an anxiolytic effect in the open field test.

Key Words: Pinealectomy, Melatonin, Open Field Test, Anxiety.

"Supported by N€UROMED, REGPOT-2009-2, FP7 of the EU".

P2.198

Involvement of the serotonergic system in the anxiogenic-like effect caused by dehydration in a semi-desert rodent Meriones shawi

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Dehydration is a powerful stimulus, which cause a disequilibrium in water and electrolyte balance, it can be defined as depletion in total body water. Most studies are generally focus on domestic and laboratory animals. However, the study of desert animals allows us to understand many things about water balance and resistance to dehydration and therefore understanding some associated behaviours mainly those related to mood disorders. As a desert rodent we used Meriones shawi, which is characterized by its resistance to long periods of thirst that could reach several months. In the present study animals were subjected to water deprivation for 1 and 3 months, and used for experimentation in order to evaluate by:
1) 5-HT immunohistochemistry: the effects of prolonged dehydration on serotoninergic system in both dorsal raphe nucleus (DRN) and median raphe nucleus (MRN), which are the main source of 5-HT input to several brain areas,
2) dark-light box test: which is useful model to predict the anxiolytic-like or anxiogenic-like effect of dehydration on Meriones shawi.
The results showed an important reduction in the 5-HT immunolabelling in both DRN and MRN following one and three months of dehydration, this diminution of serotonin immunoreactivity is accompanied by noticeable changes in anxiety behaviour of Meriones, consequently, animals spent more time in the light box, suggesting an anxiogenic-like effect caused by dehydration. Overall, the results indicate that dehydration that able to reduce serotoninergic transmission is involved in generating an anxiety behaviour disorder in this desert animal.
Keywords: Meriones shawi, dehydration, 5-HT, DRN, MRN, Immunohistochemistry, Dark-light box, anxiety

P2.199

Plasma brain-derived neurotrophic factor is modulated by dietary n-3 fatty acids in the females growing pig

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Dietary factors can affect multiple brain processes by regulating neurotransmitters, synaptic transmission and signal transduction pathways. This is particularly true for dietary lipids, which exert direct actions on brain neuronal function and represent an efficient means of modulating brain plasticity. Clinical studies indicate that low dietary consumption of n-3 long-chain polyunsaturated fatty acids (PUFAs) is correlated with a number of brain diseases and with cognitive and behavioral defects in development and aging. Dietary n-3 fatty acids may be neuroprotective by upregulating brain trophic

factors. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is involved in both energy metabolism and synaptic plasticity and has been shown to respond to food-derived signals. We therefore tested the sensitivity of BDNF expression to diet-derived n-3 fatty acids in growing pigs. Female and castrated male pigs (66) were fed isoenergetic diets containing different levels of n-3 fatty acid, depending on the vegetal source, i.e. linseed, sunflower, or palm oils for eight weeks. With all three diets, the castrated males ate more than females and this sex-effect was associated with a diet-effect (8% for sunflower, 16% for palm and 23% for linseed diet). The lean meat mass (LMM) was affected by diet in females and was higher after a linseed diet (3%), and associated with a sex-effect with the palm (5%) and linseed (7%) diets. Plasma BDNF levels ($P< 0.01$) greatly increased during growth in a sex-dependent manner. Higher circulating BDNF levels were observed in female pigs fed the linseed-diet ($P< 0.01$). These findings indicate that female growing pigs consuming higher n-3 dietary contents, exhibit a great increase in circulating BDNF leading to a decrease in feeding behavior.

P2.200

Early consumption of high-fat diet enhances odor aversion memory in rats: is it related to hippocampal dysfunction?

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Obesity is associated with adverse cognitive outcomes in humans. In animals, deficits in hippocampal-dependent spatial memory were reported in genetic models of obesity (*db/db* and *ob/ob* mice) and in diet-induced obese rodents (see poster Boitard et al.). Paradoxically, *db/db* and *ob/ob* mice show an enhancement of conditioned food aversion. It remains to be established whether diet-induced obesity induces a similar effect and what mechanisms are involved.
Wistar rats received a control diet (CD) or a high-fat diet (HFD) starting when animals were 3 weeks-old (immediately after weaning), as we recently identified particular period of development (late infancy and adolescence) as highly sensitive to the detrimental effects of HFD on hippocampal-dependent memories (see poster Boitard et al.).
After diet exposure, rats were submitted to conditioned olfactory aversion (COA), in which consumption of novel odorized water was associated with gastric malaise (induced by an i.p. injection of lithium chloride). The COA retention was assessed 3 days later by presenting the odorized water and quantifying its consumption.
Rats fed CD or HFD during 1.5 month showed similar aversion to the odor whereas rats fed HFD during 3.5 months showed greater COA (i.e. lower odorized water consumption) than animals under CD indicating that the HFD effect needs time to emerge. Interestingly, when the 3.5 months of CD or HFD consumption started at adulthood (12 weeks-old), COA was similar in both groups indicating that late infancy and adolescence are periods of higher sensitivity to the effects of HFD on COA than adulthood.
Then, we evaluated whether the COA enhancement was due to higher malaise perception or long-term memory improvement. Rats fed CD or HFD during 3.5 months (starting after weaning) showed similar short-term COA memory (assessed 4 hours after odor-malaise pairing) meaning that HFD did not change malaise perception but specifically enhanced long-term COA memory.
We are currently evaluating by imagery, biochemical and pharmacological approaches whether this COA enhancement is due to hippocampal dysfunction, as the effect of early HFD resembles those of hippocampal inactivation, i.e. spatial memory impairment and food aversion memory enhancement (Stone et al., 2005).

P2.201

A norepinephrine-mediated action on α_2-autoreceptors contributes to the consolidation of inhibitory avoidance in the rat

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The consolidation of inhibitory avoidance learning (IA) has been shown to be under the control of the α_1 and β noradrenergic system in the basolateral amygdala (BLA) Recently, post-training microinfusions of the selective α_2-adrenoceptor antagonist (Idazoxan) or agonist (UK 14,304) in the BLA were found to enhance or impair, respectively, IA performance, suggesting that the memory-modulating role of norepinephrine (NE) also involves the activation of the α_2 receptors. In reference to the preferentially presynaptic location of the α_2-receptors and their inhibitory control upon NE release in the central nervous system, the present experiment tested the effect of a local manipulation of α_2-receptors on the dynamic of NE release in the BLA during IA learning. To this aim, selective α_2-drugs Idazoxan or UK 14,304 were infused using *in vivo* intracerebral retrodialysis during IA learning. Male Long-Evans rats equipped with a dialysis probe aimed at the right BLA were perfused with Ringer solution for 2 hours before the start of the IA learning. Starting one hour before training, samples were collected every 15 minutes throughout the experiment duration. The infusions of 10 mM of Idazoxan or UK 14,304 started 15 minutes before the administration of the electric footshock and lasted for 45 minutes. Retention latencies were measured 24h later. Results showed that **i)** the administration of the footshock during IA learning induced a significant increase in NE release in control animals, **ii)** the infusions of Idazoxan and UK 14,304 induced a significant increase and decrease, respectively, of NE release, **iii)** the footshock-induced release of NE was significantly potentiated by Idazoxan infusion, an effect that was correlated with an enhancement of IA retention latencies. In contrast, the decrease of NE release induced by UK infusion was too dramatic to reveal any footshock effect and no decrease in the retention latencies was found in this group, revealing the behavioral limits of unilateral infusion technique. These data show that the presynaptic α_2 adrenoceptors in the BLA contribute to the modulation of NE release in the BLA and strongly suggest that the memory-modulated role of NE on IA consolidation is mediated by pre-synaptic α_2-adrenoceptors in the BLA.

P2.202

Learning deficits and cortical plasticity defects in Fragile-X mice

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During associative learning a neutral sensory stimulus acquires the emotional valence of an aversive event or a reward after repetitive contingent pairing. One important consequence is the enlargement of the representational area of the conditioned stimulus (CS) in the cortical map of its sensory modality. However, it is still unclear how this transformation participates to learning.
Whiskers are major sensory organs for rodents and the circuits underlying their neuronal representation in barrel cortex are particularly well studied. We showed in a previous study that fear conditioning with a whisker as CS induced plasticity in layer 4 projections of barrel cortex (Rosselet et al., 2011). Here we study the effects of fear conditioning in conditional Fragile X mutants in which *Fmr1* gene is silenced in the layer 4 of somatosensory cortex only. Fragile X mental retardation protein (FMRP), the product of *Fmr1,* is a key regulator of synaptic plasticity and Fragile X mice exhibit learning deficits. Our goal is to study the consequences of *Fmr1* knockdown on the plasticity of layer 4 projections and to understand how this perturbation affects the animal ability to learn.
Freely-moving mice are trained to associate the stimulation of a whisker row with a foot-shock. Video surveillance shows that animals that are successfully conditioned anticipate the upcoming shock by changing their motor activity when their whiskers are deflected. Animals are sacrificed and their brain

dissected and sliced immediately after. We use the technique of laser scanning photostimulation that maps neurons which are presynaptic to a cell and measures the synaptic strength of their connections with electrophysiology. Comparison of conditional mutants and wild-type littermates will help us understanding the relationships between plasticity of neuronal networks in barrel cortex and associative learning performance.
Reference: Rosselet, Fieschi, Hugues and Bureau (2011). Associative learning changes the organization of functional excitatory circuits targeting the supragranular layers of mouse barrel cortex. *Frontiers in Neural Circuits.* 4:126.

P2.203

"Hic-et-nunc" representation of a gaze target: monkeys accurately foveate a moving target after a spatiotemporal perturbation

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It has been established that the saccade system is able to compensate for changes in eye position induced by electrical microstimulation of the deep Superior Colliculus (dSC) in order to bring the eyes toward the location of a flashed target (Sparks and Mays 1983). This finding indicates that retinal signals are associated with eye movement-related signals for generating accurate saccades. However for accurately foveating a moving target, saccade generation must also take into account the changes in target position. In this study, we tested the extent to which the saccade system is able to compensate for unexpected changes in eye position (CEP) when orienting gaze toward a moving target. The following eye tracking task was used in two rhesus monkeys. After stepping 12° vertically (upward or downward) relative to the straight ahead direction, a target continuously moved in the orthogonal direction with a speed of 20-33°/s (leftward or rightward). After a duration of 150 ms, the target motion was blanked for 150-300 ms and the monkeys were rewarded for tracking the target until the edge of the visual display. In 50% trials, a CEP was induced by dSC microstimulation (20 tested sites, 30-45 ms, 400 Hz, 12-20 microA) when the target was blanked. The results show that saccades perturbed by a CEP in the direction opposite to the target motion were as accurate (at saccade end) as unperturbed control saccades. Saccade errors did not increase with the time elapsed after the CEP. An overshooting was sometimes found in saccades perturbed by a CEP in the same direction as the target motion. Finally, the amplitude and direction of electrically evoked saccades depended upon the target location and motion. The compensation for CEP indicates that the neural representation of the target is continuously updated until saccade ends. The observed overcompensation may result from an alteration in the encoding of target location by the microstimulation. Finally, the dependency of elicited saccades on target motion illustrates the interaction of target-related signals with those electrically evoked.
Supported by ANR (MAPS) and Fondation pour la Recherche Médicale (FDT 20100920165)

P2.204

Differential role of lateral entorhinal cortex and dorsal hippocampus in acquisition and flexibility of cross-modal associations in rats

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Most rodent models devoted to the study of neural basis of learning and memory are based on tasks in which the conditioned stimulus is processed by one sensory modality (a tone, an odor…). The

present work is based on a new behavioral paradigm that allows us to investigate the neural basis of cross-modal olfacto-tactile associations. In the rat, these two modalities are of prime behavioral importance for object exploration. The task consists in finding one baited cup (+) among three, each of the cups being designed by a different and specific odor (0) and texture (T) combination as following: O1T1 for cup +, O2T1 and O1T2 for the non-reinforced ones. Interestingly, most rats learned this task within 3 to 6 training sessions (20 trials/session). A second task (task 2) consisted for the animals to pair another O-T combination with the reward using a new set of stimuli (O3T3+, O4T3, O3T4). Results showed that rats managed the learn task 2 within 1 to 3 training sessions only. A third task (task 3) consisted for the animal to learn another O-T combination based on previously learned items (ex: O2T3+, O2T4, O2T3). This task is called the “flexibility task” since animals are expected neglect previously reinforced items and learn the new specific reinforced combination to solve this task. Surprisingly, most rats solved the flexibility task within 1 or 2 training sessions. The second part of our work consisted of testing the role of lateral entorhinal cortex (LEC) and the dorsal hippocampus (DH) in each of the 3 tasks. To this aim, animals bilaterally implanted with cannulas in the two structures were microinfused with lidocaine (4%; 0,4 µL) just before the test session of each tasks. The results showed that inactivation of either structure did not impair recall of task 1. Interestingly, LEC inactivation severely impaired acquisition of a new set of combinations (task 2). In contrast, DH inactivation did not affect acquisition of task 2 but selectively impaired performance during the flexibility test. Taken together, these results suggest that LEC is involved in the formation of new olfacto-tactile associations, while DH is important for flexibility of this cross-modal associations.

P2.205

New insight into the role of central serotonin: Tph2 deficiency improves stress coping in paradigms of innate anxiety and depressive-like behavior by sex specific adaptive mechanisms

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Serotonergic system have long been implicated in a wide spectrum of psychiatric disorders and behavior, development, plasticity and the mechanism of action of many drugs, but, its role remains unclear. Tryptophan hydroxylase 2 (Tph2) being the key enzyme in central serotonin (5-HT) synthesis, we have generated *Tph2* knockout (-/-) mice to lift the veil on this old mystery. Although unable to synthesize 5-HT within their brain, neurons and Tph2 -/- mice survive until adulthood. Here we analyze the impacts of central 5-HT deficiency on neuron function, emotion-linked behaviors and HPA axis regulation and adaptation to stress.
Serotonergic neurons were histologically and electrophysiologically phenotyped. Tph2 -/- mice were behaviorally characterized in different paradigms: Elevated+maze, open field, forced swimming, sucrose preference, resident intruder and fear conditioning. Their reaction to chronic mild stress (CMS) was analyzed in the same behavioral tests. HPA axis activity and adaptation were assessed by Corticosterone excretion monitoring and receptors expression analysis.
Although devoid of 5-HT, serotonergic neurons express all specific markers and display normal electrophysiological characteristics showing that 5-HT synthesis is not necessary for neuron differentiation and function. The lack of Tph2 results in reduced innate anxiety but increased fear learning; increase of depressive-like behavior but no anhedonia, and increased intermale aggression. While CMS had deleterious effect in wild type mice, Tph2 -/- mice behavior were either insensitive or even improved by stress. Moreover, a strong sexual dichotomy appears in HPA axis response as well as in the mechanism of adaptation to CMS.
Brain 5-HT deficiency induces an interesting but complex constellation of endophenotypes, with some unexpected orientation. Our results suggest that 5-HT may be the mediator of environmental adverse

effects on innate behaviors and that its absence favors resilience. Moreover, interaction with HP-gonadal axis leads to sex-specific coping strategy against chronic stress. This new insight suggests that it may well be that future research on Tph2 -/- mice will provide an entirely new view of 5-HT in brain development and function related to neuropsychiatric disorders.

P2.206

Tracking the neural fate of memories using functional Magnetic Resonance Imaging: Role of the hippocampus and neocortical areas in the retrieval of remote episodic and semantic memories

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Memory traces are not acquired in their definite state but rather undergo a time-dependent process of reorganization within brain networks. At the systemic level, this process of consolidation consists in a gradual transfer of memory traces towards neocortical sites, where they will be stored durably. For some authors, the hippocampus would play a time-limited role in this process. However evidence from human and animal studies have led to an alternative view of memory consolidation, in which hippocampal involvement depends on the nature of memory traces.
We investigated the neural substrates of memory retrieval for recent and remote episodic and semantic memories. During a learning phase, 18 young healthy subjects (mean age ± SD: 22.6 ± 1.85 years) had to memorize a series of pictures. Then, functional Magnetic Resonance Imaging data were acquired 3 days and 3 months after learning during a recognition task associated with the Remember/Know (R/K) paradigm. The same “old” pictures were used for both retrieval sessions, allowing us to track the fate of these memories, and to distinguish consistently episodic memories at both delays (RR) from semanticized ones (initially episodic memory that became later semantic; RK). Functional data were analyzed using SPM5 ($p< 0.05$, corrected for multiple comparisons).
Consistently episodic memories (RR) recruited a large neural network, including frontal and parietal areas, as well as the hippocampus without any disengagement of the posterior part of this latter structure, classically involved in retrieval processes, over time. Regarding semanticized memories (RK), despite large similarities in neocortical activations with RR memories, hippocampal activation declined with time.
Our data are important to understand how memory traces are reorganized with time within neural networks. We show here that the hippocampus maintains activation on the long term for the retrieval of episodic memories but not for semanticized ones.

P2.207

Deep brain stimulation and on-line executive control in Parkinson's disease patients

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We sought to evaluate the effects of high frequency deep brain stimulation on the ability to detect, inhibit and correct erroneous responding in Parkinson's disease (PD) patients.

On the one hand, DBS is generally considered to inactivate the subthalamic nuclei (STN) and to restore the thalamo-cortical projections impaired by striatal dopamine depletion caused by PD. This therapy dramatically improves PD motor symptoms. On the other hand, recent fMRI data collected in healthy volunteers suggest that the STN are involved in the urgent inhibition of ongoing actions. On the basis of these two lines of evidence, we reasoned that a side effect of DBS could be to impair this ability in PD patients.
Sixteen PD patients treated by DBS for at least 6 months performed a between-hand choice reaction time (RT) task in 4 treatment conditions: Stimulator ON - Medication ON, Stimulator OFF - Medication ON, Stimulator ON - Medication OFF,. Stimulator OFF - Medication OFF. The electromyographic (EMG) activity of the response agonists (*flexor pollicis brevis*) was recorded and analysed, allowing the detection of partial errors, that is of subliminal activations of the muscles involved in erroneous responses that would lead to error commission if not detected, inhibited and stopped in time.
Results show that
(i) medication exerted no notable effect on the patients' performance,
(ii) DBS shortened RT but increased the error rate and decreased the number of partial errors,
(iii) detailed analyses of EMG and RT distributions revealed that this increase in error rate was specifically due to a failure to detect, inhibit and correct partial errors before they resulted in overt erroneous responses.
Our data suggest that while it improves motor performance by shortening RT, DBS impairs the patients' faculty to suppress erroneous response activations as revealed by their inability to counteract partial errors. These results are in line with current understanding of the role played by the hyperdirect cortico-subthalamic pathway in the urgent inhibition of ongoing actions.

P2.208

Is time interval encoded in odor fear conditioning?

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Time perception is crucial to survival and goal reaching in humans and other animals and interval timing also guides fundamental animal behaviors. In pavlovian fear conditioning, an initially neutral stimulus (the conditioned stimulus) predicts the arrival of an aversive unconditioned stimulus (generally a mild foot-shock) at a fixed time interval. Accumulating experimental evidence has made it clear that in associative learning temporal relations between events are encoded. However the question of the memory of time and its underlying neural network in fear conditioning has been poorly investigated. The aim of the present study was to address this question in odor fear conditioning. Fear response is characterized by a wide range of behavioral and physiological responses. Despite the existence of this large repertoire of responses, freezing behavior is often the sole parameter used for quantifying fear response, thus limiting emotional memory appraisal to this unique index. Interestingly, respiratory changes and ultrasonic vocalizations (USVs) can occur during fear response, yet very few studies have monitored these parameters in addition to freezing. We designed an experimental setup allowing the simultaneous recording of Respiration, USVs and Behavior (RUB Cage) in rats and the offline synchronization of the collected data for fine-grain second by second analysis. These parameters, added to freezing response, greatly improve the sensitivity of the behavioral assessment of the fear response, thus increasing the probability of detecting transient anticipatory fear responses suggestive of time encoding. The RUB cage was used in rats involved in an odor fear conditioning paradigm. The data show that after a few odor(20s)-shock(1s) pairings, an anticipatory response develops, mainly characterized by a decrease in respiratory rhythm and an increase in USVs emission just prior to shock delivery. Current experiments are carried out to identify some of the key structures as well as neuromodulators involved in this time encoding. We are mainly focusing on the role of a dopaminergic transmission in the amygdala.

P2.209

A subpopulation of prefrontal putative inhibitory interneurons controls conditioned fear expression

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Accumulating evidence indicate that the medial prefrontal cortex is necessary for conditioned fear expression. Indeed, whereas inactivation of the medial prefrontal cortex prelimbic area prevents fear expression, its electrical stimulation facilitates conditioned fear responses. It is likely that neuronal changes occurring during fear expression depends on specific interactions between excitatory and inhibitory prefrontal microcircuits. However such microcircuits are still unknown. Recent anatomical and electrophysiological data indicate that cortical inhibitory circuits might regulate pyramidal cell activity at multiple levels. In order to evaluate the role of inhibitory prefrontal microcircuits during expression of conditioned fear responses we used single units recordings in behaving animals submitted to auditory fear conditioning. Our results indicate that tone-evoked neuronal responses in a subpopulation of prefrontal putative inhibitory neurons are inversely correlated with conditioned fear responses. Moreover, these changes in neuronal activity precede the behavioral expression of conditioned fear responses. This data suggest that a subpopulation of prefrontal inhibitory neurons selectively controls the expression of conditioned fear responses.

P2.210

Action mechanism underlying cannabinoids effects on food intake: focus on hypothalamic melatonin and sertoninergic systems

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Energy homeostasis is regulated by a complex interaction of molecules and pathways, for example, the endocannabinoid, melanocortin and serotoninergic systems.
The effect of intracerebroventricular or intraperitoneal administration of cannabinoid receptor agonist WIN 55,212-2 or inverse agonist AM 251 on food intake, hypothalamic melanocortin expression; and extracellular levels of serotonin (5-HT) and acetic acid 5-hydroxy-indol (5-HIAA) from presatiated rats were studied.
Compared to the vehicle-injected control, the intracerebroventricular administration of WIN 55,212-2 was associated with a significant increase in food intake, whereas the administration of AM 251 caused a significant reduction in this respect. These results were accompanied by considerable reductions or increases in serotonin and acetic acid 5-hydroxy-indol levels compared to the vehicle-injected control and the baseline values for the different experimental groups studied. Intraperitoneal administration of WIN 55,212-2 at doses of 1 and 2 mg/kg promoted hyperphagia up to 6 h after injection, whereas administration of a higher dose (5 mg/kg) significantly inhibited food intake. Administration of any of the AM 251 doses studied (0.5, 1, 2, 5 mg/kg) led to a significant decrease in the amount of food ingested from 2 h after the injection, compared to the vehicle-injected control group, with the most striking effect being observed when the 5 mg/kg dose was injected.
It looks interesting that AM251, quite significantly reduces the expression of MCH-R1, whereas WIN 55, 212-2 stimulated the expression of pro-MCH. Taken together, these data, confirm that the endocannabinoid system modulates the activity of the MCH/ 5-HT hypothalamic systems, which could underlie part of the orexigenic effects of cannabinoids.
Keywords: Cannabinoids; endocannabinoid system; Feeding; 5-HT; Melanocortin

P2.211
Place cell discharge variability predicts individual neurocognitive performance in rats

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Hippocampal place cells are pyramidal neurons that are selectively activated while the animal is in a particular location in a particular environment; their activity is therefore subject to, among other variables, environmental manipulations. In young rats, environments are reliably encoded by populations of neurons displaying a high spatial-selective activity ('place fields') that can be stable for up to months. In the time domain, however, place cells exhibit a strong discharge variability from one pass to another when a rat goes through a firing field. This variability, or 'overdispersion', is modulated by cognitive demands and may therefore reflect variations in neurocognitive abilities in individual animals. To assess relations between cognitive performance and overdispersion, we first trained old rats in a delayed non-matching-to-sample task, and then recorded hippocampal CA1 place cells while the animals foraged for food in a square arena. We found a positive correlation between the degree of variability in place cell firing and performance in the memory task at long delays, thereby providing a new way to investigate neurocognitive decline within and between individuals.

P2.212
Grid cell activity in bidimensional and unidimensional environments

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The dorsolateral part of the medial entorhinal cortex (dlMEC) contains neurons (grid cells) with multiple firing fields that form a regular triangular grid across the environment. These cells have been suggested to mediate a euclidian map-like representation of the rat's location and orientation based on movement-related information. However, whether and how restrictions on the use of movement-related information affect grid cell firing is still unknown. We studied how grid cell activity changes when the rats explore both a bidimensional and a unidimensional environment. To assess the contribution of external sensory input (allothetic information) and movement-related information (idiothetic information) to the stability of grid cell firing fields, we compared grid cell activity in both light and dark conditions. Rats were implanted with a bundle of 4 tetrodes in the dlMEC and were trained to freely explore the entire surface of a circular arena (150 cm in diameter). They were then confined to a peripheral rim of the arena in which they run unidirectional laps both in light and darkness. A new regular firing pattern appeared in the circular track in which the distance between the firing fields was increased compared to the circular arena. This novel map was maintained in the dark. These data indicate that grid cell activity is influenced by the animal's exploratory patterns. The increased distance between the fields observed in the circular track suggests a potential role of grid cells in estimating the effective distance between places (i.e. the distance travelled by the animal) rather than the absolute distance. Moreover, our data indicate that idiothetic information is sufficient to stabilize the grid map.

P2.213
Early learning and memory impairments in the 5XFAD mouse model of Alzheimer´s disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive deficits and notably by learning and memory impairments. Transgenic animal models of AD have made considerable contribution to our understanding of molecular and pathological mechanisms of AD and related cognitive dysfunctions. In this study, we focused our attention on the 5XFAD transgenic mice in which human APP and PS1 transgenes with five familial AD mutations are expressed and act together to additively increase levels of cerebral Aβ peptides. This model is one of the most early-onset and aggressive amyloid mouse models of AD, starting to develop visible amyloid deposits as early as to 2 months of age. The aim of this study is to assess the cognitive performance of the 5XFAD transgenic mice in correlation with these early molecular disturbances. For this purpose, we used two behavioral tests, the olfactory tubing maze and the olfactory H maze, to evaluate respectively hippocampus and prefrontal cortex dependant learning and memory abilities in 2, 4 and 6-month-old mice. We report that both hippocampus and frontal related deficits appear in aged 4-month-old 5XFAD transgenic mice and are more pronounced at 6-months of age in comparison to wild type mice. Strikingly, the behavioral impairment was stronger in the task involving the frontal cortex in comparison to the hippocampal dependant task. Correlation studies between these cognitive deficits and amyloid deposits, inflammation and synaptic dysfunctions in the hippocampus and the frontal cortex are in progress.

P2.214

Exploring the neural basis of cognitive and behavioral dysfunction in epileptic patients

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Recently, neuro-computational modeling has become an increasingly useful tool for understanding the diverse and complex link between brain and behavior. Epilepsy is a complex set of disorders with the commonality of recurrent seizures. Knowledge discovery and data-mining provides the substrate and support for dynamical modeling and allows model findings to be applied back to research and clinical settings. At the patient level, Markov models have been used to assess patterns of remission and relapse in pediatric epilepsy. There are at least two forms of epilepsy: Frontal lobe epilepsy (FLE) and temporal lobe epilepsy (TLE). Both disorders affect different brain structures, as their name suggests. They share similar symptoms, especially losing the contact with the environment (inability to emit behavioral responses). We tested the cognitive deficits related to memory and learning in FLE and TLE via two computerized cognitive tasks, namely Acquired Equivalence (AE) and Working Memory (WM) pre- and post-treatment. The results suggested a significant improvement in both tasks after treatment with anti-epileptic drugs. We found that the intelligence usually falls within normal limits in patients with FLE. Memory problems exist in FLE, but to a lesser degree than in TLE. However, attention and working memory seem to be equally affected by frontal and temporal seizures. Furthermore, until present day, even though there is a large body of experimental studies on cognitive dysfunction in epileptic patients, there is no computational model that explains how neural damage in these patients; leads to the various symptoms and cognitive dysfunction associated with epilepsy. This finding supports the importance of building a brain-inspired computational model, in which we will simulate the interaction between both temporal and frontal lobe as well as their neuromodulators (acetylcholine and dopamine, respectively). This computational modeling will play a key role in predicting the effect of epilepsy on cognitive deficits focusing on memory and learning. Therefore, the combination between experimental data followed by computational modeling will provide useful information on the neural and behavioral dysfunction in these patients.
(Supported by N€UROMED, REGPOT-2009-2, FP7 of the EU)

P2.215

Neurobehavioral effects after prenatal exposure to fenugreek seeds aqueous extract on mice

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The use of medicinal plant products to treat various ailments is a common practice in many developing countries. However, a lack of information on the adverse effects of these plants raises questions on their safety and possible adverse side effects. The present work studied the effect of fenugreek seeds maternal exposure on neurobehavioral endpoints in the development of mice pups. To accomplish that, the effects of fenugreek seeds aqueous extract (0, 0.1, 0.5 and 1g/kg) administered daily by gavage throughout gestational period were examined in pups. The offspring were examined for behavioral development including, cliff avoidance response (PND6) surface righting reflex at Postnatal Days (PND5 and 7), swimming development (PND8, 10 and 12) and the negative geotaxis test (PND 10 and 12). Those developmental tests evaluate innate reflex, orientation and vestibular and motor functions.
Results show a significant increase in the time of cliff avoidance response, righting reflex on PN5 and PN7, and negative geotaxis on PN10 and PN12 in all exposed groups compared to controls. The offspring in treated groups showed significantly lower success rates than controls in cliff avoidance responses. In swimming development, the offspring in the 1g/kg group, had significantly lower scores than controls for swimming (direction, angle and limb used).
These results showed a delay of early response development and motor coordination in the offspring of mice exposed to fenugreek seeds aqueous extract throughout pregnancy period. We concluded that the fenugreek seeds consumption during pregnancy could be a cause of offspring developmental toxicity.
Keywords: Prenatal exposure; behavioral teratology; fenugreek seeds extract; developmental toxicity; mice.

P2.216

Comparison of the effects of arginin-vasopressin and desmopressin on spontaneous behavior in Wistar rats in the open field

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Studies in the last two decades discovered many central effects of neurohypophyseal hormones vasopressin and oxytocin. Both hormones are implicated in the emotional and cognitive processes. Since these hormones are peptides and do not penetrate blood brain barrier (BBB), most of the research was done in studies where peptides were applied intracerebroventricularly or directly into specific brain areas known to participate in learning, memory, other cognitive functions and emotions. In our previous studies we have shown that these peptides reveal behavioral effects also after systemic application, which indicates that certain amount of drugs penetrate through BBB. The aim of this study was to compare effects of arginin-vasopressin and desmopressin, which was designed as drug with peripheral actions that have therapeutic usage as antidiuretic remedy. In spite of the fact that this drug is used therapeutically in humans, very little is known about its central effects. In our studies we used Wistar male rats (VELAZ CR; 250-270 g). Saline or desmopressin (DDAVP; l-desamino-8-D-arginin vasopressin) (Polypeptide Laboratories, Czech Republic) was administered i.p. in doses from 0.03 to 3.0 mg/kg b.w. in a volume of 2 ml/kg b.w. one hour before behavioral testing in the open field devise. Behavior of rats was videomonitored by an automated activity monitoring system (AnyMaze, Stoelting Co., IL, USA) in a circular arena with a diameter of 150cm. Total movement distance (TMD) and distance in the inner zone (diameter of 120 cm) were recorded automatically; the experimenter measured total number of rearing and total time spent in grooming. A one-way ANOVA was used and

statistical significance was accepted when $p < 0.05$. Peptide DDAVP, given intraperitoneally, reveals remarkable inhibitory effects on spontaneous behavior of rats tested in the open-field. This effect disappears on the second day after treatment and exposure to the open field test. Our results indicate that DDAVP could have some therapeutic use in the treatment of several psychiatric diseases.
Suported by MSM 0021620806.

P2.218

Relationship between adiposity-related inflammation and neuropsychiatric symptoms in diabetes patients

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Adiposity and chronic inflammation are fundamental characteristics of type 2 diabetes (T2D). Part of systemic inflammation in T2D may originate from the adipose tissue in which macrophages accumulate and potently secrete inflammatory factors. Moreover, diabetes is associated with an elevated prevalence of neuropsychiatric symptoms, including depression. Numerous experimental and clinical studies have linked chronic inflammation to the development of neuropsychiatric symptoms. In addition, recent data obtained in our group suggest that adiposity-related systemic inflammation may represent an important mediator in the development of depressive symptoms and emotional disturbances in patients with metabolic disorders. The objective of this study was thus to assess the relationship between adiposity-related inflammation and neuropsychiatric symptoms in patients with T2D.
Fifty-one diabetic patients (20 T2D *vs* 31 T1D) were included in the study and submitted to a complete neuropsychiatric evaluation assessing depressive, anxious and neurovegetative symptoms. Cognitive alterations were assessed with the neuropsychological automated battery CANTAB. Anthromorphic data were collected as well as blood samples for the measurement of inflammatory markers (e.g., C-reactive protein, interleukin-6 and leptin).
Compared to T1D subjects, T2D patients exhibited higher levels of inflammatory markers that correlated with measures of adiposity. In addition, T2D patients showed higher neuropsychiatric symptoms, notably in the dimensions of mood, fatigue and sleep. With respect to cognitive function, specific alterations were measured in T2D patients in the CANTAB tasks of spatial planning and choice reaction time. Systemic inflammation was found to contribute to sleep alterations, reduced activity and spatial planning impairment in T2D patients.
These findings support the hypothesis of the involvement of adiposity-related inflammation in the development of neuropsychiatric symptoms in T2D patients. Interventional studies are needed to further investigate the effects of variations in adiposity on systemic inflammation and related neuropsychiatric symptoms.

P2.220

Electrophysiological correlates of learning and memory of opiate withdrawal in morphine-dependent rats

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The memories of affective states associated with positive drug effects or negative withdrawal states play a crucial role both in sustaining drug consumption and in relapse. A number of studies suggest that addictive process is underpinned by a pathological recruitment of neural mechanisms underlying motivation, learning and memory. In opiate dependence, withdrawal is characterized by a motivational negative affective state as well as by the emergence of somatic signs. Interestingly the reactivation of withdrawal aversive memory can induce a motivation for both drug seeking and taking. The early withdrawal motivational component has been proposed to play a major role in addictive processes in dependent individuals, and also in relapse in abstinent individuals.
In the present study we investigated encoding and retrieval of contextual memories related to the motivational component of opiate withdrawal. To test and analyze these memories we used a conditioned placed aversion paradigm together with chronic electrophysiological recordings in a rodent model of opiate withdrawal in which motivational component can be assessed independently from the somatic one. Based on our previous work our electrophysiological recordings focused on limbic structures involved in associative learning, emotion processing and conditioned responses such as the basolateral amygdala (BLA), the nucleus accumbens (NAC) and prefrontal cortex (PFC).
Our work shows that in animals presenting a significant conditioned place aversion, neuronal activities and local field potentials in BLA, NAC and PFC present common oscillation frequencies (thêta ~8 Hz, bêta ~15 Hz et gamma ~60Hz). Moreover, these oscillations synchronize in specific frequency ranges depending on the pair of structures considered. BLA and NAC oscillate synchronously in all three modes. CPF instead, synchronizes preferentially with NAC in the theta and gamma ranges and with BLA in the beta and gamma ranges.
These results suggest that synchronous oscillations are privileged processes when it comes to communications between those limbic structures. We also demonstrated that information exchanges take place according to different frequency modes depending on the structure.

P2.221

ZIF-268 labeled cells in the hippocampus, but not in the amygdala, are more activated during memory extinction then during memory recall

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The discriminative avoidance task is a well validated animal test for memory. It consists of a modified plus-maze with its closed arms disposed in a L-configuration, where a speaker and a strong light will be installed in one of those (the aversive arm). Whenever the rat enters it, the aversive stimuli, consisting of a bright light and a loud noise, will be applied during the training session. Our aim here is investigating the ZIF-268 expression in the hippocampus and amygdala through the mnemonic processes, such as memory acquisition, recall and extinction. The experiment consists of 4 groups and 3 sessions, spaced by a 24 h interval. At first the animal will be exposed to the maze with the four arms enclosed. This will be the pre-exposition session, and will make up the control group. Then, two of the arms will be opened and training will follow, thus with the aversive stimuli in the aversive arm. Finally a test session will be conducted. On this regard, the animal behavior will determine whether the animal will enter the test-evocation group or the test-extinction group, depending if they exhibited a pure evocation (zero entrances in the aversive arm) or if they begun exploring the aversive arm after a short recall period (initiating the extinction process). The animals where perfused 1 hour after the start of the target section, their brains frozen, cut at 30 microns and then stained against ZIF-268. Our main result consists of an increase in the number of activated cells both in the hippocampus (CA1, CA3 and DG) but not in the amygdala (LA, BLA, CE) during memory extinction, in comparison to memory recall.

P2.222

Adult olfactory neurogenesis is down-regulated by parturition and interactions with young

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Olfaction is of primary importance for the onset of maternal behavior. In sheep olfactory cues are responsible for attraction to neonates and for the establishment of an individual recognition of the young. Olfactory neurogenesis, a supply of brain plasticity, could be a mechanism by which olfaction can contribute to the onset of maternal behavior and the associated learning. In this study, we investigated whether parturition and/or the first interactions with the young modulate the two main processes of olfactory neurogenesis, cell proliferation and survival. In addition we looked at hippocampal neurogenesis since this structure plays a role in some olfactory learning.
Cell proliferation was investigated by a single BrdU injection (20mg/kg) in parturient ewes interacting with their lamb for 24h (n=8) or being separated from their young after parturition (n=6) and in virgin females (n=6). A fourth group composed of ewes in oestrus interacting with a male for 24h, was studied as a control for hormonal changes and social interactions (n=5). Ewes were sacrificed 24h after BrdU injection. In the subventricular zone, in comparison with virgin ewes, parturition and interactions with the lamb decreased cell proliferation but interactions with male and mating did not. In the dentate gyrus, parturition and/or interactions with the lamb decreased cell proliferation whereas mating and interactions with male did not.
Cell survival was investigated in parturient ewes interacting with their lamb for 48h (n=5) or being separated from them after parturition (n=6) and in virgin females (n=6). Four months before sacrifice BrdU was injected daily for 4 days (20mg/kg). In the olfactory bulb, in comparison with virgin ewes, interactions with the lamb and learning of its olfactory characteristics decreased the number of new olfactory neurons whereas in the dentate gyrus parturition by itself down-regulated cell survival.
Overall, these results indicate that learning the olfactory characteristics of the young induces a decrease in both olfactory cell proliferation and survival. By decreasing cell competition, this down-regulation could favour selection of some newly-born neurons to participate to the learning of lamb olfactory signature.

P2.223

Unsupervised paradoxical sleep deprivation in Rodents using an adaptative real-time detection algorithm

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Paradoxical sleep (PS) deprivation (PSD) can be efficiently performed by using the classical "inverted flower pot" method: one or several platforms surrounded by water serve as pedestal(s) large enough to hold an animal when awake or presenting slow wave sleep (SWS), but too small when PS and its muscular atonia occurs. Animals consequently refrain from entering PS, and present a PS hypersomnia (rebound) when placed back in their home cage. In the first hours of deprivation this method also suppresses SWS; hence, it cannot be used for short selective PSD. As a non-stressful alternative, manual deprivations are possible but require an operator for the entire PSD. Here we introduce an unsupervised approach based on a real-time detection of PS coupled to a new mechanical device to awaken the animal. Our adaptative algorithm identifies PS from EEG and EMG signals, and applies a stimulus producing a sudden rise and fall of the cage´s floor. This new method was used in two paradigms, a 6-hour PSD, and a 72-h PSD.
When applied to baseline recordings, our algorithm gave a high concordance with human scoring (>95%). A first group of rats went through a 6-h manual PSD. On average, PS residual amount, i.e. the sum of PS bouts necessary PS detection was 7.6 min over the 6h-period. Manual PSD was

followed by an increase in PS amounts: 29.2±2.9% vs 12.6±2.6% in baseline (n=3, over 2h). With the unsupervised PSD, PS residual amount was lower (5.6 min), and PS rebounds of slightly less magnitude 22.1±7.6% vs 14.0±3.3%. Another group of animals went through a 72h PSD using the platform technique and, two weeks later, through a second 72h-PSD with our new device. The rats were then euthanized after the end of PSD, and brain sections were collected for Fos-labeling. On average, PS amount during the rebound was smaller after the unsupervised PSD (23.3±3.3% vs 41.2±5.1% over 3h; n=6), while SWS amount did not significantly differ (46.6±10.8% vs 43.7±4.5%). The analysis of Fos labeling (n=3) revealed similar activation levels after PS hypersomnia as those described after the classical PSD. Altogether this approach efficiently overcomes the limitations of manual detection for short PSD, and can be a valuable alternative to the classical technique for long PSD.

P2.224

Spatiotemporal dynamics of lexical access

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Selecting words for speaking is a mundane and very complex act of our daily lives. Consider for instance our ability to name a usual object placed in front of us. Psycholinguistic and neuroscientific research have showed that such word retrieval task involves different kinds of information and processing stages: conceptual, lexical, phonological. These processes are thought to engage a distributed network of brain areas in the left ventral stream, with a predominant role played by temporal lobe for lexico-semantic processing.
We investigated lexical access in healthy speakers with two picture naming tasks, where the ease of word selection was manipulated. In the “Sequence task”, participants named lists of unrepeated images while the relative positions of items within semantic categories (e.g. animals) were manipulated. In the “Block task”, participants named images repeatedly in semantically related or unrelated blocks (e.g. only animals vs. mixed categories). Behavioral and electrophysiological (EEG) data were recorded and analyzed.
A semantic interference effect was observed in both tasks, which can be attributed to lexical access processes. In the behavioral data, the Sequence task shows an increase in response latencies as a function of ordinal position within semantic categories, whereas the Block task showed longer latencies in semantically related blcoks. In the electrophysiological data, this semantic effect is present in both tasks from 250 ms onwards in left posterior electrodes; this timing is thought to correspond to the timing of lexical access [Indefrey P, Levelt W.J.M. (2004, *COGNITION*)]. The semantic effect was also observed later, around 600-700ms, i.e. at latencies corresponding to the verbal production. In this case, the effect was present in the same posterior regions but also in left anterior areas, which presumably results from the involvement of Left Inferior Frontal Gyrus in solving the conflict between the various activated candidate words [Schnur T. et al. (2009, *P NATL ACAD SCI USA*)]
These results study clarify some aspects of the spatio-temporal dynamics of lexical access in healthy speakers, which may provide a baseline for understanding word finding difficulties in pathology, e.g. in frontal and temporal lobe epilepsy.

P2.225

The nucleus reuniens contributes to memory retrieval in a simple spatial learning task

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Background: Diencephalic lesions as seen in Korsakoff syndrome or thalamic stroke can cause memory dysfunctions that resemble aspects of temporal lobe amnesia. Approaches in animals suggest that intralaminar nuclei and anterior thalamic nuclei lesions could be among the critical alterations producing diencephalic amnesia (Lopez et al., J Nsci 29, 2009, 3302). Due to its strong anatomical connections with the prefrontal cortex and the hippocampus, which are crucial structures for memory processing, damage to the nucleus reuniens (NRe) might also participate in memory deficits seen after thalamic lesions. Surprisingly, NRe has not received much attention. Therefore, using reversible inactivation, we tested, in the Rat, the role of the NRe in the retrieval of the location of a platform in the double-H maze, a novel water-escape task taxing spatial memory (Pol Bodetto et al., Behav Brain Res 218, 2011, 138).
Methods: Rats were first subjected to the implantation of a unilateral cannulae targeting the NRe. The double-H is an apparatus shaped as two contiguous "H" with 2 intermediary arms and 4 extremity arms perpendicular to a central alley. It is flooded with opaque water. The task consists in learning to swim from the start arm to the platform located at the extremity of another arm. Start points are randomly alternated to prevent any procedural strategy and rather force the animal into an approach based on place rather than response learning. After drug-free training (2 days, 1 probe trial, two additional days, 1 probe trial), the rats received either Lidocaine (Lido, 20 µg/ 0.3 µL), muscimol (MUSCI, 0.25 µg/ 0.3 µL) or PBS before each probe trial.
Results: Lido- or MUSCI-infused rats spent significantly less time in the target arm than PBS controls, showing that inactivation of the NRe before the probe after a drug-free acquisition disrupted retrieval of the platform location.
Conclusion: These data clearly show an implication of the NRe in the retrieval of a spatial memory, a finding compatible with its functional and anatomical links with the hippocampus and prefrontal cortex. Our results also provide new perspectives on a better understanding of the damage and dysfunctions underlying diencephalic amnesia.

P2.226

When and where processing in saccades generation mapped by fMRI with integrated Eye Tracking

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Introduction: Anticipatory behavior in saccade generation is based on external cues indicating the timing or position of the target movement. The aim of this study was to investigate the contribution of external cues on saccade generation compared to reflexive saccades.
Methods: Nine adults with mean age of 29.8 (dp=4.6) performed block factorial design paradigm, type ABCDE: (A)saccades with predictable time and position, (B)saccades with predictable time, (C)saccades with predictable position, (D)reflexive saccades and (E)rest condition fixation cross. 20s condition had 50 randomized trials and were repeated 6 times in 2 runs, total time 16 minutes and 20 seconds. 480 EPI images were acquired on a 3T scanner (Philips Achieva), 8 head coil with integrated MRI compatible Eye Tracker (Magconcept).TR was 2s, TE=30ms, voxels dim of 3x3x3mm. Images were analyzed using FSL software. The activation maps were obtained considering Z-voxel>2.3 and cluster-wise p-value< 0.05.The analysis of interest was to compare conditions: D>A, A>B and A>C. The eye movements were analyzed off-line for saccades on-set time and correct response.
Results: Eye movements descriptive data showed good performance with 90% of correct saccades. Saccades on-set means for each condition were: (A) m=182.87ms, dp=111.37, (B) m=350.01ms, dp=119.86, (C) m=323.36ms, dp=48.74 and (D) m=338.83ms, dp=31.9. In all the conditions, there was a common activation in occipital cortex, FEF, SEF and superior parietal cortex. Increased activity was found in reflexive saccade condition minus predictive saccades (condition D>A) in parietal superior cortex and FEF left. Second comparison of condition (A>B) found activation in left middle frontal gyrus, left precuneous cortex, angular gyrus and hippocampal gyrus. Analysis of components (A>C) showed activation in striatum, superior frontal gyrus, angular gyrus, parietal-insular vestibular cortex and precentral gyrus.
Conclusions: Saccades generated without external cues activated more sensory-related regions than saccades influenced by time and position cues. In addition the analyses of predictable components showed that external cues processing may involve different neural systems.

P2.227
Improving pitch perception by cochlear implant users

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Cochlear implants (CIs) restore hearing sensations to deaf people via direct electrical stimulation of their auditory nerve. Contemporary CIs mimic the frequency-to-place mapping performed by the cochlea by passing sounds through a bank of bandpass filters and directing the outputs of these filters to different electrodes implanted inside the cochlea. CI listeners usually perceive a change in pitch from “low” to “high” when stimulating individual electrodes from the apex to the base. However, the coding of this “place pitch” may be limited by
(1) a shallow insertion of the electrode array and by
(2) the finite number of implanted electrodes.
Here, we present and evaluate a stimulation method designed to partially overcome both of these limitations.
Most CIs stimulate the nerve fibers with trains of symmetric biphasic pulses. In the following two experiments, we tested whether *asymmetric pulses*, consisting of a short, high-amplitude phase followed by a longer and lower amplitude phase could increase the range of place pitch percepts experienced by CI subjects. This hypothesis stems from psychophysical and electrophysiological data showing that the positive phase of each pulse is mostly responsible for exciting auditory neurons in CI patients, and from the fact that, with an asymmetric waveform, neural stimulation arises from the short, high-amplitude phase rather than from the long, low-amplitude phase of each pulse.
Seven users of the CII/HiRes 90k implant were asked to compare the pitches of several stimuli presented in pairs. The stimuli were pitch-ranked using the method of constant stimuli or the optimally-efficient “mid-point comparison procedure”.
In experiment 1, we found that asymmetric pulses presented in bipolar mode with the leading phase anodic relative to the most apical electrode yielded a lower pitch than symmetric pulses presented in either bipolar or monopolar mode (as implemented in most CIs).
In experiment 2, we found that pitches intermediate to those produced by neighbouring electrodes could be created by systematically modifying the ratio of duration of the two asymmetric phases.
These results will be discussed and compared to other recent techniques designed to achieve the same goal.

P2.228
Unimpaired delay, trace and contextual fears in place learning-impaired aged rats

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Numerous studies have reported that aged rodents exhibit deficits in learning and memory tasks that require an intact hippocampus. Among these tasks, impaired place learning in the Morris water maze is consistently reported, whereas the effects of aging on several hippocampal-dependent aspects of Pavlovian conditioned fear remained a matter of debate. Young (3 months) and aged (24 months) Long-Evans female rats were first trained with a spatial reference memory procedure in the water maze and tested 24h later for spatial memory (probe test). Thereafter, they were conditioned using either a delay (10 tone- shock pairings separated by a 0s temporal gap) or trace (10 tone-shock-pairings separated by a 30s temporal gap) conditioning procedure. Contextual fear, tone fear, tone-fear extinction and tone-fear renewal were thus evaluated. When compared to young rats, aged rats were impaired in place learning and 24h probe test. In the trace and delay conditioning, aged rats globally displayed higher freezing scores than young subjects. However, aged rats were not impaired

in contextual and tone fears, fear extinction and renewal whether conditioned using a delay or a trace procedure. Moreover, contextual and tone fear were modulated by the conditioning procedure on a similar way in both young and aged rats. These results suggested that aging differentially affected hippocampal-dependent tasks and that trace conditioning was less sensitive that place learning to aging process.

P2.229

Anxiety-like and aggressive behaviors in mutant mice with fully edited 5-HT_{2C} receptors

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The 5-HT_{2C} receptor is believed to be implicated in the mechanisms of action of antidepressant drugs, and convergent studies indicated changes in 5-HT_{2C} receptor pre-mRNA editing in the brain of depressed suicide patients. 5-HT_{2C} mRNA editing consists of deamination of adenosine into inosine by specific adenosine deaminases (ADAR), which causes changes in mRNA translation, thereby generating up to 24 different isoforms of the human 5-HT_{2C} receptor protein, with subtle differences in their amino acid sequence within the second intracellular loop. 5-HT_{2C} mRNA edition has important functional consequences because the fully edited isoform (VGV) has both decreased receptor-G protein coupling and decreased constitutive activity compared to the non-edited isoform. However, behavioral consequences of such functional alterations have yet to be investigated. We thus compared mutant mice expressing only the VGV isoform (Kawahara et al., J. Neurosci. 2008) and paired wild-type (C57BL6/J) mice in the social interaction test, a validated paradigm to assess anxiety-related behavior. As expected of its anxiogenic effect, m-chlorophenylpiperazine (mCPP; a preferential 5-HT_{2C} receptor agonist), at the low acute dose of 0.1 mg/kg i.p., decreased the duration of active social interaction, but this effect was significantly more pronounced in VGV mice. In untreated VGV mice, about half of social interactions between congeners were fighting behaviors, whereas, in contrast, no fighting behaviors were observed in socially interacting wild-type mice. Because both 5-HT and DA are implicated in anxiety and aggressive behavior, and 5-HT_{2C} receptors play a key role in the regulation of both DA and 5-HT neurotransmissions (Mongeau et al., J. Neurochem., 2010), we are currently investigating whether VGV editing affects 5-HT and/or DA turnover in various brain areas. Interestingly, VGV mice have been shown to overexpress 5-HT_{2C} receptor binding sites in brain (possibly as a compensation for the decreased functional coupling of edited receptors), and whether or not this change contributes to their anxious/aggressive traits is also under investigation.
Supported by INSERM, UPMC and ANR (Sercedit, 2006).

P2.230

Neural substrates of self memory system: a meta-analysis

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Autobiographical memory (AM) is a complex concept encompassing various different types of knowledge about the self (Conway, 2005). The **episodic component of AM** contains personal specific events in a particular time and space, for which the subject can mentally travel back through subjective time, reliving the encoding context (Piolino et al., 2009; Tulving, 2002). Otherwise, the **semantic component of AM** stores the general knowledge of a person's past for which the subject can be aware of information in the absence of specific recollection. According to Conway's AM model, it contains **general self knowledge** of significant persons, common locations, general events encompassing both repeated events and extended events, and at the most abstract representation

level, the **conceptual self** which contains personal beliefs, evaluations and currently active self-images and goals.
The aim of this study was to investigate the distinct neural substrates of different form of AM self-knowledge. With this goal, we conducted a meta-analysis based on Activation Likelihood Estimation (ALE) of available neuroimaging studies on **episodic component of AM**, **general self knowledge** and **conceptual self**.
Our results demonstrate a disengagement of limbic and posterior medial regions corresponding to the gradual abstraction process of autobiographical material. As such, self-knowledge only activates lateral temporal structures with no recruitment of posterior structures corresponding to scene reconstruction and specific remembering. Moreover, the conceptual self only activates anterior structures(dorsolateral and medial prefrontal cortex) linked to the evaluation of self-referential stimuli (VanDerMeer et al. 2010).Finally our results shed light on the progressive disengagement of posterior and hippocampal regions with the semantization of memories linked to the activation of anterior regions.

P2.231

The Feeding Switch: endocannabinoid-mediated hypophagia in the paraventricular nucleus of the hypothalamus

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Cannabinoid receptor type-1 (CB1)-dependent signaling in the brain exerts a bimodal control on stimulated food intake, by different regulation of excitatory and inhibitory synaptic transmission. However, the brain areas where this complex control is exerted are not yet fully dissected. Here, using behavioural and electrophysiological approaches, we show for the first time that CB1 blockade in the paraventricular nucleus (PVN) of the hypothalamus further increased the fasting-induced hyperphagia. Furthermore, in animals with food *ad libitum* CB1 blockade in the PVN potentiated the orexigenic effect of the gut hormone ghrelin. At electrophysiological level, bath application of the CB1 antagonist, AM251, in slices pre-incubated with ghrelin, potentiates the decreasing in PVN cellular activity induced by this hormone. Hence, the PVN is one of the sites where signals associated with the individual´s energy status determine the direction of the effects of endocannabinoid signaling.

P2.232

Combined lesions of the entorhinal cortex and basal forebrain cholinergic neurons in mice as a novel model of memory deficits in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by a progressive alteration of cognitive functions, including spatio-temporal disorientation, ending up in dementia. Histological hallmarks such as amyloid plaques,

neurofibrillary tangles and neurodegeneration spread over several structures during the course of AD. Ongoing neuronal loss in the retrohippocampal gyrus, the hippocampus and, in later stages, the basal forebrain cholinergic nuclei (BF) certainly plays a critical role in the progressive degradation of memory functions. Traissard and coll. (Neuropsychopharmacol 32, 2007, 851) showed that combined lesions of the entorhinal cortex (EC) and the BF in rats induced massive spatial memory deficits, whereas each lesion separately had limited effects. It was hypothesized that the synergistic effects of the two lesions could play an important role in the memory decline of AD patients. Here, we investigated the effects of each lesion alone (EC or BF) or combined (EC + BF) in several memory tasks in mice. In the water-maze task, EC and BF lesions alone had small effects on acquisition and no effect on retention in a 24-h probe test. The BF lesion selectively affected object recognition performances and the latency to reach the target hole during training in the Barnes maze. Spatial recognition and other aspects of the Barnes maze task were not affected by single lesions. In contrast, the double lesion (EC+BF) induced massive acquisition and retention deficits in the water maze task. It affected both latency and repetitive errors during training and the 16-day probe test in the Barnes maze. Object recognition deficits were similar to those obtained with the BF lesion alone. Interestingly, spatial recognition was preserved. Thus, we confirmed in mice an additive effect of EC and BF lesions on spatial memory performances in both the water maze and the Barnes maze. The double lesion did not provoke additional defects in other tasks (recognition tasks) engaging mostly adjacent structures (perirhinal cortex, hippocampus). This simplistic though realistic model combines neurodegenerative patterns and reproduces symptoms of AD. It could be tested in transgenic mice with early plaque or tangle onset but delayed evidence for neurodegeneration.

P2.233

Mathematical modeling for brain state dependence of post-inhibitory rebound

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Post-inhibitory rebound (PIR) is considered an important mechanism in the genesis of biological rhythms. PIR has been demonstrated in different brain structures both *in vitro* and *in vivo* in response to external stimuli. However, it has not been reported *in vivo* during a brain state in the absence of any external stimulus.
We recorded extracellular local field potentials and isolated spikes of putative interneurons and principal neurons in the dorso-medial entorhinal cortex of the anaesthetized rat during two brain states - the theta oscillation state (~4 Hz) and the slow oscillation (SO) state (~0.5 Hz). |Construction of cross-correlograms of the spike trains of these neurons revealed rebound excitation in some target neurons following inhibition induced by the firing of presynaptic interneurons. Further, more pairs showed PIR during the theta state than during the SO state, and in particular, the pairs which displayed PIR only during theta also displayed a firing preference for a phase of the theta oscillation. In order to understand this observation, we constructed a mathematical model of two neurons with a unidirectional, inhibitory synpatic coupling that can induce rebound excitation in the target. We then coupled each neuron to a periodic rhythm representing the brain state. We observed that, for a given choice of parameters, the lower frequency rhythm could modulate the firing rate of the presynaptic neuron more than the higher frequency one, which, in turn, decreased the rebound firing rate of the postsynaptic neuron. We propose this as an explanation for the brain-state dependency of PIR observed in the data.

P2.234

Dynamical solution for aperture problem using motion-based predictive coding

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It is still unclear how information collected locally by low-level sensory neurons may give rise to a coherent global percept. This is well demonstrated in the aperture problem both in visual or haptic senses. Experimental findings on its biological solution in area MT show that local motion measures are integrated to see the dynamical emergence of global motion information [1]. We develop a theory of spatio-temporal integration defined as implementing motion-based predictive coding. This takes the form of an anisotropic, context-dependent diffusion of local information [2]. Here, we test this functional model for the aperture problem in the visual and haptic low-level sensory areas.
In our model, spatial and motion information is represented in a probabilistic framework. Information is pooled using a Markov chain formulation, merging current information and measurement likelihood thanks to a prior on motion transition. This prior is defined so that it is adapted to smooth trajectories such as are observed in natural environments.This dynamical system favors temporally coherent features. Differently to neural approximations [3], we use a particle filtering method to implement this functional model. This generalizes Kalman filtering approaches that were used previously by allowing to represent non-gaussian and multimodal distributions.
We observe the emergence of mechanisms that reflect observations made at psychophysical and behavioral levels. First, the dynamical system shows the emergence of the solution to the aperture problem and show dependence to line's length [4]. Then,when presented with an object with a regular translation, the dynamical system grabs itsmotion independently of its shape and exhibits motion extrapolation. This shows that prediction is sufficient for the dynamical build-up of information from a local to a global scale. More generally it may give insights in the role of spatio-temporal integration on neural dynamics in the emergence of properties that are accredited to low-level sensory computations.
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P2.235

Neural coding in the early stages of the somatosensory pathway: a metrical information theory analysis of human microneurography data

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Neural information processing involves multistage transmission mechanisms in which neurons act as stochastic communication channels. We set forth an information theoretical measure to quantify neurotransmission reliability while taking into full account the metrical properties of spike trains. We stress the importance of considering the properties of actual neural decoders (as opposed to ideal observers as in Shannon theory) when studying neurotransmission. We derive a novel spike train

metrics based on a parametric non-linear distance inspired by the probabilistic behavior of the Spike-Response Model (SRM).
We apply the proposed information theoretical analysis to study neurotransmission along the ascending somatosensory pathway. We focus on the encoding/decoding of primary tactile signals (fingertip mechanoreceptor responses) recorded via human microneurography experiments. We show that first-spike latencies of mechanoreceptor responses contain enough information to perform perfect discrimination of 81 distinct stimuli within 40 ms of the first afferent spike. We also demonstrate that the relative spike times of entire primary spike trains permit to go beyond stimulus discrimination and encode isometric representations of the stimulus space, a likely basis for generalization in haptic perception.
Then, we consider the propagation of mechanoreceptor signals along peripheral nerve fibers up to second-order cuneate neurons (CNs). We hypothesize that CNs mediate optimal decoding/re-encoding of primary haptic information. We model CN as SRM neurons and tune the model parameters according to in vivo intracellular CN recordings from non-anesthetized but decerebrated cats. We modulate the efficacy of mechanoreceptor-CN synapses by spike-timing-dependent plasticity. Our simulations of the integration of the sensory quanta in single CNs predict that optimal information transfer occurs when only as few as 1-3 % of CN synapses are non-silent. This prediction is verified by in vivo recordings in cats showing that inputs from just a few of the primary afferents have a high synaptic weight, whereas the vast majority have very low or undetectable synaptic efficacy.

P2.236

Neural population decoding: a study towards real time decoding of cognitive variables in the non-human primate

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While most of the research effort in neural prosthetics has concentrated on the use of motor signals to drive external devices, new directions in the field of brain-computer interfaces (BCIs) are emerging. In particular, internal cognitive variables are starting to be used to modulate and improve the performance of current BCIs. Here, we will present a twin theoretical and experimental study paving the way to the development of a real-time cognitive BCI in the non-human primate.
1) Experimental study. Monkeys were engaged in a cued detection task that allowed for dissociation in time of two types of cognitive variables, namely the visuospatial attention and perception. We show that these cognitive variables were both reliably decodable *on a trial by trial basis*, from the activity of Frontal Eye Field (FEF) neurons. The decoding performance of these cognitive variables is quantitatively comparable to those of the sensory attributes of the stimuli and is over the 80% correct predictions of stimulus status or monkey behavior. Decoding performance decreases on incorrect trials, indicating that the spikes hold less information about the main elements of the task on these trials.
2) Theoretical study. Before starting multichannel recordings, we wished to quantify the dependence of the readout on
i) the intrinsic properties of a population of visually responsive neurons and
ii) active attentional and perceptual processes. We thus built a population of visual neurons drawn from a theoretical higher order visual area. For each cell, the receptive field was represented by a 2D Gaussian tuning curve and instantaneous spiking neural activities were generated according to a Poisson process. Targets were virtually presented to this subpopulation and the performance of the same neural network decoder was evaluated at reading out this target location from the neuronal population responses. We specifically analyzed the dependence of this readout performance on
a) subpopulation size;
b) cell density function of eccentricity;
c) receptive field size function of eccentricity and
d) focal enhancement of visual processing mimicking spatial attention modulations.
These two studies pave the way to real-time decoding of cognitive variables in the non-human primate.

P2.237

Novel flexible implantable neural probes for long term application

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We present the fabrication, characterization and utilization of new flexible implantable neural probes. These probes are a sandwich of polyimide, platinum, conducting polymer and SU8 layers providing excellent biocompatibility, good electrical characteristics and high mechanical flexibility. The new probes are made to create a better interface with soft biological tissues and decrease damage caused by the insertion and the brain-probe micromotions.
These new materials are a good compromise between flexibility and stiffness needed for implantation and long term in vivo recording and the electrodes design is selected to provide simultaneous recordings (LFP and units) of several neurons in rat hippocampus.

P2.238

Functional anatomy of respiratory networks in ventrolateral medulla inferred using time-series analysis

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For over a hundred years, neuroanatomical studies have hinted at the complexity of neuronal networks. To understand how densely interconnected neuronal networks compute, the dynamics of neuronal signaling must be observed in living tissue. Here, a medium-affinity Ca^{2+} indicator (fluo8L K_D= 1.86 µM) was used to record networks of respiration-modulated neurons involved with central chemosensation and respiratory rhythm generation. These networks were exposed at the cut surface of neonate rat (P0-P4) hindbrain in vitro preparation cut at a compound angle along its saggital axis. Optical recordings (n=6) were made at 45-50 Hz for 600 s under quasi-stationary conditions. Regions of interest (ROIs) were identified algorithmically, and respiration-modulated cells were selected by inspection of traces of time-varying luminance values. Spike times were extracted from Ca^{2+} signals using a fast deconvolution algorithm that generated spike probabilities, which were thresholded using the Hannan-Quinn criterion; signal quality was then quantified by estimating the goodness-of-fit of convolved spikes to the optical traces. Coupling between respiratory network constituents was inferred by quantifying the information transfer, using the normalized transfer entropy (NTE) metric applied to spike times, which permitted driver/follower relations to be identified. Because the NTE metric estimates coupling based on the degree to which the future activity of cell A can be accounted for by the past activity of cell B, for a given NTE, an interval of past activity (τ_p), and future activity is defined (τ_f). Statistically significant coupling relations were identified by comparing actual data to shuffled data. Each cell's coupling to all other recorded cells was characterized by 3 parameters: maximal NTE value, and its associated τ_p and τ_f. Graphs summarizing the functional topology of respiratory networks under steady state conditions reveal the following qualitative features: predominantly follower neurons were more common than driver neurons; most follower neurons were converged upon by multiple drivers; a small subset of neurons were found to have strong coupling relations (both driver and follower) with many other neurons, consistent with hub-like function.

P2.239
Optical recording of chloride current by Digital Holographic Microscopy

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Chloride transport across the plasma membrane of cells plays a crucial role in several cellular processes such as regulation of cell volume, trans-epithelial transport of fluids, muscular and neuronal excitability. Dysfunctions of the chloride channels are associated with several human diseases including cystic fibrosis, congenital chloride diarrhea, myotonia congenita, deafness and epilepsy. While a number of chloride channel modulators have been discovered for the treatment of some of these diseases, their development has been less intense than for the other ionic channel modulators, in part because of the limitations of currently available imaging techniques amenable to screening novel compounds. Digital Holographic Microscopy (DHM) is a non invasive optical imaging technique able to reveal changes in the intracellular refractive index such as those occurring with water fluxes that accompany the trans-membrane movement of ions, in particular chloride ions. Here, we show that DHM can provide the quantitative determination of transmembrane chloride fluxes mediated by the activation of chloride channels associated $GABA_A$ receptors. Indeed through a simple equation, chloride currents elicited by application of appropriate agonists of the $GABA_A$ receptors can be derived from the phase shift recorded with DHM. Finally because of the optical nature of the signal, chloride currents can be determined and pharmacologically characterized non-invasively and simultaneously on large number of cells thus indicating that DHM could be a useful tool for High Throughput Screening (HTS).

P2.240
The emergence of dendritic mosaics via STDP

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Synapse location, dendritic active properties and synaptic plasticity are all known to play some role in shaping the different input streams impinging onto a neuron. It remains unclear however, how the magnitude and spatial distribution of synaptic efficacies emerge from this interplay. Here, we investigate this interplay using a biophysically detailed neuron model of a reconstructed layer 2/3 pyramidal cell and spike timing-dependent plasticity (STDP). Specifically, we focus on the issue of how the efficacy of synapses contributed by different input streams are spatially represented in dendrites after STDP learning. We construct a simple feed forward network where a detailed model neuron receives synaptic inputs independently from multiple yet equally sized groups of afferent fibers with correlated activity, mimicking the spike activity from different neuronal populations encoding, for example, different sensory modalities. Interestingly, ensuing STDP learning, we observe that for all afferent groups, STDP leads to synaptic efficacies arranged into spatially segregated clusters effectively partitioning the dendritic tree. These segregated clusters possess a characteristic global organization in space, where they form a tessellation in which each group dominates mutually exclusive regions of the dendrite. Put simply, the dendritic imprint from different input streams left after STDP learning effectively forms what we term a "dendritic efficacy mosaic." Furthermore, we show how variations of the inputs and STDP rule affect such an organization. Our model suggests that

STDP may be an important mechanism for creating a clustered plasticity engram, which shapes how different input streams are spatially represented in dendrite.

P2.241

Computational analysis of nociceptive information processing in the spinal cord: impact of multiple firing of deep dorsal horn neurons

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Deep dorsal horn relay neurons (dDHNs) primarily located in lamina V of the spinal cord, are known to integrate innocuous and nociceptive signals before relaying the information to the somato-sensory thalamus through the contra-lateral spino-thalamic tract. Interestingly, dDHNs exhibit multiple firing patterns under the control of local metabotropic neuromodulation: tonic firing, plateau potential, and spontaneous oscillations. Expression of regenerative properties such as plateau in dDHN participates to central sensitization and a local short-term form of sensitization called Wind Up.
To investigate the role of interactions between voltage-gated channels and the occurrence of different firing patterns and then to correlate these two phenomena with their functional role in sensory information processing, we designed a conductance-based model of dDHN. This model successfully reproduces the classical features of plateau in dDHNs, including a wind-up of the neuronal response after repetitive stimulation. This modeling approach allowed us to systematically testing the impact of conductance interactions on the firing patterns.
Our simulation results showed that the expression of multiple firing patterns could be reproduced by changes in the balance between two currents (L-type calcium and Kir conductances). By investigating a possible generalization of the firing state switch, we found that the switch can also occur by varying the balance of any hyperpolarizing and depolarizing conductances. This result extends the control of the firing switch to other neuromodulators or to network effects such as synaptic inhibition.
Interestingly, we observed that the switch between the different firing patterns occurs as a continuous function in the model, revealing a particular intermediate state called the accelerating mode. Using correlation analysis between a model of peripheral nociceptive afference and the dDHN model, we found that the newly identified accelerating mode was the optimal firing state for information transfer.
Our work sheds light on new aspects of nociceptive integration and provides a theoretical framework to investigate the different mechanisms involved in nociceptive integration and both short and long term plasticity mechanisms.

P2.242

Design and validation of electrodes for human multiscale electrophysiological recordings

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We propose in this study to design new types of electrodes, which will try to solve the challenges presented by simultaneous recordings at multiple scales in humans.

In a first project, we will adapt small dry electrodes, already used for multisite recordings on the auditory cortex in animals (Takahashi 2003), single site on the cochlear nerve during surgery of skull base (Schmerber 2004) and polysomnographic recordings. Dry electrodes enable indeed good quality recordings for a very long time.
A fixation system composed of a flexible mesh must be developed for a fast installation of a large number of electrodes (64) during the operation. The flexible property will avoid putting electrodes near the SEEG electrodes. Tests will be performed in healthy subjects, like conventional EEG recordings (evoked potentials) and in patients during implantation.
We will also study the electrodes based on conducting polymers. Besides the fact that these materials are naturally compatible with flexible substrates, they are able to decrease electrode impedance and improve the quality of recordings of neuronal activity (Abidian 2009). We work on polythiophenes that are commercially available and are deposited from solution. Patterning is achieved either by photolithography or by inkjet printing. We optimize morphology by varying the deposition conditions, in an effort to minimize impedance and maximize signal to noise ratio of our recordings.

P2.243
Spatial learning and action planning in a prefrontal cortical network model

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The interplay between hippocampus and prefrontal cortex (PFC) is fundamental to spatial cognition. Complementing hippocampal place coding, prefrontal representations provide more abstract and hierarchically organized memories suitable for decision making. We model a hippocampal-prefrontal network mediating distributed information processing for spatial learning and action planning. We couple an existing hippocampal model producing dense and redundant place field representations [1] to a novel PFC architecture suitable for learning sparser topological-metrical maps. Specific connectivity and synaptic adaptation principles shape the recurrent dynamics of the PFC network arranged in cortical minicolumns. The recurrent nature of the network supports multilevel spatial processing, allowing structural features of the environment to be encoded. An activation-diffusion mechanism spreads the neural activity through the column population leading to trajectory planning. We illustrate the link from single unit activity to behavioral responses. The results suggest plausible neural mechanisms subserving the cognitive "insight" capability originally attributed to rodents by Tolman & Honzik [2]. Our time course analysis of neural responses shows how the interaction between hippocampus and PFC can yield the encoding of manifold information pertinent to spatial planning, including prospective coding and distance-to-goal correlates. The model provides a vantage point to interpret PFC eletrophysiological data in terms of quantitative clustering of population activity. On the basis of a set of statistical measures, we perform a principal component analysis on both simulated and real data sets of PFC recordings. This study gives rise to comparative results based on the identification of clusters of characteristic discharge properties. We discuss a series of testable hypotheses about the functional meaning of the observed clusters |in terms of their role in spatial localization and planning, reward coding, and prospective memory.
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P2.244
Finding optimal tactile stimuli ensemble of populations of cortical neurons

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The studies of the relationship between the cortical responses and the sensory stimuli can greatly benefit from an Information Theory approach, which quantifies the information conveyed by the neuronal response about the stimulus set. Within this approach, one can select a set of stimuli called the “optimal sensory ensemble” that maximizes the mutual information between the stimuli and the

neuronal response. An adaptive algorithm (Blahut 1972, Arimoto 1972, Mackens et al 2005) allows one to solve this problem in an iterative manner.
Based on previous works, we have constructed a model of cortical network (Wang 1999, Mackens 2002) in order to generalize the application of adaptive algorithm, from single cell to the activity of populations of cortical neurons. The network is controlled by afferent connectivity from thalamus. This connectivity is described by a Poisson process with a rate depending on time. The off-line optimization procedure of sensory stimuli is applied both to cortical network model and experimental data of sensory cortex (multi-units activity from somatosensory cortex). In both cases, the stimulus have two control parameters: modulation and amplitude of external input for network model, frequency and locations for tactile stimulus.
In the network model we obtained an optimal stimulus that corresponds to low spiking rate, which corresponds to the maximal information carried when the spontaneous activity is lightly influenced by the stimulation. This behavior is also observed in the optimization of multi-units activity from somatosensory cortex.
We show that the optimal stimulus ensemble is characterized by a distribution where the stimulus which induces a change in the dynamics of the network is highly represented. These results suggests that bifurcation points in the networks dynamic are highly informative and are in accordance with observations of electrophysiological recordings. The developed algorithms can be used to explore the process of sensory information integration in sensory cortex.

P2.245

A neurocomputational study of the role of the cerebellum in spatial cognition

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Recent experimental findings have begun to unravel the implication of the cerebellum in high-level functions such as spatial cognition [1]. We modelled the main information processing components of the cerebellar microcomplex and focus on behavioural genetic data suggesting that L7-PKCI mice (lacking LTD at parallel fibre-Purkinje cell synapses) are impaired in learning the procedural component of spatial navigation [1]. In order to validate this hypothesis, we compared the performance of simulated control and L7-PKCI mice in both Morris watermaze and the Starmaze [2] tasks. Our findings show that a purely local impairment of the procedural component cannot explain all the experimentally observed differences between the goal-searching behaviour of mutants and controls [1]. We therefore put forth a new hypothesis according to which mutants' spatial learning impairment may reflect a deficit in trading-off exploration and exploitation strategies. Based on this assumption, we were able to reproduce the entire set of behavioural results [1]. Furthermore, we argued that the deficit in the exploration-exploitation balance might be due to suboptimal spatial representations in L7-PKCI mice, which would result in increased exploration in novel environments. We evaluated this assumption by coupling our cerebellar model to an existing model of hippocampal spatial learning [3,4]. Our simulation results suggest that the cerebellum may play an important role in integrating proprioceptive information to infer future state variables such as body orientation and position [5]. This ability might be impaired in L7-PKCI mutants, which would affect hippocampal multisensory integration mediating stable spatial representations learning [3]. This work gave rise to a testable prediction (currently under experimental investigation in our laboratory) on the difference between the free exploratory behaviour of control animals [6] and L7-PKCI mutants.
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P2.246

The role of phasic and tonic dopamine for long-term plasticity in the rat prefrontal cortex: a computational model

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The prefrontal cortex (PFC) is a key structure mediating executive long-term memory. Dopamine (DA) input to the PFC has been shown to modulate the magnitude and direction (i.e. potentiation, LTP; or depression, LTD) of long-term plasticity induced by tetanic stimulation in vitro [1]. Moreover, the DA action is different depending on whether tonic (background) or phasic (stimulation-induced) DA levels are manipulated. Whereas a fair amount of theoretical work addresses short-term DA action and its importance for working memory, no theoretical models address the role of DA for long-term plasticity in the PFC.
The present work attempts to fill this gap by proposing a computational model of induction and maintenance of LTP and LTD in the PFC under the influence of DA.
We use a Hodgkin-Huxley-type computational model of a single PFC layer V pyramidal cell and we study neuronal properties that may be responsible for the changes in synaptic efficacy following tetanic stimulation in the presence of DA. We use a variant of Tag-Trigger-Consolidation framework [2] as a model for LTP and LTD induction and maintenance. Distinct properties of our model are a DA-dose-dependent switch from LTD to LTP during induction, and an inverted-U-shape dependence of protein synthesis threshold on the level of background DA. Protein synthesis is responsible for maintenance and late phase of LTP/LTD in the model.
The model has been tested by stimulating the simulated neuron with spike trains of different duration under different doses of DA and has been found to reproduce well the results of the in vitro studies. Our simulations suggest that in order to comply with the in vitro data, prefrontal synapses must contain a protein that is slowly (on the timescale of minutes) activated in the presence of DA in a dose-dependent manner. More generally our results support the hypothesis that phasic release of endogenous DA is necessary for the induction of long-term changes in synaptic efficacy, while the concentration of tonic DA determines the direction (i.e. LTP or LTD) of these changes [1].
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P3.001

Functional role of the postsynaptic Guanylate Kinase Domain-Associated Protein (GKAP) interaction with a light chain of myosin-V and dynein (DLC_2)

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The scaffolding protein GKAP binds the guanylate kinase-like domain of PSD-95 and the PDZ domain of Shank, thus organizing the specific scaffolding protein complex that physically and functionally links the postsynaptic glutamate receptors. The molecular mechanisms regulating the trafficking and assembly of postsynaptic density (PSD) proteins to the synapses are still widely unknown. Interestingly, yeast two-hybrid screen revealed that GKAP also binds directly to DLC_2, a light chain shared by myosin-V and cytoplasmic dynein. DLC_2 would thus be a pertinent motor protein candidate involved in GKAP trafficking. In this study, we assessed a direct interaction between GKAP and DLC_2 in living cells, by the non-invasive Bioluminescence Resonance Energy Transfer (BRET) technique. To this aim, we constructed GKAP and DLC_2 fusion proteins with renilla luciferase (RLuc, donor) and YFP (acceptor), respectively. Importantly, the tag did not impair known properties of GKAP and DLC_2.

Interestingly, we found that Rluc-GKAP partially colocalized with YFP-DLC_2. Furthermore, BRET experiments point to a specific interaction between the two partners in living cells. We finally identified by punctual mutations the amino acid sequences involved in the interaction of the two proteins. Using this GKAP punctual mutant that lacks the ability to interact with DLC_2, we investigated the role of the interaction in the trafficking of GKAP and its postsynaptic partners to the spine, as well as the modulation of glutamate currents. We show that DLC_2 is involved in GKAP accumulation to dendritic spines during enhanced neuronal acivity. Interestingly, preliminary results point to a role of this interaction in the synaptic accumulation of PSD-95 and in the modulation of NMDA currents. In accordance with previous publications showing non synaptic clusters of postsynaptic proteins, these results put emphasis on the role of GKAP in the correct targeting and organization of postsynaptic proteins in the spine apparatus.

P3.002

Bassoon and Piccolo, two proteins implicated in nigrostrial system in Parkinson disease

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Bassoon and Piccolo are two proteins specifically localized at the active zone of presynaptic nerve terminals of conventional synapses in the rat striatum. They are involved in the structural organization of the neurotransmitter release site. In this study we used immunocytochemistry to examine at the ultrastructural level the distribution of Bassoon and Piccolo proteins in the rat striatum and determine which type of presynaptic terminals contain these proteins. Using double labelling for Bassoon or Picollo and tyrosine hydroxylase we studied more specifically the distribution of Bassoon and Piccolo in the nigrostriatal afferent fibers. Bassoon and Piccolo labelling was identified in symmetric and asymmetric synapses. Bassoon was seen in 46 6.3% symmetric and 49 5.6% of asymmetric synapses, whereas Piccolo was seen in 53 6.8% of symmetric and 43 6.3% of asymmetric synapses. Moreover we observed Bassoon and Piccolo labelling in 5 1.1% and 4 0.3% of asymmetric perfored synapses respectively. These observations suggest that Bassoon and Piccolo are present in very active synapses. In term of localization, the two proteins were detected in asymmetric terminals making contacts with the head of the spines of gamma-aminobutyric acid (GABA)-ergic neurons suggesting the presence of these proteins in cortico and thalamo striatal terminals. We also detected Bassoon in some tyrosine hydroxylase-positive terminals and fibers. In contrast, Piccolo was localized only in some dopaminergic terminals but was not seen in fibers. These data suggest that Bassoon and Piccolo play complementary but distinct roles at the active zone of the synapses. Future plans are to extend the studies to rats with 6-OHDA lesion of the nigrostriatal pathway to analyze the putative involvement of these proteins in parkinsonian syndromes.

P3.003

Necdin protects embryonic motoneurons from TNF-dependent programmed cell death

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Necdin belongs to the type II Melanoma-Associated Antigen Gene Expression (MAGE) gene family and has been located in the Prader-Willi Syndrome (PWS) critical region. Mice with targeted deletion of *Necdin* develop symptoms of PWS, including sensory and motor deficits. The molecular mechanisms underlying these motor deficits remain however elusive. Here, we show that the genetic ablation of *Necdin*, which expression is restricted to post-mitotic neurons in the spinal cord during development, leads to a significant 31% increased loss of specified motoneurons, during the period of naturally-occurring cell death. To better understand the role of *Necdin* during the development of motoneurons we used embryonic spinal cord explants and primary motoneuron cultures from *Necdin*-deficient mice. Interestingly, while *Necdin*-deficient motoneurons presented the same survival response to neurotrophic factors, we demonstrate that the deletion of *Necdin* leads to an exacerbated susceptibility of motoneurons to neurotrophic factor deprivation. We further demonstrate that by neutralizing TNFα this increased susceptibility of mutant motoneurons to trophic factor deprivation can be restored to the level observed in WT motoneurons deprived of trophic factors. We propose that Necdin contributes through the TNF receptor pathway, which might include NucB2 and Arts-1 as signalling intermediates, to the developmental death of motoneurons.

P3.004

A hypomorphic mutation in *Lpin1* induces progressively improving peripheral neuropathy in the rat

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The *Lpin1* gene encodes the phosphatidate phosphatase (PAP1) enzyme Lipin 1, which plays a critical role in lipid metabolism. In this study we describe the identification and characterization of a rat with a mutated *Lpin1* gene (*Lpin1*^{1Hubr}), generated by *N*-ethyl-*N*-nitrosourea mutagenesis. *Lpin1*^{1Hubr} rats are characterized by hindlimb paralysis that is detectable from the second postnatal week. Sequencing of *Lpin1* identified a missense mutation in the 5'-end splice site of exon 18 resulting in mis-splicing, a reading frame shift and a premature stop codon. As this mutation does not induce nonsense-mediated decay, it allows the production of a *truncated* Lipin 1 protein lacking PAP1 activity. As a consequence, *Lpin1*^{1Hubr} rats develop hypomyelination rather than the pronounced demyelination defect characteristic of *Lpin1*$^{fld/fld}$ mice, which carry a null allele for *Lpin1*. Furthermore, histological and molecular analyses revealed that this lesion improve in older *Lpin1*^{1Hubr} rats as compared to young *Lpin1*^{1Hubr} rats and *Lpin1*$^{fld/fld}$ mice. The observed differences between the murine *Lpin1*$^{fld/fld}$ mutant, with a complete loss of Lipin 1 function, and the *Lpin1*^{Hubr} rat, with a truncated PAP1 activity-deficient form of Lipin 1, provide additional evidence for suggested non-enzymatic Lipin1 function residing outside of its PAP1 domain. While we are cautious in making a direct parallel between the presented rodent model and human disease, our data may provide new insight into pathogenicity of recently identified human *Lpin1* mutations.

P3.005

Role of calcium-stimulated adenylate cyclase 1 (AC1) in the targeting of the corticospinal tract and in regeneration after a lesion

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The mammalian corticospinal tract (CST), arising from layer V neurons in the Somatosensory and motor cortex, is the only direct cortical pathway to the spinal cord. Guidance molecules such as Ephrins and Slits are involved at various decision points for guiding the CST axons. However, previous analyses of the corticospinal tract guidance defects in mutant mice lacking these molecules have suggested that there are other molecules involved in CST axon guidance that are yet to be identified. The role of the calcium-stimulated adenylate cyclase 1 (AC1) has been revealed in the fine patterning of the retinal maps. Further studies indicated that lack of AC1 disturbed the repulsive response of retinal axon growth cones to ephrin A5.
Because the AC1 gene is highly expressed in cortical neurons and spinal cord during the development of the CST,we questioned whether AC1 is involved in the targeting of the CST and regeneration after a lesion .We used the barrelless (brl) mouse strain which carry a spontaneous mutation of the AC1 gene and investigated the projections of the CST in the cervical spinal cord using anterograde tracers. We analysed corticospinal neurons in the motor cortex using retrograde tracers.To investigate the effects of AC1 on axon regeneration in vivo, the barrelless (brl) mice were tested in a model of spinal cord injury (SCI): dorsal hémisection at (T7-T9)
Our study shows an increase in the number of contralateral and ipsilateral projections in the cervical spinal cord in brl mice. Moreover, the density of labelled neurons in the motor cortex is significantly higher in the brl mice. However no major abnormalities of the CST were detected.The brl mice showed greater functional improvement compared to controle mice.
The targeting defects could be linked to activity dependent remodeling of the CST, and maintenance of exuberant axonal branches. Increase in the number of corticospinal may explain the enhanced functional recovery after a spinal cord injury.

P3.006

Motor neuron survival but not axonal mRNA transport is impaired in an ES cell based model of Spinal Muscular Atrophy

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Proximal Spinal Muscular Atrophy (SMA) is caused by the loss of the gene encoding the Survival of Motor Neuron protein (SMN), an ubiquitously expressed protein essential for neuromuscular junction formation and motor neuron survival. SMN is part of an RNA binding complex critical for mRNA splicing, recently suggested to also regulate axonal mRNA transport. Importantly, axonal translation has previously been implicated in axon guidance, synapse formation and neuronal survival. However, difficulty in isolating primary motor neurons in large quantities precluded analysis of axonal mRNAs and their function in motor neuron disease or axon guidance. To overcome these limitations we have employed an in vitro ES cell based system. We have derived an embryonic stem cell lines from the type I SMA mouse. We demonstrate that motor neurons derived from these ES cells project axons which respond to guidance cues in a protein synthesis dependent manner and that this response is not affected in SMA motor neurons. Expression profiling of axonal mRNAs demonstrates that axonal

mRNA transport is not globally deregulated in SMA. Interestingly, neurofilament heavy chain mRNA is selectively increased in SMA motor neuron axons. While neurofilament accumulation in neuromuscular junctions is one of the earliest pathological phenotype in SMA mice, ES cell derived SMA motor neurons transplanted in vivo or cultured in vitro did not exhibit defects in NMJ formation or in presynaptic differentiation. In contrast, we discovered that a subset of SMA motor neurons dies in vitro. Based on these observations we conclude that axonal mRNA transport, protein synthesis dependent responses to guidance cues as well as early steps of synaptogenesis are not deficient in SMA. However, motor neuron cell death might be an important in vitro phenotype of SMA motor neurons, which could be exploited for further dissection of pathogenic pathways and for cell based drug screening.

P3.007

The JAK-STAT pathway is involved in synaptic plasticity

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The Janus kinases (JAKs) are protein tyrosine kinases involved in processes such as cell survival or inflammation. Their main direct downstream target is the signal transducer and activator of transcription (STAT). In neurons, the JAK/STAT pathway is involved in leptin receptor signalling but also in regulation of synaptic receptors like the AMPA or NMDA receptors. Recently, this pathway has also been involved in memory formation and Alzheimer's disease. However, its role in synaptic plasticity, the process that is widely believed to underlie memory formation in the brain, was still not known.
Here we provide the first evidence that the JAK/STAT pathway plays a role in NMDA-receptor dependent long-term depression (LTD). Indeed, in hippoccampal slices from two-week old rats, JAK inhibitors can block LTD but not long-term potentiation or depotentiation, when the schaffer-collateral commissural pathway is stimulated. The JAK family contains 4 isoforms of which JAK2 is the main one expressed in the post-synaptic density. We have confirmed that JAK2 is present in synapses and we have shown that the knockdown of this isoform with shRNA completely blocks the induction of LTD, on organotypic slices. We have then shown that JAK2 was activated after LTD in an NMDAR receptor-dependent manner but not after DHPG or carbachol-induced LTD. We have also shown that STAT3 is activated after LTD and translocated to the nucleus. Furthermore, STAT3 activation is required for LTD since a STAT3 inhibitor can block LTD on acute slices.
These data provide strong evidence that the JAK/STAT pathway is involved in synaptic plasticity.

P3.008

Contribution of matrix metalloproteianses (MMPs) to migration and neurotrophic properties of nasal olfactory stem and ensheathing cells

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Recent studies in our laboratory have characterised ectomesenchymal stem cells (EMSCs) from nasal olfactory lamina propria as a subtype of mesenchymal stem cells with osteogenic and neurogenic properties. We have also demonstrated these stem cells differentiate into neurons and promote learning and memory recovery in a mouse model of amnesia. In these conditions, EMSCs reach the

lesioned cerebral areas when they are transplanted into the controlateral unlesioned hemisphere, the cerebrospinal fluid or the blood, but their mechanisms of migration remain elusive. Among the possible molecular factors involved in EMSCs motility MMPs are particularly well suited because of their ability to regulate the pericellular environment. We have previously demonstrated the implication of MMPs in the motility of neural cells, including, neurons, astrocytes, and ensheathing glial cells. In this study, we have characterised the profile of expression of several MMPs currently associated with cell migration and invasiveness and their role in cell migration using 2D and 3D *in vitro* cell migration models. We also investigated the role of MMPs secreted by EMSCs and by olfactory ensheathing cells from the same region in neurite outgrowth and axodendritic differentiation. Our data indicate that MMP-2, MMP-9 and MT1-MMP are involved in EMSCs transmigration in transwell chambers. Moreover, the distribution of these proteinases reveals a partial colocalisation with the actin cytoskeleton. All tested MMPs are present in cell fractions, including membrane, cytoskeleton, cytoplasm and, more surprisingly, nucleus. Their homogenous distribution across the cell becomes rapidly polarised towards the migration front in migrating cells. Finally, we demonstrate that conditioned supernatants of EMSCs promote neurite outgrowth and axodendritic differentiation, an effect that is abrogated by depletion of MMPs from the media. Altogether, these results provide the first evidence of MMP-related functions for EMSCs that could account for at least some of their therapeutic properties.

P3.009

Contribution of 5-HT to locomotion: the paradox of Pet-1$^{-/-}$ mice

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Serotonin (5-HT) plays a critical role in locomotor pattern generation by modulating the rhythm and the coordinations. *Pet-1*, a transcription factor selectively expressed in the raphe nuclei, controls the differentiation of 5-HT neurons. Surprisingly, inactivation of *Pet-1* (*Pet-1*$^{-/-}$ mice) that causes a 70% reduction in the number of 5-HT positive neurons in the raphe, does not impair locomotion in adult mice. The goal of the present study was to investigate the operation of the locomotor central pattern generator (CPG) in neonatal *Pet-1*$^{-/-}$ mice. We first confirmed, by means of immunohistochemistry, that there is a marked reduction of 5-HT innervation in the lumbar spinal cord of *Pet-1*$^{-/-}$ mice. Fictive locomotion was induced in the in vitro neonatal mouse spinal cord preparation by bath application of N-methyl-DL-Aspartate (NMA) alone or together with dopamine and 5-HT. A locomotor pattern characterized by left-right and flexor-extensor alternations was observed in both conditions. Increasing the concentration of 5-HT from 0.5µM to 5µM impaired the pattern in *Pet-1*$^{-/-}$ mice. We tested the role of endogenous 5-HT in the NMA-induced fictive locomotion. Application of 5-HT$_2$ or 5-HT$_7$ receptor antagonists affected the NMA-induced fictive locomotion in both heterozygous and homozygous mice although the effects were weaker in the latter strain. This may be, at least partly, explained by the reduced expression of 5-HT$_{2A}$R as observed by means of immunohistochemistry. These results suggest that compensatory mechanisms take place in *Pet-1*$^{-/-}$ mice that make locomotion less dependent upon 5-HT.

P3.010

Spike-timing-dependent plasticity rules differ between somatosensory and prefrontal corticostriatal areas

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GABAergic interneurons exert a strong feedforward inhibition on output neurons and therefore control their input-output gain function by modulating their spike timing. Such control of the spike timing could lead to a major modulation of the induction of long-term synaptic plasticity and particularly the spike-timing-dependent plasticity (STDP). STDP, a Hebbian synaptic learning rule, is considered as the first

law of synaptic plasticity. Here, we analyzed the impact of GABAergic circuits on STDP depending on the corticostriatal territories: somatosensory vs prefrontal. The corticostriatal pathway, the main entrance of basal ganglia, process inputs from the whole cerebral cortex and is engaged in procedural learning and memory. The corticostriatal long-term plasticity provides a fundamental mechanism for the procedural learning. Feedforward and feedback inhibition, from respectively GABAergic interneurons and the striatal output neurons, the medium-sized spiny neurons (MSNs), are the main sources of GABAergic inhibition in striatum. The feedforward interneuronal system exerts the strongest effects on MSN spike timing. Using dual patch-clamp recordings with a biophysical synapse model, we demonstrated that GABAergic interneurons govern the STDP-timing rule in the somatosensory area. Indeed, blockade of ionotropic GABAergic receptors reversed the temporal order of STDP potentiation and depression. With immunohistochemistry associated with a 3D-model of corticostriatal territories, we analyzed the distribution of GABAergic interneurons (parvalbumin-, calretinin- and NO-synthase-expressing cells). Here, we report a heterogeneous distribution of parvalbumin cells: predominant in the somatosensory territory while barely detectable in the prefrontal area. Therefore, we are currently investigated the STDP-timing rules in MSN in the prefrontal territory of the striatum innervated by the cingulate area 2 of the cerebral cortex; preliminary results show that STDP-timing rule differ between prefrontal and somatosensory striatal areas. These findings establish a central role for GABAergic circuits in shaping STDP and suggest that disorders affecting inhibitory circuits may change differently the polarity of plasticity depending the corticostriatal territories.

P3.011

Numerical changes in the visual V2 lateral cerebral cortex of the developing rat, induced by proteic malnutrition

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Background: The central nervous system in developing rats has structural and functional variations during the maturation period, until it reaches the characteristics of an adult animal. This was the reason why, comparing the structural and morphometric variations during the growing process of developing rats under malnutrition in different growing and development stages, was proposed.
Material and methods: Samples were taken from the visual V2L brain area of Sprague- Dawley rats distributed on three groups: the group under malnutrition condition (**MN**), under hypo protein experimental condition (a 6% protein diet), the control group, with 25% protein diet (**N**), and the renourished group (**RN**), which was divided in those that began renourishing from birthday (**P0**), and those that began renourishing from day 21 (**P21**). Staining was carried out with hematoxiline-eosine for measuring the volume of cortex and volume V2L. Additionally, immunohistochemistry for NeuN and GFAP for densities and absolute number of neurons and glia was implemented.
Summary of results: CEREBRAL VOLUMEN AND V2L CEREBRAL CORTEX:
The alimentary condition affects the cerebral cortex growth and development. So, there was a significant difference of the cerebral volume and V2L cortex volume between N and RN; and between N and rats under MN.
NUMERICAL DENSITY: NEURONAL (**N_V neun**) AND GLIAL (**N_V glia**):
With respect to **N_V neun**, a significant difference ($p< 0,05$) was found between N and DN groups, and between N and RN groups; and this was in an independent manner. This difference was not seen between RN and DN.
With respect to **N_V glia,** these significant differences were seen only in the group of rats under 30 days old.
ABSOLUT NEURONAL NUCLEUS NUMBER (**Nneu)** AND ABSOLUTE ASTROCYTE NUMBER (**Nglia**):
No significant differences were found, with respect to these parameters, between the studied groups; except with the group of rats under 30 days old, which evidenced an Nglia of the N group lesser than in MN.
Conclusions:

1. Proteic malnutrition affects the width of the cortex, Nvneun, Nneu and Nvglia.
2. These observations are more evident on the group of rats under 30 days old, but **N_V neun** is lesser in the renourishing since P0 than since P21.

P3.012

A lateral belt of cortical LGN and NuMA guides mitotic spindle movements and planar division in neuroepithelial cells

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To maintain tissue architecture, epithelial cells divide in a planar fashion, perpendicular to their main polarity axis. As the centrosome resumes an apical localisation in interphase, planar spindle orientation is reset at each cell cycle. We used 3-dimensional live imaging of GFP-labelled centrosomes to investigate the dynamics of spindle orientation in chick neuroepithelial cells. The mitotic spindle displays stereotypic movements during metaphase, with an active phase of planar orientation and a subsequent phase of planar maintenance before anaphase. We describe the localisation of the NuMA and LGN proteins in a belt at the lateral cell cortex during spindle orientation. Finally, we show that the complex formed of LGN, NuMA, and of cortically located Gαi subunits is necessary for spindle movements and regulates the dynamics of spindle orientation. The restricted localization of LGN and NuMA in the lateral belt is instructive for the planar alignment of the mitotic spindle, and required for its planar maintenance.

P3.013

Early migrating GABA neurons become functional "hub cells" in the developing hippocampus

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Connectivity in the developing hippocampus displays a functional organization particularly effective in supporting network synchronization, as it includes superconnected hub neurons. We have previously shown that hub network function is carried out by a subpopulation of GABAergic interneurons that display dense and widespread axonal arborisations. However the fate of hub neurons remains unknown. Specifically it is unclear whether these hub cells are only transiently present or later develop into distinctive subclasses of interneurons. These questions are difficult to assess given the complexity of the GABAergic neurons and the poor expression of interneuron markers at early developmental stages. To circumvent this conundrum we used "genetic fate mapping" that allows for the selective labelling of interneurons based on their place and time of origin. We show that early generated GABAergic cells form a subpopulation of hub neurons in the developing hippocampus, which in adulthood acquire classical markers for long-range projecting GABAergic neurons.

P3.014

Scribble1 is a new regulator of NMDA receptor endocytic sorting

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The number of NMDA receptors at the synapse plays a critical role in message transmission thought LTP and LTD mechanisms. Several studies have shown that PDZ-domain proteins such as PSD95 are able to modulate NMDAR traffic, through direct interaction with the NR2 subunits. To further investigate the functional relationship between the PDZ proteins and NMDARs, we used yeast two-hybrid screening and we identified Scribble1 (Scrib1) as a new partner of NR2 subunits. Scrib1 is a LAP (Leucine and PDZ) protein involved in cell proliferation, migration and polarity. Scrb1 was previously implicated in presynaptic vesicle dynamic and synaptic plasticity in drosophila and mammals. Recently, our laboratory found a role for Scrib1 in dendritic spine formation and memory processes.
In the present work, we used a combination of molecular, cellular and biochemical approaches to understand the role of Scrb1 in NMDARs trafficking. RT-PCR and western blot show that Scrib1 RNA and protein are highly expressed during development. The interaction between NR2 subunits and Scrb1 was further validated using yeast two-hybrid assay, GST pull-down and co-immunoprecipitations. We showed that NR2 subunits bind directly to the PDZ domains of Scrib1, and form a protein complex in the brain. In adult rat, Scrib1 is primarily located in pyramidal cells in hippocampal areas, and strongly expressed in dendrites and spines. Our results show that the interaction between NR2 and Scrib1 increases NMDARs recycling to the cell surface in heterologous cells (COS-7) and in hippocampal neurons in culture. We were able to correlate these results with a decrease in NMDA receptors levels at the synapses of circletail mutant, a mouse lacking full length Scrib1. Taken together, our data suggest that the NMDAR-Scrib1 complex is an important component of NMDAR trafficking.

P3.015

A test to assess the development of elementary visuo-spatial perceptual skills

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Objective: To study typical and atypical development of visuo-spatial perception during childhood, a simple and short test was lacking. We designed one, inherited from adult neuropsychology.
Methods: The idea was to target elementary visuo-spatial tasks suspected to involve the dorsal occipital cortex (comparison of lengths and sizes) or the dorsal posterior parietal cortex (midline localization, angle processing and relative dot/square localizations) and compare their evolution with age between 4 and 12 years old. A group of adults was also tested to estimate whether the ability was fully acquired at twelve.
Results: First, none of the elementary visuo-spatial abilities tested was acquired as early as 4 years old. Second, as expected, the results distinguished the subtests Length and Size, for which performance abruptly increases between 4 and 6 years old and reach the adult performance level, and the others for which the performance ameliorates more progressively during childhood and adolescence.

Conclusion: This test has proved useful to distinguish the speed of development of primary and more cognitive abilities of visuo-spatial perception. The results obtained in this population of healthy children can serve as control for an age-specific comparison to children with developmental disorders in which visuo-spatial perceptual deficits are suspected.

P3.016

Gravity differentially affects the transcription of reticulum dependent calcium signaling proteins in pial arteries from late adolescent and adult male C57Bl/6J mice

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Neuronal metabolism almost essentially implies glucose oxidation. Indeed, in all animal species, cerebral and cognitive functions closely depend on oxygen availability. Oxygen is brought to neurons by cerebral blood flow, which is regulated by vascular smooth muscle cells (VSMC) contractility/reactivity. The reactivity of these VSMC is regulated by calcium signaling, including calcium release by reticulum stores through ryanodine (RyR) and inositol-trisphosphate (IP3R) receptors. Among all environmental parameters that can alter VSMC reactivity, variations in the level of gravity have been proposed as a candidate by re-equilibrating blood perfusion. Another inescapable parameter that can alter vascular function is aging, in which arterial reactivity is decreased in humans and rodents. We then proposed that increasing the level of gravity could differentially modify the contractility of cerebro-VSMC by changing the levels of expression of RyRs, IP3Rs and associated proteins, as a function of the age in male mice.
Late adolescents (8 weeks) and adults (6 months) C57Bl/6J male mice were bred under different levels of gravity (1G, 2G, 3G) for three weeks. Cellular responses were then studied by determining the level of expression (RT-qPCR) of reticulum dependent calcium signaling implicated proteins in anterior and middle pial arteries VSMC.
In late adolescent, increasing the level of gravity induced a decrease of expression of RyR3 associated with an increase of expression of Sarco-Endoplasmic Reticulum ATPase 2A, 2B and 3 pumps, as well as Phospholamban, responsible for the refilling of calcium stores. This could increase calcium-induced-calcium-release processes and potentially cerebro-vascular myogenic reactivity. Contrarily, adult mice bred under high gravity conditions (3G) displayed decreased RyR2 and IP3R2 expression associated with elevated CD38 expression, suggesting lower calcium signals and potentially a decrease of myo-vascular reactivity.
Altogether, our results highlight the differential effects of gravity environment alteration on cerebro-vascular reactivity in late adolescent and adult mice. Further experiments of calcium imagery are in process to investigate whether our molecular data are effectively correlated with functional alterations.

P3.017

Estradiol-induced plasticity in hippocampal neurons requires NMDA receptor surface redistribution

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The sex hormone, 17β-estradiol (E2), modulates the neuronal communication in brain regions involved in cognitive processes. In the hippocampus, E2 modulates multiple aspects of the morphological and functional plasticity, such as the dendritic spine density, the NMDA receptor (NMDAR)-dependent long-term plasticity and the content of AMPA receptor (AMPAR) and NMDAR in

synapses. However, the cellular pathways involved in these key processes remain unknown. To tackle this issue, we investigated the impact of E2 on spine density, glutamatergic signaling markers and surface trafficking of GluN2A- and GluN2B-NMDAR. For this, a combination of immunocytochemistry and high-resolution single particle tracking (quantum dots) approaches was used in hippocampal cultured neurons (22 days *in vitro*). As previously reported, we confirmed that E2 (10 nM) long-term treatment (24h) increases significantly the spine density and the GluA1-AMPAR synaptic content. Interestingly, E2 modulated the synaptic content of GluN2A-NMDAR (+173% of control) and GluN2B-NMDAR (-82% of control). These effects were blocked by ICI (100 nM), a complete estrogen receptor antagonist. In parallel, E2 significantly altered the surface diffusion of GluN2A- and GluN2B-NMDAR, favoring the synaptic anchoring of GluN2A-NMDAR and the lateral escape of GluN2B-NMDAR. More importantly, we report that the artificial immobilization of surface GluN2A- and GluN2B-NMDAR (using an antibody-based cross-link protocol) fully prevented the E2-induced spine density increase. Together, our data unveil that E2 modulates the surface distribution of GluN2A- and GluN2B-NMDAR by acting on their surface diffusion. The identification of this cellular pathway shed new lights on the relationship between hormone, glutamate receptor dynamics and synaptic plasticity in the hippocampus.

P3.018

Expression of guidance molecules in the nigrostriatal pathway in animal model of Parkinson's disease

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Functional involvement of guidance molecules in mesencephalic dopaminergic (mDA) axon guidance during development has been recently described. For instance, semaphorin3A and 3F participate to the navigation of mDA axons to their final synaptic target, DCC (deleted in colorectal cancer) loss of function was found to induce malformed DA innervation of the ventral striatum, and in the slit1/2 robo1/2 knock-out mice, mDA axons were shown to spread out of the medial forebrain bundle (MFB) into the diencephalon to form aberrant tract. In our laboratory, we recently described the expression of a striatal gradient of ephrinA5 having a repulsive action on the mDA axons through its interaction with EphA5 receptor.
In animal model of Parkinson's disease, the anatomical and functional repair of the nigrostriatal pathway using dopaminergic neuroblasts transplantation suggests that (i) specific guidance cues exist and (ii) transplanted embryonic cells are able to respond to theses cues, guiding them to their final target. As guidance mechanisms occurring in the lesioned adult brain after graft may mimic those established during development, we are currently investigating the expression of these guidance molecules in the vicinity of the mDA pathway in animal model of Parkinson's disease with intranigral transplantation. Identifying the guidance molecules involved in the navigation of grafted cells may help to improve the efficiency of neuron re-connexion in Parkinson's disease cell therapy.

P3.019

Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) in the metabolism of APP/Abeta

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Amyloid beta (Abeta) degrading MMPs, and putative alpha-secretases adamalysins (ADAMs), which convert the amyloid precursor protein (APP) to its non-toxic truncated soluble form (sAPPalpha), are

zinc dependent endopeptidases. These enzymes are both physiologically inhibited by the tissue inhibitors of metalloproteinases (TIMPs), upregulated in Alzheimer's disease. Little is known about the role that the metalloproteinase/TIMP balance may play in the metabolism of APP/Abeta. Our aim is to investigate in cell culture how the metalloproteinase/TIMP system affects the sAPPalpha/Abeta balance, and how in turn Abeta overproduction affects the metalloproteinase/TIMP system. In a model of HEK cells carrying the Swedish mutation in the APP gene (APPsw), we modulate metalloproteinase activity using recombinant and synthetic molecules displaying different specificities against MMPs and ADAMs. We use molecular and cell biology approaches to evaluate sAPPalpha/Abeta production and metalloproteinase/TIMP expression and activity. We found that MMP-2 expression but not MMP-9 is upregulated in APPsw expressing cells, as compared with mock controls, and MMP-2 increase is reduced by NF-kB inhibitors. Moreover, a specific inhibitor of MMPs that does not inhibit ADAMs or neprilysin, differently alters the production of Abeta and sAPPalpha, as compared with TIMP-1. The overexpression of TIMP-1/GFP or TIMP-2/GFP in their active and inactive forms reduces the intracellular content of APP. On the contrary, the overexpression of membrane-bound MT1-MMP (also known as MMP-14) increases the overall content of APP species, generating an APP breakdown product 20 kDa smaller, compatible with the size of an APPalpha-like protein. These data suggest that MMP-dependent and independent pathways operate in the control of APP/Abeta metabolism, and that in turn MMP levels are regulated by APP/Abeta, suggesting altogether a functional interplay between the latter and one or several MMPs. Ongoing experiments should provide further clarify the nature of the interactions between the MMP/TIMP system and APP/Abeta metabolism in neural cells and the physiopathological meaning of these interactions.

P3.020

Usefull of neuromuscular junction electrophysiological studies in diagnosis of acute steps of organophosphate intoxications

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Introduction: Acute organophosphate (OP) intoxications, accidental or voluntary, are frequent in our countries and are responsible for a high mortality. They cause extensive muscular paralysis by acetyl cholinesterase activity inhibition at the neuromuscular junction level.
Cases report:
Case 1: A 28-year-old woman was admitted to the medical intensive care unit for Malathion acute intoxication with signs of glandular hypersecretion, complicated tetraparesis, and respiratory distress. The cholinesterase activity was 17%. The electroneuromyography showed multiple motor responses to the same stimulation, which is characteristic of the cholinergic crisis. Other electrophysiological parameters, in particular low-frequency repetitive stimulations, were normal. The evolution was favourable after symptomatic treatment and respiratory assistance.
Case 2: A 25-year-old woman admitted to intensive care unit with muscarinic signs and respiratory failure after attempted suicidal organophosphate poisoning. Cholinesterase activity was low (12.5%) and the electrophysiological study disclosed the characteristic aspect of intermediate syndrome (decrement-increment phenomenon). The patient died due to septic complications.
Discussion and conclusions: Organophosphate intoxications evolve in three phases: acute cholinergic crisis, intermediate syndrome, and delayed neuropathy. While the electrophysiological aspects of delayed neuropathy are best characterized, those of crisis and intermediate syndrome remain very little studied. The persistence of acetylcholine in the synaptic slit would explain the multiple motor responses to single stimulation during the crisis. In intermediate syndrome the pathophysiological aspect remain unclear.
We underling the rarity but characteristic electrophysiological aspect in acute organophosphate intoxication and we purpose this as another criteria for diagnosis of this phases of intoxication.

P3.021

Action potential waveform regulates presynaptic calcium entry in CA3 pyramidal cell axon terminals

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Neuronal activity in brain circuits is shaped into specific temporal patterns with a millisecond precision. At elementary connections, the neuronal timing is essentially described by the conduction time along the axon and the synaptic delay (SyD). SyD is often considered to be a constant parameter. However, our team has recently showed that synaptic latency at cortical synapses is determined by the presynaptic release probability (Boudkkazi et al. Neuron 2007) and the waveform of the presynaptic spike (Boudkkazi et al., J Physiol 2011). For instance, the extension of action potential (AP) duration prolongs SyD whereas its reduction in amplitude shortens the SyD.
Using confocal microscopy combined with patch-clamp recording from CA3 pyramidal neurons, we examined how calcium signals measured in axon terminals are modulated by changes in presynaptic AP waveform induced pharmacologically. The axon of CA3 neurons in organotypic slice cultures of mouse hippocampus was identified as the only neurite displaying a cylindrical morphology without spines in which a train of APs evoked a sodium entry visible with CoroNa green dye. Neurons were filled with a solution of Fluo-4 & Alexa 594, and the kinetics of the spike-evoked calcium signal, measured in en passant boutons, was analyzed at a rate of 2.5 kHz. Blocking voltage-gated potassium channels with 4-AP (20 µM) or DTx-K (100 nM), reduced the rising phase of the spike-evoked calcium signal by 30%. We conclude that modulation in presynaptic calcium signal kinetics may account for the increased SyD measured at CA3-CA3 connections.

P3.022

Role of PACAP-induced tPA release in the regulation of granule cell migration during cerebellar development

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Postnatally, cerebellar granule neurons (CGN) change the mode and rate of migration when they translocate from the external granular layer (EGL) to the internal granular layer (IGL) but the fine mechanisms underlying this process are not completely established. Both pituitary adenylate-cyclase activating polypeptide (PACAP) and tissue-type plasminogen activator (tPA) have been shown to regulate independently mouse CGN migration. In the present study, we showed that PACAP stimulates *in vitro* the expression (2 fold) and the secretion (3 fold) of tissue-type plasminogen activator (tPA) from rat CGN. Application of exogenous PACAP significantly slowed by 70% the migration of isolated rat CGN in the microexplant culture of P2-P4 cerebella. In contrast, tPA, plasminogen activator inhibitor-1 (PAI-1) and plasminogen were devoid of effect on CGN movement. These results suggest that PACAP, in contrary to tPA, acts directly on CGN. Immunohistochemistry labelling revealed an intense signal of tPA-like immunoreactivity in the PCL and the IGL of the cerebellum of P10 rats. The ML was also moderately labelled with tPA antibodies while the EGL was virtually devoid of immunoreactive signal. The tPA-like immunoreactive material was observed in the extracellular matrix but also in cells including large soma and dendrites of Purkinje cells and small cell

bodies of DAPI-positive CGN. The data suggest the existence of several sources of tPA in the developing rat cerebellum. In the ML of P10 rat cerebellar slices, application of exogenous PACAP significantly inhibited by 72% CGN migration while exogenous tPA had no effect. In contrast, application of the PACAP antagonist PACAP6-38 increased by 23% the rate of GCN migration in the PCL while application of PAI-1 reduced CGN movement in the ML and the PCL by 70 and 27% respectively. These data indicate that both endogenous PACAP and tPA are involved in the radial glia-independent migration at the PCL level. Collectively, these results suggest that PACAP-induced tPA release could allow the resumption of CGN migration toward the IGL after the transient brake induced by PACAP in the PCL.
Supported by PRIMACEN, FEDER, AdMIN, Inserm U982 and the Région Haute-Normandie

P3.023

Rich2 a new binding partner of shank3 that regulates endosomal and AMPA receptor recycling

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Synaptic activity and plasticity are the principal mechanisms implicated in learning and memory, and their alteration is associated with mental disorder. Shank3 is a major post-synaptic scaffolding protein that orchestrates dendritic spine morphology. Shank3 mutations lead to autism spectrum disorders. A recent study has shown that mice carrying an heterozygous deletion of Shank3 display a reduced level of Glur1 glutamate receptors at the plasma membrane and impaired LTP, suggesting that Shank3 is implicated in AMPA receptors trafficking. In a proteomic screen, we identified a new Shank3 binding partner, the Rho-GAP interacting CIP4 homologue (Rich2), whose function was uncharacterized so far. We found that Rich2 is an endosomal recycling protein. In hippocampal neurons, Rich2 overexpression increased endosomal recycling and AMPA receptor exocytosis and its depletion by siRNA inhibited the exocytosis of Glur1. A paradigm of chemical LTP applied to hippocampal cultures showed that depletion of Rich2 by siRNA inhibits LTP and GluR1 trafficking. Moreover, we determined that Shank3-Rich2 interaction is required for GluR1 exocytosis and LTP. These results suggest that the Shank3-Rich2 complex is implicated in AMPA receptor trafficking and synaptic potentiation.

P3.024

Genetic dissection of ratiometric EphA receptor signaling in the formation of a retinotopic map

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Nervous systems frequently organize representations of the external world into 'topographic maps' - systems of synaptic connections in which the positional coordinates of a set of input neurons are maintained in their wiring to target neurons in the brain. The most intensively studied of such sensory maps is the projection of the ganglion cells of the vertebrate eye to their synaptic targets in the superior colliculus (or tectum) of the midbrain. The retinocollicular map is Cartesian: the orthogonal nasal-temporal and dorsal-ventral axes of the retina are mapped onto the orthogonal caudal-rostral and lateral-medial axes, respectively, of the superior colliculus.

In vitro and mouse genetic 'knock-out' studies have demonstrated that EphA receptors and ephrinA ligands are essential for topography formation. In the retina, ganglion cells express EphA receptors in a low-nasal-to-high-temporal gradient, and in the target, the superior colliculus, ephrin-As are expressed in a low-caudal-to-high-rostral gradient. These gradient distributions and mapping relationships are consistent with a role for EphA receptors in the development of retinotopy, however, in order to directly demonstrate an instructive role for the *gradient* of EphA receptors, one must systematically alter the magnitude, periodicity or slope of the gradient, without ablating it, and demonstrate a subsequent change in the retinocollicular map.
We report an update on the 'Relative Signaling' model of retinocollicular mapping, which quantitatively predicts how EphA gradients in the retina instruct the development of the retinotopic map in the superior colliculus. We have generated a set of combined EphA3 knock-in/EphA5 knock-out mouse mutants in which the EphA receptors gradient in the retina is altered, but not ablated, and measured the maps of these mutants. The rules of the 'Relative Signaling' model accurately predict retinocollicular map configuration demonstrating that
(1) the shape and magnitude of EphA gradients are essentially informative for the development of retinotopic maps and that
(2) EphA receptors, in this context, are functionally equivalent.
This work confirms the general utility of the 'Relative Signaling' model as a method for translating EphA gradients into topographic maps.

P3.025

Plexin-A1 and Semaphorin 6D control retinal axon targeting in the dorsal lateral geniculate nucleus

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Proper neuronal function relies on precise wiring of axonal projections during development. Axons are guided by axon-axon interactions and by extrinsic cues along their route at intermediate targets and in their final synaptic targets. The visual system is ideal for investigating these guidance and targeting mechanisms. At the optic chiasm, axons from each eye distribute to the same (ipsilateral) and opposite (contralateral) side of the brain. In the optic tract (OT), retinal axons fasciculate and reorganize according to retinotopic origin, implicating presorting of retinal axons before reaching their target. Retinal axons must defasciculate and branch to enter their targets such as the dorsolateral geniculate nucleus (dLGN) and the superior colliculus (SC). Within the dLGN and SC, retinal axons terminate in a topographic and eye-specific manner, relying on molecule gradients and activity-dependent mechanisms. The role of axon organization and fasciculation as axons approach and enter targets is less well understood.
We investigated the role of Plexin-A1, a receptor for Semaphorins, in the establishment of retinal projections. Plexin-A1 is expressed in retinal ganglion cells (RGCs) and dLGN during development. Both *Sema6D*$^{-/-}$ and *Plexin-A1*$^{-/-}$ mutants display an increased ipsilateral projection and defasciculation defects at the optic chiasm. Strikingly, in the dLGN of *Plexin-A1*$^{-/-}$ mutants, RGC axons misproject to ectopic locations. Furthermore, *Plexin-A1*$^{-/-}$ RGC axons aberrantly defasciculate in the optic tract. We are now investigating the topographic organization of retinal projection in the dLGN and SC to determine if PlexinA1 is important for retinotopic map formation through fasciculation and presorting of axons prior to target innervation.

Plexin-A1 is known to interact with Sema3A, Sema6C and Sema6D. At postnatal ages, Sema6C and Sema6D are expressed in RGCs and Sema6D is expressed in the dLGN. The *Sema6D* $^{-/-}$ mice display a phenotype similar to *Plexin-A1*$^{-/-}$ although milder, with defects in fasciculation of the optic tract and in retinogeniculate targeting.
Together these results show a role for Plexin-A1 and Sema6D for retinal axon guidance, fasciculation and targeting.
Supported by the NIH-NEI, INSERM and Gatsby Foundation.

P3.026

Genomic imprinting disruption of the mouse Necdin gene: consequences and causes

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The Prader-Willi Syndrome (PWS) is a rare neurodevelopmental genetic disorder. It is a complex disease for which the symptoms, evolving according to the age, present a high variability whose severity varies among patients. Several genes located in the 15q11-q13 region, including NECDIN gene, are involved in the PWS. These genes are regulated by the genomic imprinting mechanism: only the paternal allele of these genes is expressed, their maternal allele being silenced. Our team has generated a mouse model in which the paternal allele of the Necdin gene has been inactivated (+m/-p). This model presents phenotypical similarities with PW patients, notably respiratory distress which is responsible for lethality in 30% of pups. We observed that mortality affects more -/- pups than +/-p mice (n survivors = 15 -/- vs 25 +/-p ie 40% mortality) suggesting in a functional role of the maternal allele in the mutant mice survival. We then investigated the presence of the Necdin maternal allele with alleviation in respiratory distress and consequently a reduced lethality. Interestingly, we recently showed an unexpected expression of the maternal imprinted allele of the Necdin gene in our Necdin-KO mouse model (detection of protein and transcript). 0n the basis of that observation, we wondered firstly when and where that maternal Necdin expression occurs in Necdin-KO mutant mice, and then we quantified it. Necdin expression in mutants versus wild type mice was analyzed by immunohistochemistry, *in-situ* hybridization, western-blot and RT-qPCR. A sorely variable maternal Necdin expression was observed in the nervous system, particularly in the brain (hypothalamus and medulla), at E12.5, P1 and adult Necdin-KO mutant mice, issued from the same litter. For the first time, we observed in our mutant mice a maternal expression of the Necdin gene which is normally silent. It raises an important question about the existence of a loss of imprinting and its consequences in the pathology, notably in the variability in the expression of the symptoms. It also allows to contemplate a therapeutic pharmacological prospect by stimulating the maternal allele.

P3.027

Short-term memory impairment in mice caused by hippocampal downregulation of the neuronal monocarboxylate transporter MCT2

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MCT2 is the predominant neuronal monocarboxylate transporter involved in the transport of lactate, an energy substrate which seems to play a key metabolic role in the CNS. Previously, we found that brain-derived neurotrophic factor (BDNF), an essential element in the mechanism of synaptic plasticity, enhances MCT2 expression *in vitro* in cultured cortical neurons and in synaptosomal preparations (Robinet and Pellerin, 2010) as well as *in vivo* by acute injection in the CA1 hippocampus (unpublished data). In addition, it was demonstrated that MCT2 and GluR2 (an AMPA receptor subunit) are colocalized in the post-synaptic density (Bergersen et al., 2005) and associated in a common trafficking route (Pierre et al., 2009). We therefore hypothesize that changes in MCT2 expression participate in the process of synaptic plasticity. In the present study, we assessed the effect of hippocampal MCT2 downregulation on the proficiency of learning and memory of spatial tasks. Bilateral stereotaxic injections of lentiviral vectors were performed into mouse hippocampal CA1 areas. Twelve mice were injected with a lentivirus expressing the siMCT2 (MCT2 knockdown mice) and 12 mice with a lentivirus expressing a non-sense siRNA. (control siRNA mice) . Spatial working and reference memory abilities were evaluated on a radial arm- and a Morris water maze, while the dynamics of episodic memory formation was assessed in a passive avoidance task. MCT2 knockdown resulted in clear short-term memory impairments, yet with a mild impact on long-term spatial reference memory. These results indicate that MCT2 is involved in the mechanisms of working memory, and support the notion that short-term and long-term memory can be supported by independent molecular mechanisms.

P3.028

Effect of haemorrhage on the catecholindolaminergic activity of neurohypophysis and the haematological parameters in mal wistar rat

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Catecholindolamines in the brain participate in dipsic behavior control in response to hypovolemia, release of vasopressin and angiotensin-II induced thirst. The aim of the present work is to study the effect of haemorrhage on the neurohypophysis neurotransmitters and their implication in the homeostasis .The experiment focused on:
1) The study of physiological parameters of the water balance.
2) The study of catecholindolaminergic activity in the neurohypophysis.
The bleeding has caused significant changes in the plasma parameters. We noted a decrease in hematocrit, a result that demonstrates haemodilution, probably caused by the antidiuretic activity of vasopressin. Thus, statistical analysis showed an increase in kaliemia, the plasma osmolarity and a non significant decrease in natremia. The results obtained during the study of systemic parameters, gave a reliable indication of the relative change in plasma volume. In the second part of our work, neurochemical study which concerns the determination of catecholindolamines rates (DA dopamine, norepinephrine NA and serotonin 5-HT) and their metabolites (DOPAC, HVA, 5HIAA) on neurohypophysial tissue, after separation by HPLC-DEC, showed an increase of DA and 5-HT, but rate of NA decreases.The increase in the DA and the decrease in its turnover reveal an increase of anabolism of this neurotransmitter. Thus, the accumulation of DOPAC, resulting from the increased activity of tuberohy-pophyseal dopaminergic neurons**.** Although the rate of serotonin and its metabolite 5-HIAA were not significantly affected by the hemorrhage. Serotonin turnover is altered, thus demonstrating a localized effect of bleeding in the catabolism serotonin. We suggested that this decline could be resulted from an accelerated transfer of this neurotransmitter into the blood. In conclusion, our results suggested that changes in neurochemical and haematological parameters could result from a failure of hydromineral homeostasis which activates several neuroendocrine mechanisms including the release of catecholindolamines, ANG II and VP. These pituitary hormones work with the autonomic nervous system to decrease water and sodium loss, adjust the distribution of water between intra and extracellular fluid compartments.
Supported by N€UROMED, REGPOT-2009-2, FP7 of the EU

P3.029

Regulation of autophagy reduces caspase 3 activity and improves the survival of GABAergic progenitors: transposition to grafted precursors

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Hypoxia-ischemia is among the main causes of brain damages in preterm and full-term neonates. Even if several molecular mechanisms have been identified, excitotoxicity resulting from a massive release of glutamate appears as a major process leading to cell death. Consequently, blockade of glutamate receptors has been proposed as a possible neuroprotective strategy. However, there are more and more evidences that, in the immature brain, NMDA antagonists are deleterious for GABAergic precursors through activation of the apoptotic mitochondrial pathway (Desfeux, cereb cortex, 2010). Autophagy is a catabolic process involving the degradation of cell´s own components through the lysosomal machinery. Because autophagy and apoptosis can interact *via* members of the Bcl2 family, the aim of this study was to characterize the effect of autophagy regulation on apoptosis of grafted GABAergic precursors in the neocortex of newborn mice. Visualization and quantification of cell death indicated that 80% of the GABAergic precursors obtained from GAD67-GFP mice were viable before the graft. After transplantation, a time course study showed that length and number of processes progressively increased from P2 to P30 suggesting that precursors differentiated into the host tissue. Consistent with these data, grafted GadGFP cells expressed GAT-1 and synaptophysine at P15. In addition, glutamate induced an increase of intracellular calcium levels in the grafted cells. However, density of transplanted cells strongly decreased from P2 to P5 and numerous cells were caspase-3 positive indicating that most of grafted cells died by apoptosis within the first days following transplantation. Pretreatment of ganglionic eminences by rapamycine increased LC3 cleavage but had no effect on apoptotic death. In contrast, 3-MA significantly decreased LC3 cleavage, bax expression and caspase-3 cleavage and activity. Altogether, morphological, neurochemical and functional studies indicate that transplanted GABAergic progenitors differentiate *in vivo* with a low survival rate. Inhibition of autophagy using 3-MA improved the survival rate of grafted cells by decreasing apoptosis. This works was supported by Rouen University, Inserm, ANR, the Region Haute-Normandie, the French Research Ministry and FEDER.

P3.030

Mechanisms underlying muscle spasticity: alterations of the neural network and inhibitory synaptic transmission after spinal cord injury

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Spasticity is a disorder associated with muscular hypertonicity, which occurs in association with spinal cord injury (SCI) and multiple sclerosis or metabolic diseases. We will focus on inhibitory synaptic transmission, which appears to be decreased in several pathologies of the nervous system.
Adult rats with thoracic spinal cord transection (SCT) are used as model of spasticity. The occurrence of spasticity is tested by monitoring the rate-dependent depression of the H reflex (RDD) 3 weeks and 4 months after SCT. Concomitantly the alterations of the reciprocal inhibition between antagonistic muscles (ankle flexor tibialis (Tib) *versus* ankle extensor gastrocnemius (GS) are measured in vivo. Three weeks after SCT, a significant decrease of the reciprocal inhibition is observed. After SCI, the reciprocal inhibition is suppressed and switched to reciprocal facilitation in some cases. This deterioration may explain the co-contraction of antagonist muscles observed in spastic patients.

The suppression of the reciprocal inhibition can account for an alteration of network of inhibitory interneurones projecting to motoneurons and/or to modifications of membrane properties of the motoneurons (MNs).
Changes in GABAA and Glycine receptors (GABAAR and GlyRα1) expression in muscle specific motoneurons identified with retrograde dyes, were investigated by immunohistochemistry 1 and 4 months after a complete mid-thoracic spinal cord transection .
One month after SCT, a significant upregulation of both GlyR and GABAAR occurred in Gs MNs. This state was transitory since a global significant decrease of expression of GlyR and GABAAR was observed in both pools of MNs 4 months after SCI.
The upregulation of the expression of GABAAR observed after SCI, can be considered as a post-reactionnal compensatory mechanism of the inhibitory synaptic transmission to lumbar Mns, in response to the suppression of the descending pathways.
Changes in the inhibitory premotor networks were mapped by use of Rabies Virus as a transynaptic retrograde tracer in order to compare the groups of last order interneurons (loINs) connected to pools of Mns innervating antagonist muscles (Tib versus GS). The preliminary results suggest a transversal reorganization of the premotor network after SCI.

P3.031

SubVentricular zone proliferation and migration of endogenous neuroblasts following cortical lesion

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The SubVentricular Zone (SVZ) and the Dentate Gyrus of the Hippocampus are the two main proliferative niches in adult mammalian brain. In the rodent adult brain, neuroblasts generated in the SVZ migrate along the rostral migratory stream (RMS) to the olfactory bulb. Previous studies have demonstrated that in several diseases and in brain injury, newly generated neuroblasts from the SVZ can migrate ectopically to the affected areas, which might constitute an endogenous repair mechanism. The aim of our study is to investigate changes occurring in the SVZ following lesion in the motor cortex and to study the ectopic migration of neuroblasts from the SVZ and their fate. We observed a transient increase in cell proliferation in the SVZ, which peaks at day7, following lesion. Neuroblasts start to migrate out of the RMS to the lesion site after 3days. This migration appears to be multimodal as we found migrating cells in close association with either blood vessels or glial cells. Some cells also migrate without any such association. We have not observed any 'chain' formation, a hallmark of neuroblasts migration along the RMS. In normal adults, neuroblasts migration is restricted within the RMS by the astrocytic glial tube surrounding the RMS. Following cortical lesion, we observed that the tube morphology was altered and appeared to be more 'open'. Blood vessels, which are organized in a reticular fashion in the normal brain, appeared to be re-oriented and arranged in parallel direction to the lesioned area. Cells were also migrating from the contralateral side; some cells migrated caudally along the corpus callosum. This study shows that cortical lesion stimulates SVZ cell proliferation and migration of neural progenitor cells to sites of cortical injury. Ongoing study is focused on the fate of the cells moving to cortex as well as their integration into the host circuitries.

P3.032

The glutamate electrodiffusion in regulating activation of metabotropic glutamate receptors

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Electric fields of synaptic currents could influence diffusion of charged neurotransmitters inside the synaptic cleft. However, the adaptive significance of such effects in physiological circumstances remains poorly understood. Here we will ask whether and how the postsynaptic activity could modulate activation of synaptic glutamate receptors through electric interactions in the cleft. Our simulations revealed that postsynaptic action potentials should significantly, albeit only briefly, retard an escape of glutamate from the synaptic cleft. However, this is unlikely to affect activation of local

AMPA or NMDA receptors. In contrast, activation of metabotropic glutamate receptors (mGluRs) could be significantly influenced by postsynaptic spikes coincident with glutamate release events. To study such modulation of metabotropic glutamate receptors, we combined the methods of electrophysiological experiment, theoretical physics, and computer simulations. Our results revealed that coincidence of the postsynaptic action potential and presynaptic glutamate release increase activation of mGluRs expressed perisynaptically at these connections.

P3.033

Regulation of adult olfactory bulb neurogenesis in a hyposerotonergic mouse model

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Neurogenesis persists in two regions of the adult mammal brain: the hippocampus and the subventricular zone (SVZ), which contributes interneurons to the olfactory bulb (OB), via the rostral migratory stream (RMS). Regulation of adult neurogenesis by extrinsic signals has been thoroughly studied in the hippocampus and to a less extent in the SVZ. For instance, the role of serotonin (5-HT) has been essentially characterized in hippocampal neurogenesis, whereas very few studies have analyzed its role in SVZ and OB neurogenesis. However, adult bulbar neurogenesis is an interesting model since OB receives an important innervation from 5-HT neurons located in the brainstem raphe nuclei. Moreover, the recent discovery of a modulatory role of 5-HT in odor processing in the OB (Petzold et al, 2009, Nature Neuroscience 12: 784-793) highlights the importance of characterizing its neuromodulatory effect on the production, integration and survival of olfactory newborn neurons. Here, we used the Pet1-knockout, as a serotonin-deficient model, to investigate the role of 5-HT on adult bulbar neurogenesis. Pet1 is a transcription factor, required to control the gene cascade that define the 5-HT phenotype (Hendricks et al 2003, Neuron 37: 233-247). Nevertheless, a group of raphe neurons still acquires a 5-HT phenotype in the Pet1-KO and targets brain regions involved in stress responses (Kiyasova et al, Journal of Neuroscience, in press). By contrast, a complete depletion in the hippocampus is observed and the consequence of this chronic depletion is currently analyzed by our group.
To determine the extent of 5-HT depletion in the other adult neurogenic region in this mouse model, 5-HT and SERT labeling was performed and preliminary results showed a strong but incomplete depletion of 5-HT innervation in the SVZ, the RMS and the OB in Pet1 KO. We are currently investigating the effect of a hyposerotonergy on cell proliferation in SVZ, neuroblast migration in RMS, and newborn neuron maturation and survival in OB.

P3.034

Microtubule-associated protein 1B (MAP1B) is necessary to overcome myelin inhibition and promotes axonal regeneration from adult neurons

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Following a traumatic lesion to adult central nervous system, axonal regeneration is generally very poor due to a neuronal state inapt for regeneration, and inhibition by environmental cues (glial scar, myelin components). Still, axons directly or indirectly affected by the lesion undergo important

morphological remodeling, indicating that permissive and/or non-permissive signals are eventually translated into a reaction of the axonal cytoskeleton.
Our team has demonstrated in vivo and in vitro that MAP1B, a “juvenile” microtubule-associated protein, plays in fact an important role also during axonal remodeling in the adult. Interestingly, it has been reported that MAP1B can be expressed at the plasma membrane, where it might interact with the myelin protein MAG (Myelin Associated Glycoprotein). Inhibition of axon growth by MAG actually depends on the neuron-intrinsic concentration of cAMP that switches from high/permissive during development to low/non-permissive in the adult. Here, we thus analysed the function of MAP1B in axonal regeneration in relation to neuronal cAMP concentration. In vivo, prior to a dorsal column injury on adult wildtype and MAP1B-ko, we performed a conditioning lesion by sectioning the sciatic nerve, known to induce elevation of cAMP levels. Central projections of sensory neurons, visualized by CTB-tracing, were then able to re-grow across the injury site into wildtype spinal cord. In MAP1B-ko mice, axon regrowth is much less vigorous, and only few fibers grow past the lesion site. In vitro, high cAMP levels seem to enhance MAP1B localization at the plasma membrane of adult DRG neurons. When plated on myelin extracted from adult brain of wildtype or MAG-ko mice, regeneration of wildtype DRG neurites is poor, but significantly enhanced when cAMP is added to the medium. In the absence of MAP1B, however, elevated cAMP levels have no effect on neurite growth. These data strongly suggest that MAP1B is a key player in axonal regeneration, in particular to overcome myelin inhibition in the adult central nervous system.

P3.035

Glycinergic signaling during postnatal neurogenesis in the SVZ

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Neurogenesis persists in the brain after birth in two main regions, the hippocampal dentate gyrus and the subventricular zone (SVZ). The SVZ contains the largest pool of dividing neural progenitors in the adult mammalian brain. These Neural progenitors give birth to immature neurons (called neuroblasts). Neuroblasts leave the SVZ, migrate along the rostral migratory stream (RMS) while being ensheathed by the process of SVZ astrocytes and reach the olfactory bulb where they differentiate into GABAergic interneurons. We have shown recently that expression and activation of specific neurotransmitter receptors, i.e. glutamate receptors, is critically involved in migration, proliferation and survival of migrating neuroblasts (Platel et al. 2008; Platel et al. 2010). Likewise, activation of GABA receptors has been shown to control many aspects of neurogenesis (Bordey 2007; Ge et al. 2007). These effects of GABA are due to the fact that the chloride gradient differs in immature versus mature neurons, causing GABA receptor activation to depolarize the membrane potential early in development. During embryonic development, a second chloride-permeable receptor, the glycine receptor, has also been shown to cause membrane depolarizations because of this chloride gradient (Flint et al. 1998). We are investigating the role of glycine receptors in different process of the postnatal neurogenesis: proliferation, migration, survival and integration of newborn neurons.
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P3.036

Prenatal exposure to fluoxetine increases spontaneous activity in lumbar spinal networks in mice

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We recently showed that the excitability of spinal networks highly depends on the expression of the K^+, Cl^- co-transporter (KCC2) in the plasma membrane. The up-regulation of the outward-directed pump, the neuron specific KCC2, is known to underlie the shift from GABA/glycine-induced depolarization to hyperpolarization in several regions of the central nervous system including the spinal cord. However, the role of neuromodulators in the regulation of KCC2 expression is not well understood. Among neuromodulators, serotonin may play a critical role during development in regulating the balance between the excitability of motoneurons and that of interneurons. Projections arising from the raphe nuclei are among the earliest axons to reach the upper lumbar segments in rats. They are the source of almost all the 5-HT in the rat lumbar spinal cord. Here, we disturbed the serotoninergic system by fluoxetine treatment and we investigated the effects on spinal motor networks. We recorded spontaneous activity in lumbar ventral roots in pups born from mother treated by fluoxetine and from control. The frequency of episodes of spontaneous activity was significantly higher in litter that received fluoxetine compared to control. The period of fictive locomotion induced in vitro was also altered in animals exposed to fluoxetine. We measured by means of western blot analysis the expression of KCC2 in the lumbar spinal cord. Pups exposed to fluoxetine during the prenatal period expressed a reduced level of KCC2 (25%), in agreement with the increased excitability of spinal cord networks. Taken together our data suggest that exposure to fluoxetine during the prenatal period may be at risk and may lead to motor dysfunctions.

P3.037

Alcohol, neuroplasticity and epigenesis in mice

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Although alcohol is well known to induce neurotoxic effects on hippocampal neurogenesis, chronic voluntary ethanol intake has been shown, surprisingly, to stimulate dentate gyrus (DG) cell proliferation in C57BL/6J mice. In order to unveil the molecular mechanisms involved in alcohol-induced neuroplasticity in the hippocampus, we studied the expression of markers involved in cell differentiation and plasticity. In addition, and because alcohol consumption has been shown to induce epigenetic modifications, we analyzed the expression of epigenetic markers such as HDACs, which are implicated in transcriptional state and neuronal maturation, and histones, whose post-translational modifications are representative of active transcription state.
To this aim, we used qRT-PCR and immunohistochemistry approaches in C57BL/6J mice that had been exposed to either ethanol in a free choice paradigm or only tap water during 3 weeks. Under these experimental conditions, voluntary alcohol consumption increased the hippocampal expression of the two neuronal markers NeuroD1 (+20.00 ± 0.05 %, mean ± SEM, n= 9) and MeCP2 (+23.76 ± 0.06 %). Furthermore, MeCP2 immunoreactivity was also increased (+37.18 ± 5.66 %) in the DG of mice which drank ethanol. Global expression of BDNF gene was also increased (+26.00 ± 0.06 %) in the hippocampus after ethanol intake. However, the precise analyze of BDNF exons showed that mRNA expression of exons II, III and VI was increased (respectively + 29.7 ± 0.08, 50.00 ± 0.13 and 33.34 ± 0.10 %) whereas that of exon VIII was decreased (-23.76 ± 0.08 %) and the others not modified. On the other hand, alcohol consumption was also found to reduce immunoreactivity of HDAC2 (-17.43 ± 4.38%) and increase that of histone H4 acetylated on lysines 5, 8, 12 and 16 (H4K5K8K12K16Ac) and histone H3 tri-methylated on lysine 4 (H3K4me3) indicating an increase of transcriptional activity.

Altogether, these results suggest that alcohol induced epigenetic modifications, which, in turn, affected the expression of proteins involved in neuroplasticity in the hippocampus. In order to explain the differential modifications in BDNF exons related to alcohol intake, chromatin immunoprecipitation applied to different BDNF gene promoters is under way.

P3.038

Wnt4 controls formation of vertebrate neuromuscular junction

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Neuromuscular junction (NMJ) formation requires a highly coordinated communication via several reciprocal signaling processes between motoneurons and muscle targets. Identification of the local and early cues in target recognition at the NMJ is still poorly documented in mammals. Wnt signaling is one of the key pathways regulating synaptic connectivity. Here, we report that Wnt4 controls NMJ innervation *in vivo*. Results from a microarray screen and quantitative RT-PCR demonstrate that Wnt4 expression is regulated during muscle cell differentiation *in vitro* and muscle development *in vivo*, being highly expressed when the first synaptic contacts are formed and subsequently downregulated as muscle differentiation occurs. Analysis of the mouse Wnt4-/- NMJ phenotype reveals profound innervation defects with motor axons overgrowing and bypassing AChR aggregates and perturbation of AChR cluster distribution resulting in 30% of AChR clusters left unapposed by nerve terminals. Lack of Wnt4 function does not likely perturb muscle differentiation, prepatterning prior to innervation as well as the localization of several synaptic proteins including acetylcholinesterase, MuSK and rapsyn. We show that Wnt4 is able to attract developing motor axons *in vitro* suggesting that Wnt4 acts as a guidance cue for motor neurons. Finally, we identify MuSK as a Wnt4 receptor: not only Wnt4 interacts with MuSK ectodomain but also mediates MuSK activation. Taken together these data demonstrate that Wnt4 is involved in NMJ recognition by nerve terminals during mammalian NMJ formation.

P3.039

Limk2c, a new isoform of LIM kinase 2, expressed during neuronal differentiation

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Background: Lim kinase proteins (LIMK1 and LIMK2) have been implicated in actin dynamics. LIMK1/LIMK2 KO mice present increased phosphorylated ADF/cofilin levels and more altered hippocampal excitatory synaptic transmission and plasticity than LIMK1 KO mice. These observations suggest a role for LIMK2 during brain development and in cognitive processes.
Methods: We analyzed the expression of LIMK2 gene by real-time RT-PCR during neurogenesis *in vitro* and in adult rat brain regions (frontal cortex, hippocampus and cerebellum). We next expressed GFP-LIMK2c protein in hippocampal neurons of rat embryos and monitored its localization. We finally compared the cell cycle repartition of HeLa cells expressing GFP or GFP-LIMK2c protein.

Results: LIMK2 isoforms present important structural variations both intra and interspecies. Databases (Ensembl; NCBI) predicted the existence of 6 isoforms of LIMK2 in rat. The longest isoform contained two LIM domains, a PDZ domain and a kinase domain (LIMK2a). The other isoforms showed deletions or variations of the LIM (LIMK2b, f) or the kinase domains (LIMK2c, d, e). We analyzed by real-time RT-PCR the expression of three isoforms of LIMK2 in rat adult brain and in culture of neural stem cells (E14) from rat embryos. We observed the expression of LIMK2 isoforms in all analyzed brain regions. The expression of LIMK2a and LIMK2c increased during the differentiation of neural stem cells *in vitro.* We next monitored the cellular localization of the fused GFP-LIMK2c protein in HeLa cells and primary hippocampal neurons. Into both cell types, GFP-LIMK2c was expressed in the cytoplasm but not in the nucleus. As LIMK2 proteins are also involved in cell cycle control, we compared the cell cycle repartition of HeLa cells expressing either GFP or GFP-LIMK2c protein. We found that GFP-LIMK2c expression did not affect the cell cycle in our cell model.
Conclusion: We showed that LIMK2 isoforms are differentially expressed during neuronal differentiation. To better understand the role of LIMK2 in the central nervous system, we are studying the consequences of an increase or a decrease of expression of each isoform on proliferation and on neurite outgrowth or dendritic spine shape in HeLa cells and primary hippocampal neurons.

P3.040
Synaptic plasticity in the brainstem: role of endocannabinoid receptors

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The nucleus tractus solitarius is the first sensory relay for visceral information in the CNS. At this level, incoming information is processed by local mechanisms before being sent to higher brain regions. We have re-evaluated the mechanisms of induction of long-term synaptic depression (LTD) at excitatory synapses. As previously found (Zhou et al; 1997), LTD induction is partially blocked by NMDAr antagonists. However, quantal analysis reveals that LTD is better explain by a reduction of glutamate release probability rather than a post-synaptic alteration of AMPAr. Accordingly, pharmacological experiments show that LTD is prevented by antagonists of CB1 receptors thought to be located on pre-synaptic terminals. Although direct activation of CB1 receptors by available agonists mimics LTD, quantal parameters are unaltered leaving open questions on the exact mechanisms at play during LTD.

P3.041
Prefrontal cortex synaptic deficits and rescue in a mouse model of partial trisomy 21

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Down syndrome (DS), the most common genetic source of mental retardation (500000 cases in Europe), is caused by the presence of an extra copy of human chromosome 21 (ie trisomy 21, HSA21). Genotype/phenotype correlations have defined candidate regions. The smallest region contains 13 genes including a pleiotropic serine-threoine kinase named Dyrk1a, which chiefly plays a role in the control of gene expression and synaptic transmission. Mouse models of partial trisomy containing Dyrk1a present a number of DS-relevant central nervous system phenotypes including changes in neuronal morphology and functional deficits such as cognitive impairments in cortical-dependent learning tasks. Here we characterized synaptic transmission and plasticity at deep layers

prefrontal cortex (PFC) excitatory synapses in a newly constructed DS mouse model (BACtgDyrk1a) with an extra copy of the complete murine Dyrk1a gene. We used *ex vivo* electrophysiological techniques: extracellular and whole-cell patch-clamp recordings on BACtgDyrk1a mice PFC slices preparation. We found that these mice presented an overexcitability of deep layer PFC pyramidal neurons. In addition, endocannabinoid dependent long-term depression (eCB-LTD) and post-synaptic NMDA-dependent long-term potentiation (NMDA-LTP) were abolished. To rescue BACtgDyrk1a mice deficits, they were given a green tea extract in their drinking water because green tea contains a natural Dyrk1a inhibitor, epigallocatechin gallate (EGCG). Following 6 weeks treatment, there was a recovery of neurons excitability and a reinstatement of the NMDA-LTP, but not the eCB-LTD. These results suggest that the DS mouse model BACtgDyrk1a presents impairments of synaptic transmission and plasticity mechanisms, which could be partially restored with green tea extracts.

P3.042

Effects of chronic intra cerebro-ventricular infusions of GABAA receptor agonist or antagonist on the reactive neural cell proliferation and the recovery of vestibular functions in the adult cat after vestibular neurectomy

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We have evidenced in the adult cat that unilateral vestibular neurectomy (UVN) induces an intense cell proliferation in the deafferented vestibular nuclei (VN). Most of these newborn cells survive, differentiate into astrocytes and GABAergic neurons and contribute to the recovery of the posturo-locomotor functions. The GABAergic system is known to modulate cell proliferation, survival, differentiation and functional integration of the newborn neurons in pre-existing neural networks. In this study we aimed to determine the effects of the GABAergic system on the regulation of cell proliferation observed in the deafferented VN after UVN. With a cellular approach, we have investigated the effects of chronic intracerebroventricular infusions of NaCl and GABA (A) receptor agonist (muscimol)/antagonist (gabazine) on the different steps of neurogenesis in the VN. Cell proliferation and differentiation into astrocytes and GABAergic neurons were revealed in the VN using bromodeoxyuridine (BrdU), glial fibrillary acidic protein (GFAP) and glutamate decarboxylase 67 (GAD67) immunoreactivity. At the behavioral level, we have determined the effects of such pharmacological drugs on the recovery of posturo-locomotor and oculomotor function using behavioral tests. Results showed a high number of BrdU-immunoreactive (Ir) cells in the deafferented VN 3 days after UVN in the UVN-NaCl, UVN-muscimol and UVN-gabazine groups. At 30 days post-UVN, BrdU-Ir cells survived exclusively in the UVN-gabazine group and lesser in the UVN-NaCl group. A stronger astroglial reaction was observed in the deafferented VN in the UVN-muscimol group compared to the UVN-NaCl and UVN-gabazine groups. The highest number of newly generated GABA-Ir neurons was located in the deafferented VN of the UVN-gabazine group compared to the UVN-NaCl group. Relative to the UVN-NaCl group, cats infused with gabazine showed a strongly accelerated behavioural recovery. In contrast, in the UVN-muscimol, this recovery was drastically delayed. This study shows that GABAergic signaling controls the regulation of the reactive neural cell proliferation in the deafferented VN and influences the vestibular function recovery in the adult cat.

P3.043

Chronic prenatal hypoxia affects respiratory rhythmogenesis at birth in rats

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Breathing is one of the earliest motor behaviours of the foetus. It depends on the activity of a brainstem respiratory rhythm generator (RRG). At birth, peripheral structures and the RRG contribute to homeostatic regulation of breathing to maintain constancy of blood gases. The foetus can experience very early insults during its embryonic development such as chronic hypoxia/hypoglycaemia, exposure to drugs, different types of medicine etc., with the potential to modify the homeostatic regulation of breathing. We investigated the effect of chronic prenatal hypoxia on respiratory rhythm during the first days of life. Effects of hypoxia (10% O_2) from the 5^{th} to the 20^{th} day of gestation were studied on offspring at birth (P0) and on postnatal day three (P3). Respiratory frequency was evaluated *in vivo* using whole body plethysmography. Central rhythmogenesis was assessed using *in vitro* brainstem spinal cord as well as slice preparations. Two brainstem spinal cord preparations were used, medullary and ponto-medullary, to evaluate after the prenatal hypoxia, the influence of the pons on the discharge of the fourth cervical ventral root (C4) used as index of the activity of the RRG. Slice recording techniques focalise on the Pre-Bötzinger Complex (Pre-BötC) containing inspiratory breathing pacemaker cells, important to the neuronal network of the RRG. *In vivo*, prenatal hypoxia increases respiratory frequency at both ages. *In vitro*, medulla and slice preparations also showed an increased burst frequency in the prenatal hypoxic group. These results indicate that the medulla RRG is modified by the prenatal hypoxia, and may be partly responsible of the basal increase of ventilation *in vivo.*

P3.044

A blueprint for the spatiotemporal origins of hippocampal interneuron diversity

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Though vastly outnumbered inhibitory interneurons critically pace and synchronize excitatory principal cell populations to coordinate cortical information processing. Precision in this control relies upon a remarkable diversity of interneurons primarily determined during embryogenesis by genetic restriction of neuronal potential at the progenitor stage. Like their neocortical counterparts, hippocampal interneurons arise from medial and caudal ganglionic eminence (MGE and CGE) precursors. However, while studies of the early specification of neocortical interneurons are rapidly advancing, similar lineage analyses of hippocampal interneurons have lagged. A "hippocampocentric" investigation is necessary as several hippocampal interneuron subtypes are poorly represented in the neocortex. Thus, we investigated the spatiotemporal origins of hippocampal interneurons using transgenic mice that specifically report MGE- and CGE-derived interneurons either constitutively or inducibly. We found that hippocampal interneurons are produced in two neurogenic waves between E9-12 and E12-16 from MGE and CGE respectively and invade the hippocampus by E14. In the mature hippocampus CGE-derived interneurons primarily localize to superficial layers in strata lacunosum moleculare and deep radiatum while MGE-derived interneurons readily populate all layers with preference for strata pyramidale and oriens. Combined molecular, anatomical, and electrophysiological interrogation of MGE-/CGE-derived interneurons revealed that MGE produces parvalbumin, somatostatin, and nitric oxide synthase expressing interneurons including fast-spiking basket, bistratified, axo-axonic, oriens-lacunosum moleculare, neurogliaform and ivy cells. In contrast CGE-derived interneurons contain cholecystokinin, calretinin, vasoactive intestinal peptide, and reelin including non-fast-spiking basket, schaffer collateral associated, mossy fiber associated, trilaminar and additional neurogliaform cells. Our findings provide a basic blueprint of the developmental origins of hippocampal interneuron diversity.

P3.045

Characterization of short term plasticity during high frequency bursts at parallel fiber - Purkinje cells synapse

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The cerebellum integrates sensorimotor information coming from the whole organism through 2 pathways, one of which involving the mossy fibers (MF) entry, granular cells (GC) via parallel fibers (PF), and finally Purkinje cells (PC), the only output of the cerebellar cortex. In vivo experiments show a high variability in the pattern of discharge of the MF, including high frequency transmission. Likewise, GC can discharge with high frequency bursts, from 300Hz (1) to 1kHz (2). Understanding the integration of the signal by PC in the whole physiological range is fundamental to apprehend the cerebellar processing of the information
We set out to characterize short term plasticity at PF - PC synapse during high frequency bursts. Small stimulations in GC layer allowed us to avoid recruitment of fibers during high frequency bursts, a process that interfere with PPR analysis. By this way, we determined the profile of short term plasticity in a wide range of stimulation frequencies (from 0.5 to 1000 Hz). Both extracellular stimulations and pair recordings showed that synaptic transmission is efficient up to 500Hz, and can be sustained in 100Hz trains. Using variance mean analysis (3), we analyzed which parameters of neural transmission (N, P, Q) could explain the properties of this synapse during high frequency transmission. These results suggest that several mechanisms (fast refilling, increase in the number of ready-to-release sites) could interact to allow this fast transmission.
To conclude, high frequency bursts ensure high reliability of neural transmission at GC - PC synapse, allowing a few GC to transmit a very reproducible and powerful stimulation to PC, even during sustained trains.
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P3.046

Characterization of developing dendritic synaptic transmission from adult born granule cells in the mouse olfactory bulb

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The adult olfactory bulb circuit is constantly rearranged through the death and replacement of interneurons. This replacement of interneurons indicates a replacement of synapses; therefore this system offers the opportunity to study the process of synaptogenesis in the context of an adult neural circuit. In the olfactory bulb, a majority of adult born interneurons mature to become granule cells that form GABAergic synaptic contacts onto mitral cell output neurons. Recent evidence indicates that GABAergic output develops late in the process of neuronal maturation, emerging weeks after the first synaptic inputs. Here, we probe the physiology of these developing output synapses using patch-clamp recordings from mitral cells and channelrhodopsin stimulation of granule cells *in-vitro*. We compare the reliability of GABAergic transmission from newly formed synapses and established synapses. Furthermore we examine the requirements for synaptic release, focusing in particular on the novel ability of granule cell dendrites to release in the absence of sodium spikes. Finally, we examine the plasticity of these outputs and its relation to the age of the adult born neuron.

P3.047

Expression of FXYD2 in normal and injured nociceptive neurons depends on Runx1 and Ret signaling

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FXYD2, which codes for the gamma-subunit of the Na,K-ATPase, is expressed in the normal mouse DRG, where it is progressively restricted exclusively to the TrkB-expressing and Ret-positive/IB4-lectin binding neuronal populations. By analyzing Ret and Runx1 mutant mice, we demonstrate that these genes are necessary for the normal expression of FXYD2 in non-peptidergic nociceptors during the maturation of the somato-sensory system. Finally, we establish that FXYD2 is down-regulated in axotomized neurons, and that in vivo application of the Ret ligands NRTN or GDNF can rescue FXYD2 expression in axotomized neurons of the Ret+/IB4-lectin binding population. These results reveal FXYD2 as a target of Ret signaling during normal maturation of the non-peptidergic nociceptive neurons and after sciatic nerve injury.

P3.049

Early, time-dependent disturbances of hippocampal synaptic transmission and plasticity after in utero immune challenge

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Maternal infection during pregnancy is a recognized risk factor for the occurrence of a broad spectrum of psychiatric and neurologic disorders, including schizophrenia, autism and cerebral palsy. Prenatal exposure of rats to lipopolysacharride (LPS) leads to impaired learning and psychotic-like behavior in mature offspring, together with an enduring modification of glutamatergic excitatory synaptic transmission. The question which arises is whether any alterations of excitatory transmission and plasticity occurred at early developmental stages after in utero LPS-exposure. In order to test this hypothesis, electrophysiological experiments were carried out on hippocampal slices from prenatally LPS-exposed offspring from 4 to 190 day-old to study long-term depression (LTD) triggered by delivering 900 shocks either single or pulsed (50ms interval) at 1Hz and the developmental profiles of NMDA receptor contribution to synaptic transmission. Maternal stress leads to an accelerated developmental decline of LTD and LTD transiently converted into a slow-onset LTP between 16 and 25 day-old. This LTP depends on Group I mGlu receptor and PKA activation and is independent of NMDA receptors. In prenatally LPS-exposed animals a rapid developmental decline of the NMDA receptor contribution to synaptic transmission in both CA1 and CA3 hippocampal areas is observed. It is associated with a reduced expression of GluN1 in these areas, without any detectable alteration in the developmental switch of NMDA receptor GluN2 subunits. The hypofunctionning of the NMDA receptor in the hippocampus associated with aberrant forms of synaptic plasticity can be detected at early developmental stages after prenatal LPS stress. This may result in later-occurring brain dysfunctions.

P3.050

Role of PAK3, a kinase involved in X-linked mental retardation, in cortical interneuron migration and differentiation

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Pak3 has been shown to be mutated in patients with non-syndromic forms of X-linked mental retardation. Members of the Pak (p21-activated kinase) family are effectors of Rac and Cdc42 and can thus play a role in several aspects of the CNS development and maturation (Bokoch et al, 2003). A change in Pak3 expression level has been observed in future cortical interneurons during embryonic development (Cobos et al, 2007). Future interneurons proliferate in the Median Ganglionic Eminence (MGE) and then migrate tangentially to the cortex (from stages E12.5 to E15.5 in mouse embryo). Pak3 is repressed under the control of Dlx1/2. Thus, MGE cells start to express Pak3 only after entering the embryonic cortex and downregulating Dlx1/2. Pak3 overexpression in these neurons leads, in vitro, to excessive neurite outgrowth and inhibits cell migration (Cobos et al, 2007).
Our work is aimed at identifying the molecular and cellular mecanisms through which Pak3 regulates the migration and differentiation of the future cortical interneurons.
We are combining in vitro and in vivo approaches to examine the dynamic properties, trajectories and final positioning of Pak3 KO and Pak3 mutant interneurons. The dynamic properties of Pak3 KO MGE cells like migration speed and neuritic outgrowth have been analyzed in co-cultures using time lapse videomicroscopy. Using *in utero* electroporation, we are studying *in vivo* the trajectories and cortical distribution of MGE cells transfected with plasmids encoding eGFP fused with wild-type or mutant Pak3. Finally, crosses between Pak3 KO mice and transgenic mice expressing the GFP in MGE cells are done to compare the distribution of GFP positive MGE cells in wild-type and Pak3 KO mice at adult stage. Our first results show that Pak3 KO does not strongly alter the dynamic properties of cortical interneurons. In contrast, we show local alterations in the cortical distribution of Pak3 KO interneurons at adult stage.
Those studies shall bring new data allowing a better understanding of the role of Pak3 in the migration and differentiation of future cortical interneurons. They may give new insights on how signal transduction through Rho GTPases and PAK3 may be critical for human cognitive function.

P3.051

The INMED post-genomic platform (PPG): an innovative platform for the creation and study of animal models with cortex malformations

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Among human neurological diseases, cortical malformations (such as lissencephaly, polymicrogyria, periventricular heterotopias, microcephaly …) represent a broad spectrum of developmental disorders resulting from genetic mutations and/or environmental perturbations that can affect cellular proliferation, migration and/or differentiation. These cortical malformations are often associated with severe epilepsies and cognitive defects, and as such, are a major public health concern.Much of our knowledge about the cellular and molecular functions of the genes associated with cortical malformations comes from the study of rodent models carrying naturally occurring or engineered mutations. However, in the last few years, *in utero* electroporation (IUE) of rodent embryos (mouse and rat) has become a more efficient and very rapid method to perform gain and loss of function

studies of embryonic cortex development. It should also be noted that the *in utero* technology is more cost effective as it does not require the expensive maintenance of mouse or rat transgenic lines. The main goal of our platform is to offer to the scientific community, a combination of technical skills and equipments to allow the creation and study of rodent models mimicking human cortex malformation pathologies. Our strategy is based on the transduction or transfection of neuronal progenitors, at specific embryonic stages, by *in utero* intra-cerebroventricular injection of viral particles (lentiviral or AAV particles) or *in utero* intra-cerebroventricular injection and electroporation of appropriate plasmid vectors in order to either inactivate (by RNA interference) or express mutated or wild-type forms of specific proteins, respectively. Our facility proposes:
1) the conception and *in vitro* validation of your constructs (shRNA expressing plasmids for example) in cell lines or primary neuron cultures;
2) the *in utero* intra-cerebellar injection and electroporation of the rat or mouse embryos;
3) the dissection of the embryos and fixation of the brains;
4) the vibratome sectioning of the brains;
5) councelling and supervising for morphological and electrophysiological analyses.
Several examples of studies performed in our facility will be exposed.

P3.053

Surface dynamics of the astroglial glutamate transporter GLT-1

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Glutamate is the major excitatory neurotransmitter in the brain. Its concentration in the synaptic area is highly and locally regulated in order to maintain point-to-point transmission and prevent excitatoxicity associated with chronic activation of glutamate receptors. As there is no endogenous extracellular enzyme to degrade glutamate, glutamate clearance is ensured by transporters located at the surface of astrocytes. Among these, GLT-1 ensures almost 90% of glutamate uptake in synapses. However, whether the surface distribution of this transporter is dynamically regulated at the surface of astrocytes is simply unknown. To unravel the surface trafficking of GLT-1 in these glial cells we used a combination of high-resolution imaging, such as single particle (Quantum Dot) tracking, and molecular biology approaches. Schematically, tagged-GLT-1 were expressed in cultured astrocytes and tracked at the astrocytic surface using single Quantum Dot coupled to antibodies against GLT-1extracellular tag. We report that surface GLT-1 are highly dynamic, exploring large area of the astrocyte plasma membrane. The diffusion pattern indicates that GLT-1 do not diffuse freely, suggesting the existence of regulatory processes at the membrane level. Consistently, GLT-1 surface diffusion is different between compartments of the astrocyte. Together, this work unravels that GLT-1 are highly dynamic at the surface of astrocytes and further suggests that the regulation of extracellular glutamate and synaptic adaptations is linked to GLT-1 surface trafficking.

P3.054

Evaluation of the effect of tobacco smoking on the chromosome breaks of hematopoietic stem cells in mice and differentiated human cells of the immune system. Study of the antigenotoxic possible effect of Propolis

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The addiction to smoking remains one of the biggest threats for the human health today at the world level. According to the World Health Organization, there are 1.1 billion smokers in the world; five

million of them die every year because of the consumption of the tobacco. In fact, the tobacco smoking is the main cause of death on the world. Tobacco smoking is highly toxic for the human body as much in the smokers as in the non-smokers. It acts in various regions at the level of the body by provoking several disorders such as addiction to cigarette, grave respiratory and cardiovascular diseases even cancer. The aim of the present study was to evaluate the effect of tobacco on the chromosome breaks of hematopoietic stem cells in mice. In human, we calculated differentiated cells of the immune system (PBMC) in smokers and no smokers subjects. We also estimated the effect of Propolis on the genotoxicity induced by cigarette smoking by studying its preventive and curative effect. The evaluation of these effects was revealing by using the micronuclei (MN) test. We calculated the percentages of cells having shown 1, 2 or more than 2 MN. In mice, results showed that the number of (MN) is significantly higher in treated mice compared to controls. This result suggests a genotoxic effect of tobacco smoking. Besides, this effect is decreased by the administration of the propolis which showed a curative but not a preventive effect. In human, our result showed that the number of micronuclei of PBMC in smoker subjects is more important than those of the no-smokers. These results bring to light the clastogenic and maybe tumoral effects of tobacco smoking directly on hematopoietic stem cells and Human PBMC.

P3.055

Cerebrospinal fluid contacting neurons in the brainstem detect pH variation through polycystic kidney disease 2-like 1 protein (PKD2L1 or TRPP2)

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Cerebrospinal fluid (CSF) contacting neurons (CSF-cNs) are part of the circumventricular organs and are present in the walls of ventricles, along the central canal and in the parenchyma. Although the presence of CSF-cNs in several brain regions has been known for decades, their properties and functional role remain unclear mainly because of the difficulty to visualize and record them in brain slices. However, it has been shown that spinal CSF-cNs express the PKD2L1 protein, a member of the transient receptor potential (TRP) channel family.
Using a transgenic mice model where EGFP is selectively expressed in $PKD2L1^+$ cells, we demonstrate that $PKD2L1^+$ cells are present in the brainstem and hypothalamus. Within the brainstem, $PKD2L1^+$ CSF-cNs can be found around the central canal in close contact with vimentin positive ependymal cells.
At the functional level, we recorded in acute brain stem slices identified EGFP:$PKD2L1^+$ cells using the patch-clamp technique in whole-cell configuration. We show that these cells are neurons expressing functional ionotropic receptors for GABA, glycine, and acetylcholine but not for ATP or glutamate. Further, along with a spontaneous synaptic activity of GABAergic and glycinergic nature, CSF-cNs exhibit a unitary ionic current activity. This channel activity, recorded in the whole-cell configuration, is of large conductance (~140 pS), of cationic origin and is blocked by amiloride. Finally, the channel open probability is strongly reduced by acidification (pH 3) and increased by alkalinization (pH 9) of the extracellular medium. These results strongly suggest that the recorded unitary current is carried by PKD2L1 since all the identified properties remind those of PKD2L1 channels.
The results presented are the first characterization of CSF-cNs in the brainstem and show that they express PKD2L1, a channel sensitive to protons. They further suggest that CSF-cNs could act as sensor for the CSF pH and would integrate both sensory and synaptic information.
This work has been suported by : CNRS-INSB PEPS 2010 (NEURACIL, NW), Région PACA & CG13

P3.056

Properties of TTX-resistant sodium currents and firing behavior of myenteric neurons in wild-type and Nav1.9-null mutant mice

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We have shown previously that the TTX-resistant (TTX-R) Nav1.9 sodium channel is expressed in rat and guinea-pig myenteric neurons (Rugiero et al, 2003; Coste et al, 2004; Padilla et al, 2007; Maingret et al, 2008). To address the role of the Nav1.9 channel isoform in myenteric neurons, we characterized for the first time the TTX-R Na^+ currents in WT and Nav1.9-null mutant mice.
Whole-cell patch-clamp recording from *in situ* myenteric neurons revealed the presence of two TTX-R voltage-gated Na^+ currents: 1/ a rapidly-activating/inactivating current (fast INa), with a relatively high threshold of activation (-40 mV) and 2/ a slow INa with a relatively low threshold of activation (-60 mV) and a persistent component. These two TTX-R components were differentially distributed as the fast INa was recorded in nearly all myenteric neurons (95%) whereas the slow TTX-R INa was restricted to a subpopulation of myenteric neurons. Immunolabelling with anti-Nav1.9 antibody indicated that only large myenteric neurons, identified as intrinsic sensory neurons, expressed Nav1.9 whereas anti-Nav1.5 antibody stained most of the population. The slow TTX-R INa, but not the fast INa, was absent in Nav1.9-null myenteric neurons.
Moreover, current-clamp experiments in these neurons showed that the presence of Nav1.9 was associated with subthreshold depolarizations and an increase in the action potential half-width. Experimental findings were supported by computer modelling showing that action potentials can drive Nav1.9 activation.
In conclusion, our data show that Nav1.9 is the molecular correlate of the slow INa in mouse myenteric neurons and suggest that Nav1.5 sustains the fast TTX-R INa.
Rugiero F, Mistry M, Sage D, Black JA, Waxman SG, Crest M, Clerc N, Delmas P, Gola M. *J Neurosci.* 2003; 23: 2715-25.
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Maingret F, Coste B, Padilla F, Clerc N, Crest M, Korogod S, Delmas P. *J Gen Physiol*. 2008; 131: 211-25.

P3.057

Pharmacological inhibition of PKR in APPswePS1dE9 mice transiently prevents inflammation but increases amyloid load at advanced stages of the disease

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The double-stranded RNA-dependent protein kinase (PKR) is switched on by a wide range of stimuli, including the amyloid peptide. Then, PKR transmits signals to the translational machinery, apoptosis and inflammatory signaling pathways by interacting with some adapters. In virus-infected cells, PKR engages the nucleus factor κB (NF-κB) pathway. In many models of Alzheimer's disease (AD) and patients with AD, PKR was activated. Furthermore, there is strong evidence involving the inflammatory process in the AD brain. However, the PKR involvement in inflammatory responses in AD is not elucidated. Based on our previous in vitro results, the aim of this study was to evaluate the effects of a pharmacological inhibition of PKR in inflammation in APPswePS1dE9 transgenic mice. Our results showed that PKR inhibition prevented the NF-κB activation and production of tumor necrosis factor alpha (TNFα) and interleukin (IL)-1β at 12 months of age without improvement of amyloid load and memory deficits. Surprisingly, PKR inhibition failed to prevent IL-1β-mediated inflammation and

induced a great increase in β-amyloid peptide (Aβ42) levels at 18 months of age. In this model, our findings highlight the lack of relationship between inflammation and amyloid load. Moreover, the age-dependent inflammatory response must be carefully taken into account in the establishment of an anti-inflammatory therapy in AD.

P3.058

Astrocyte, an endogenous regulator of basal synaptic transmission

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Basal synaptic transmission is fundamental for information processing in the brain. It involves the release of neurotransmitters at individual synapses evoked by single action potential. For a long time, it has been considered that the transmission of information in the brain was only a “neuronal story”. Recently, a new actor has been added in this story to form “un ménage à trois”. Indeed, astrocytes, the most abundant type of glial cells in the central nervous system, detect and in turn modulate synaptic transmission during intense and sustained neuronal network activity. However, the ability of these glial cells to regulate basal synaptic transmission remains unclear and controversial. Here we show that astrocytes in CA1 region of hippocampus detect synaptic activity induced by single synaptic stimulation at functional compartments along the astrocytic process. This detection is mediated by metabotropic glutamate receptors subtype 5. Moreover, we uncover that following their activation by basal synaptic transmission, astrocytes increase the efficacy of transmission in CA1 pyramidal cells through activation of presynaptic adenosine A_{2A} receptors. This work provides a new perspective of fundamental brain function since astrocytes are now intimately involved with neurons in the regulation of elementary synaptic communication in the brain.

P3.059

CK2 phosporylation regulates the interaction between SCHIP1-1a and ankyrin G

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The axonal initial segment (AIS) is a sub-domain of neurons that plays a central role in the action potential genesis and the neuronal polarity. This compartment presents a complex molecular organisation that includes sodium and potassium channels, soluble cytosolic proteins such as growth and transcriptional factors and cytosqueletal associated proteins. Among these proteins ankyrin G (Ank G) orchestrates the formation and maintenance of the AIS. In particular, AnkG is responsible for the accumulation of sodium channels to the AIS. The association between the two proteins is regulated by channel phosphorylation by CK2, a kinase that also segregates at the AIS (Brechet et al., 2008).
We showed that IQ-CJ SCHIP1, one isoform of the SCHIP1 family, is enriched in the AIS. This protein that binds calmodulin in the absence of calcium, interacts with AnkG, but its role is presently unknown (Martin et al. 2008). Here, our study focuses on SCHIP1-1a, one other member of the SCHIP1 family. An antibody directed against SCHIP1-1a stained the AIS of hippocampal neurons in culture indicating that SCHIP1-1a segregates in this compartment. Using pull-down assays, we showed that AnkG-GFP

expressed in COS-1 cells associated with GST-SCHIP1-1a phosphorylated by CK2 but not with non-phosphorylated GST-SCHIP1-1a. The effect of CK2 phosphorylation on the interaction between SCHIP1-1a and Ank G was further analyzed by Surface Plasmon Resonance (SPR). Phosphorylated SCHIP1-1a interacted with Ank G with a Kd of nanomolar range while no binding was observed between the two partners without CK2 treatment. Both pull-down and SPR approaches using SCHIP1-1a mutants truncated in the C-terminus tail revealed that a segment included several potential CK2 phosphorylation sites located down-stream to the dimerization domain, is directly involved in the interaction with AnkG. Altogether these observations strongly suggest that SCHIP1-1a segregates at the AIS through its interaction with the scaffolding protein AnkG and that this interaction is strongly regulated by SCHIP1-1a phosphorylation by CK2.
Bréchet A, et al. J Cell Biol. 2008, 183(6):1101-14
Martin PM, et al. J Neurosci. 2008 28(24):6111-7

P3.060

Cocaine-induced Extracellular-signal Regulated Kinase (ERK) activation is a key event to drive cell-type synaptic plasticity in the nucleus accumbens

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Addictive drugs, such as cocaine, cause a surge of extracellular DA levels in the ventral part of the striatum. This may evoke synaptic plasticity that ultimately hijacks the reward circuitry leading to long-lasting behavior changes and addiction. Within minutes cocaine leads to an activation of the ERK pathway in the dopamine D1 receptor (D1R)-expressing neurons of the striatum that has been linked to behaviors modeling core components of addiction (Girault et al., 2007).
Here we test the hypothesis that the cocaine-induced activation of the ERK pathway contributes to the drug-evoked synaptic plasticity of excitatory transmission onto medium spiny neurons (MSNs) in the nucleus accumbens (NAc). To this end, we performed whole-cell patch clamp recordings in MSNs in acute brain slices while stimulating excitatory afferents at the prelimbic cortex - NAc border.
We demonstrate that HFS-induced N-methyl-d-aspartic acid (NMDA)-dependent long term potententition (LTP) at glutamatergic synapses requires ERK activity.
In *slices* from *cocaine*-treated mice, HFS failed to induce LTP in 30 to 40 % of the recorded neurons, while under control conditions less than 10 % of failures were observed. Using transgenic mouse lines with enhanced green fluorescent protein (EGFP) expression under the control of the D1R (Drd1a-EGFP) and or D2R promoter (Drd2-EGFP) we showed that this effect of cocaine is restricted to the direct pathway neurons. Administration of the ERK pathway inhibitor (SL327) prior to cocaine restored the ability of HFS to induce LTP in D1R-neurons.
Taking together these results suggested that cocaine-induced ERK activation drives synaptic plasticity specifically in D1R-expressing MSNs of the NAc.

P3.061

A synthetic amino acid substitution of Tyr10 in Abeta peptide sequence acts as a dominant negative in amyloidogenesis

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Alzheimer´s disease (AD) represents the most common form of dementia in the elderly. A hallmark of AD is the accumulation of amyloid in the brain, which is composed mainly by the amyloid beta-peptide (Ab) in the form of oligomers and fibrils. Ab1-42 shows enhanced misfolding and aggregation propensity, resulting in increased neurotoxic effects in AD pathogenesis. The molecular mechanisms underlying the assembly of Ab species is not yet fully determined and it is unknown how soluble Ab begins to assemble and deposit into the brain triggering neurotoxicity. Several evidences indicate that specific amino acids in the Ab sequence play a key role in Ab aggregation and neurotoxicity. However, it is not yet fully elucidated how soluble Ab starts to assemble and deposit in the brain in a age-dependent manner and how it initiates its neurotoxic effect. Rodent Ab shows impaired aggregation. Rodent Ab presents 3 amino acid substitutions: Arg3Gly, Tyr10Phe, and His13Arg. Molecular modelling studies have suggested that Tyr10 is important for the interaction of Ab with neutral glycosphingolipids such as GM1. GM1 acts as seed inducing Ab aggregation and GM1 level increases with age. GM1 is significantly increased in amyloid-positive synaptosomes extracted from AD brains. Tyr10 is also required for Ab-induced oxidative stress and neurotoxicity. We explored the function of Tyr10 in Ab aggregation *in vitro* and *in cellulo.* We substituted Tyr10 with the synthetic amino acid para-amino-Phenylalanine. This mutant (mut) Ab1-42 shows sensibly increased velocity of binding to GM1 *in vitro* compared to wild type (wt) Ab, and has impaired aggregation capability *in vitro* and *in cellulo*. Mut Ab partially inhibits the binding of wt Ab to the plasma membrane of differentiated SHSY5Y neuroblastoma cells and affects the aggregation of wt Ab *in vitro* and *in cellulo*. Mut Ab fails to induce oxidative stress and inhibits wt Ab-induced neurotoxicity. The present study shed new light in the understanding of Ab-membrane interactions in Ab-induced neurotoxicity. It demonstrates the relevance of Tyr 10 in
(i) Ab-membrane interaction,
(ii) Ab aggregation,
(iii) Ab-induced oxidative stress.
Our results open the way for the design of peptides aimed to inhibit Ab aggregation and neurotoxicity.

P3.062

Cortical connexins mRNA levels following sleep deprivation in young and old mice

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In addition to synapses, gap-junctions (GJ) play an active role in the neuronal as well as in glial cell coupling. GJs are formed by apposition of intercellular channels composed by hexameric assemblies of intra-membrane proteins named connexins (Cx). In the brain, different subtypes of Cxs are present with a cellular specific distribution. The Cx43 is mainly expressed by astrocytes but also by developing neurons, while Cx30 is only present in astrocytes. The Cx36 are almost exclusively present in neurons and Cx47 and Cx32 in oligodendrocytes. Moreover, it has been recently described that in astrocytes Cxs can also form hemichannels, possibly involved in gliotransmission. Interestingly, gliotransmission has been recently involved in sleep regulation (Halassa et al. 2009), suggesting the potential involvement of GJs in sleep mechanisms. To test this hypothesis, we assessed the effects of a “gentle” sleep deprivation (GSD) on the levels of mRNA encoding the Cx30, Cx32, Cx36, Cx43, Cx47 and the in the cortex of young (2 months) and old (15-16 months) C57Bl6/j mice by quantitative RT-PCR. To avoid any stress-confounding effects, we also determined these levels in mice that have been restrained for 1 hour. The GSD was realized during 6 hours from the beginning of the light period (zeitgeber time 0, ZT0). Sleep-deprived mice were sacrificed at the end of the GSD (ZT6). Undisturbed mice, corresponding to the control group were sacrificed at ZT6 as well as stressed mice. After normalization relative to actin mRNA levels, we observed that Cx30 mRNA was increased after GSD (+73±3% relative to control group, n=6, $p< 0.0001$, t-test) while no significant changes were observed for the other tested genes. Stress control did not induce any significant change (+8±14% relative to control group, n=6, n.s., for Cx30) suggesting a specific sleep deprivation effect. Interestingly, basal as well GSD-induced Cx30 expression is maintained in old mice while basal expression of Cx32 is decreased in old mice (-18±6%, $p=0.022$). Since Cx30 is specifically expressed by astrocytes, these

results suggest an impact of a prolonged wakefulness on the functionality of astroglial networks and/or hemichannels.
(Halassa et al., Neuron 61:213, 2009)
Supported by FNRS grant 3100AO-108336/1 to PJM.

P3.063

5-HT2A receptor functional selectivity ruled by mGlu2 receptor activation states

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New concepts emerged in the field of G-protein coupled receptor (GPCR): GPCRs can induce different signaling (and physiological) pathways depending on the nature of the ligand they are activated by (ligand trafficking); and the signaling properties of a given receptor can be altered by its association with other GPCRs.
The serotoninergic 5-HT_{2A} receptor is involved in mental disorders (schizophrenia, anxiety, and depression), and is the target of antipsychotic and antidepressant compounds. This GPCR mediates the psychotropic effects of hallucinogens, like LSD and DOI, while other 5-HT2A agonists, like lisuride or ergotamine, lack psychoactive effects. Hallucinogen agonists trigger the expression of specific transcription factors via Gi/o proteins, whereas non hallucinogenic compounds only activate the Gq/PLC pathway. The glutamatergic mGlu2 GPCR would form a complex with 5-HT2A in frontal cortex pyramidal neurons, might explain why mGlu2 agonists decrease DOI-induced hallucinogenic behaviors in rodents. Co-expression of both receptors enhanced the 5-HT2A-driven DOI-induced Gi activation, while application of a mGlu2 agonist suppressed this effect. These data suggested that mGlu2 would rather control 5-HT2A-mediated response, than inhibit glutamate release from cortical terminals.
However, it is not clear whether the endogenous ligand (5-HT) induces coupling to Gi/o proteins, that would explain why it generates hallucinogen symptoms in some conditions, whether both Gi and Go pathways are involved in hallucinogen action and are regulated by mGlu2 the same way, and whether mGlu2 is necessary for hallucinogen action and how it regulates their Gi/o coupling.
We then investigated the 5-HT_{2A} ability to activate both Gi and Go pathways in living cells and the modulation induced by mGlu2. We showed that 5-HT, like DOI, was able to activate Gi/o heterotrimeric proteins even in absence of mGlu2. Moreover, we showed a differential action of mGlu2 on 5-HT2A coupling: both 5-HT and DOI-induced Go activation was abrogated by mGlu2 activation, whereas only the DOI-induced Gi activation was blocked. Thus, depending on its activation state, mGlu2 induced ligand trafficking (5-HT vs DOI) and functional selectivity towards the 5-HT2A ability to activate the Gi and Go pathways.

P3.064

Proteomic analysis of the partners of a presynaptic metabotropic Glutamatergic Receptor (mGluR4) in rat cerebellum

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The eight pre- or/and post-synaptic glutamatergic G protein-coupled receptors, so called mGluRs, modulate rapid excitatory transmission produced by ionotropic receptors. They are classified in 3

families according to their percentage of sequence identity and their pharmacological properties. The presynaptic mGluR4 belongs to the group III (with mGluR6, 7 and 8), it is mainly localized pre-synaptically. In the rodent cerebellar cortex, depression of excitatory transmission at Parallel Fiber-Purkinje Cell (PF-PC) synapse by group III mGluRs is provided exclusively by mGluR4, via an inhibition of presynaptic calcium flux that controls glutamate release (Abitbo*l et al,* 2008). In order to improve the understanding of the mechanisms of action of this receptor, we decided to identify the mGluR4 interacting proteins. In this purpose, anti-mGluR4 antibodies directed against C-terminal part were cross-linked to Protein A Sepharose magnetic beads and used for immunoprecipitation by incubation of total lysate of rats cerebellum. A control was performed with non specific antibodies. Numerous proteins specifically co-immunoprecipitated with anti-mGluR4 antibodies : 185 putative partners were identified by mass spectrometry and classified according to their cellular function. Among them, we obtained Munc18-1, which has already been reported to interact with mGluR4 in GST pull-down experiments (Nakajima *et al*, 2009). We are currently validating our results by affinity chromatography using peptides of intracellular loop and C-terminal tail of mGluR4. In addition this approach will allow us to determine the interaction area between the receptor and its protein partners.
Abitbol K., Acher F., Daniel H. (2008) Depression of excitatory transmission at PF-PC synapse by group III metabotropic glutamate receptors is provided exclusively by mGluR4 in the rodent cerebellar cortex. *JNC 105:2069-2079*
Nakajima Y., Mochiba S., Okawa K., Nakanishi S. (2009) Ca^{2+} -dependent release of Munc18-1 from presynaptic mGluRs in short-term facilitation. *PNAS 43:18385-18389*

P3.065

Western blot detection of brain phosphoproteins after performing Laser Microdissection and Pressure Catapulting (LMPC)

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The Central Nervous System (CNS) is constituted of complex and specific anatomical regions that cluster together and interact with each other with the final objective of receiving and delivering information. This information is characterized by selective biochemical changes that happen within specific brain sub-regions. Most of these changes involve a dynamic balance between kinase and phosphatase activities. Thus, the fine-tuning of this kinase/phosphatase balance is critical for neuronal adaptation, transition to long-term responses and higher brain functions including specific behaviors. Data emerging from several biological systems may suggest that disruption of this dynamic cell signaling balance within specific brain sub-regions leads to behavioral impairments. Therefore, accurate and powerful techniques are required to study global changes in protein expression levels and activities in specific groups of cells. Laser-based systems for tissue microdissection represent a method of choice enabling more accurate proteomic profiling. The goal of this study was to develop a methodological approach using Laser Microdissection and Pressure Catapulting (LMPC) technology combined with an immunoblotting technique in order to specifically detect the expression of phosphoproteins in particular small brain areas.

P3.066

L-type voltage dependent calcium channels in molecular layer interneurons

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Fast release of neurotransmitter is elicited by depolarization of the presynaptic compartment and Ca2+ entry into the terminal through voltage dependent Ca2+ channels (VDCCs). In cerebellar molecular

interneurons (MLIs), only P/Q and N -types high threshold VDCCs have been shown to play a pivotal role in the release of GABA (Forti et al., 2000; Stephens et al., 2001). Although pharmacologically identified at the membrane of these neurons, L-types channels have not been extensively studied. They are currently thought to mediate Ca2+-induced Ca2+ release (CICR) processes at MLIs somata through a conformational association with ryanodine receptors (Chavas et al., 2004) and involvement in neurotransmitter release has been ruled out either by electrophysiology and Ca2+ imaging (Forti et al., 2000; Stephens et al., 2001).
Our electrophysiological data confirm that P/Q (Cav2.1) , N (Cav2.2) and L-type (Cav1.2/3) VDCCs are functionally expressed in MLIs. Moreover, mRNA encoding Cav2.1, Cav2.2, Cav1.2 and Cav1.3 have been detected in MLI by single-cell RT-PCR. The use of various concentrations of Isradipine suggests the presence of both L-type VDCCs Cav1.2 and Cav1.3 which are also positively modulated in the presence of BayK8644. Interestingly, BayK8644 (1-10 µM) dramatically increases mIPSCs frequency indicating that L-type VDCCs could be present in presynaptic sites. Action potential-evoked Ca2+ transients recorded in axonal varicosities exhibit a larger amplitude and slower decay kinetics in the presence of BayK8644. Moreover, our immunochemistry experiments show that Cav1.2 and Cav1.3 colocalise with synaptophysin and V-GAT in MLIs.
Together, our data indicate that L-type VDCCs are located in the presynaptic compartment of MLIs and that they are involved in GABA release process.

P3.067

$GABA_B$ receptor: a complex allosteric machine to tune up synaptic transmission

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The G-protein coupled receptor activated by the neurotransmitter GABA is made up of two distinct subunits, $GABA_{B1}$ that binds agonists, and $GABA_{B2}$ that activates G-proteins. Each subunit comprises an extracellular domain and a transmembrane heptahelical domain. How the two subunits communicate in the heteromeric receptor remains unknown and understand these processes will enable to develop better drugs. Here, we used a combinaison of biophysical, biochemical and bioinformatics approaches to investigate the molecular functioning of the $GABA_B$ receptor. First, by using a glycan wedge scanning approach, we demonstrate that a relative movement the two extracellular domains is required for receptor activation. Second, we set up a novel multi-labeling approach compatible with time-resolved FRET based on the use of ACP- and SNAP-tag technologies to verify the heteromeric assembly of $GABA_B$ receptors made of wild-type and mutated subunits. We show a direct allosteric coupling between the two heptahelical domains is a key step for receptor activation. Our data are challenging the actual view of the activation process of the $GABA_B$ receptor.

P3.068

Mechanism of action of the Smo antagonist MRT-83 at the primary cilium and in vivo

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Trafficking of Sonic Hedgehog (Shh) signaling components at the primary cilium is proposed to regulate Shh pathway activities. In the absence of its ligand, Patched (Ptc) is localized at the primary cilium. Upon Shh binding, Ptc traffics out of the cilium and Smoothened (Smo) translocates into it (Traiffort et al, 2010). Small molecules acting at Smo have been shown to modify Smo trafficking at the primary cilium, and the mechanism of antagonist action to block Smo translocation at the primary cilium might be relevant in brain cancer therapies (Rohatgi et al., 2009).
To examine the mechanism of action of MRT-83, a novel potent Smo antagonist (Roudaut et al., in press), we investigated its ability to modulate the trafficking of endogenous mouse or human Smo to the primary cilia of mesenchymal pluripotent C3H10T1/2 or of NT2 cells. After treatment with the Smo agonist SAG, Smo accumulation was induced at the primary cilium as shown by the co-localization of acetylated tubulin and Smo positive immunolabellings. This effect was blocked when cells were incubated in the presence of MRT-83 whereas it did not promote Smo-positive signals at the primary cilium alone. Cyclopamine, a reference Smo antagonist, was as effective as SAG to induce accumulation of Smo signals at the primary cilium. Therefore, it might be anticipated that MRT-83 interacts with Smo in a manner different from that of cyclopamine, suggesting that while the binding sites are overlapping they are not identical.
Stereotaxic injection into the lateral ventricle of adult mice of MRT-83 but not of a structurally-related compound inactive at Smo, abolished upregulation of Ptc transcription induced by Shh in the neighboring subventricular zone, one of the main neurogenic area of the adult brain. These data demonstrate that MRT-83 efficiently antagonizes Shh signaling *in vivo*. All together, these studies provide evidence that MRT-83 acts at Smo. Thus, this novel Smo antagonist and structurally-related molecules (Manetti et al., 2010) should be useful to clarify the molecular and biochemical mechanisms underlying the resistance to Smo inhibitors, particularly at the level of the primary cilia in cancer cells and may help develop new therapies against Shh pathway-related brain diseases.

P3.069

Human Merkel cell in culture express thermo- and mechano-sensitive ion channels

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Skin is the body organ of touch and thermo-sensation. Slowly adapting type I receptors in vertebrate are identified with Merkel cells connected to Ab nerve endings. It has been suggested that Merkel cells are mechano- and thermo-transducers that signal to adjacent nerve. Nevertheless, the molecular identity of the Merkel cell ion channels and the functioning of these complexes remain poorly understood. Our objective was to develop a method for culturing Human Merkel cells to characterize ion channels sensitive to mechanical and thermal stimuli.
We used the expression of surface protein to sort on affinity column Merkel cells from other cells of the epidermis. Cell extract contained 80% of Merkel cells immuno-positive for cytokeratine 20, 18 and 8. Using patch-clamp and calcium imaging, we determined the response of Merkel cells to mechanical and thermal stimulation. Merkel cell swelling, hot and cold temperature provoked calcium entry. TRPM8 and TRPV3 mRNAs were identified by PCR and channel activities were induced by the agonist menthol, eucalyptol and 2-APB. These results demonstrate that human Merkel cells express thermo-TRP channels that may act as transducer of skin sensation.

P3.070

Quinelorane-induced transient activation of Akt/GSK-3β signaling and mTOR pathway in the rat striatum and nucleus accumbens: direct involvement of D_2 and D_3 receptors

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Increasing evidence suggests a role for Akt and glycogen synthase kinase-3β (GSK-3β) in the etiology of schizophrenia. Dopaminergic transmission regulates the phosphorylation of Akt and GSK-3β[1], and recent work suggests a direct influence of D_2 and D_3 receptors in the recruitment of Akt/GSK-3β signaling both *in vivo*[2], in primary cultures of striatal neurons[3] and in CHO cells[4]. The present study evaluated the effects of short-term administration of quinelorane, a D_2/D_3 receptor agonist, on Akt/GSK-3β and mammalian target of rapamycin (mTOR) pathways in rat dorsal striatum and nucleus accumbens (NAcc). Adult rats received a single, acute, subcutaneous injection of quinelorane (0.16 mg/kg) and 5, 10 and 20 min afterwards, the brain was rapidly dissected for analysis by western blot. Quinelorane induced a significant increase of Akt and GSK-3β phosphorylation in both dorsal striatum and NAcc at 10 min but not at 5 or 20 min. Pre-treatment with raclopride (0.16 mg/kg, sc), a mixed D_2/D_3 receptor antagonist, 30 min before quinelorane abolished its action, suggesting a role for of D_2 and/or D_3 receptors in Akt/GSK-3β phosphorylation. In both structures, quinelorane also increased levels of phospho-p70S6K and phospho-4E-BP1, two downstream targets of mTOR complex 1. Recruitment of this mTOR pathway was likewise mediated by D_2 and/or D_3 receptors since it was blocked by raclopride. Moreover, selective antagonists at D_2 and D_3 receptors, L741,626 and S33084 respectively, both attenuated the actions of quinelorane. In mice genetically lacking D_3 receptors, the effect of quinelorane upon phosphorylation of Akt, GSK-3β, p70S6K and 4E-BP1 in striatum and NAcc was reduced but not abolished, in line with a role for both D_2 and D_3 receptors. These results indicate that D_2 and D_3 receptors recruit both Akt/GSK-3β and mTOR signaling pathways in the dorsal striatum and NAcc of rats, a regulation that may participate in the therapeutic activity of antipsychotics.

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P3.071

PROKR2 missense mutations in Kallmann syndrome: a relationship between genetic and signaling pathway

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Kallmann syndrome (KS) combines anosmia, related to defective olfactory bulb morphogenesis, and hypogonadism due to gonadotropin-releasing hormone deficiency. In a large series of KS patients, mutations have been identified in the gene encoding the G protein-coupled receptor prokineticin receptor-2 (PROKR2) (1, 2). Mutations in PROKR2 were found in the heterozygous and homozygous state of KS patient. Many of these mutations were also detected in clinically unaffected individuals, thus raising the question of their actual implication in the KS phenotype. Using a combination of molecular biology, pharmacology and biochemistry approaches, we characterized some of these mutations in a recombinant murine Prokr2, and tested their effects on the signalling activity in transfected HEK-293 cells.

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P3.072

Heterodimeric mGlu receptors: pharmacological properties revealed by a novel time-resolved FRET approach

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Metabotropic glutamate receptors (mGluRs) are family C G-protein-coupled receptors that participate in the modulation of synaptic transmission and neuronal excitability throughout the central nervous system, and thus are promising therapeutic targets to treat neurological and psychiatric disorders such as Alzheimer's and Parkinson's diseases, anxiety, depression, pain or schizophrenia. The 8 mGluR subtypes are divided into 3 groups based on their sequences, pharmacological properties and signaling pathways, and they are usually described as constitutive homodimers.
By combining the advantages of time-resolved FRET, and the use of two tags, SNAP- and CLIP-tags, to specifically label two distinct proteins, we develop here a new multiple labeling approach to examine the formation of heteromeric mGluRs at the cell surface. We show that group II and III mGlu subunits (mGlu2, 3, 4, 7, 8) can form heterodimeric receptors at the cell surface, whereas they cannot assemble with group I mGlu subunits (1 & 5). The existence of such mGlu heterodimers is further confirmed by biochemical and functional complementation studies.
Our FRET strategy also allowed us to study the molecular functioning of such heterodimers, by monitoring the conformational changes induced by subunits specific agonists, antagonists and allosteric modulators. Using the mGlu2-mGlu4 heterodimer as a model system, we provide new information on how the signal is transmitted from the binding of the agonist in the extracellular domain, to the activation of the intracellular G-protein.
Whether such mGluR heterodimers exist *in vivo* or have a physiological role remains an open question. However with a strategy that could be applied to many other membrane proteins, we have identified for the first time their existence at the surface of living cells, and even developed a method to study their functioning, thus offering new possibilities to develop new drugs targeting these heterodimers specifically.

P3.073

Native $GABA_B$ receptors are heteromultimers with a family of auxiliary subunits

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$GABA_B$ receptors are the G-protein coupled receptors for γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain. They are expressed in virtually all neurons of the brain where they regulate synaptic transmission and signal propagation by controlling the activity of calcium and potassium channels. Molecular cloning revealed that functional $GABA_B$ receptors are formed by the heteromeric assembly of $GABA_{B1}$ with $GABA_{B2}$ subunits. However, cloned $GABA_{B(1,2)}$ receptors failed to reproduce the functional diversity observed with native $GABA_B$ receptors. We now show by functional proteomics that $GABA_B$ receptors in the rat brain are high molecular weight complexes of

$GABA_{B1}$, $GABA_{B2}$ and members of a subfamily of the ´potassium channel tetramerization domain-containing´ (KCTD) proteins. KCTD proteins 8, 12, 12b and 16 exhibit distinct expression profiles in the brain and tightly associate with the C-terminus of $GABA_{B2}$ as tetramers. This co-assembly changes the properties of the $GABA_{B(1,2)}$ core receptor in a KCTD subtype-specific manner. Our results establish the KCTD proteins as auxiliary subunits of $GABA_B$ receptors that determine the functional properties of the receptor. We will provide an update on our analysis of the effects of KCTD proteins on recombinant and native $GABA_B$ responses.

P3.074

Notch signaling in glia modulates sleep homeostasis and learning after sleep deprivation in *Drosophila*

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The role of the trans-membrane receptor *Notch* in the adult brain is poorly understood. In wild-type *Drosophila*, both *Notch* and *i*ts ligand *Delta* are transcriptionally down regulated after sleep deprivation. Preventing the down regulation of *Delta* by over-expressing a wild-type transgene in the mushroom body neuronal center reduces sleep homeostasis and, importantly, prevents learning impairments induced by sleep deprivation. Similar resistance to sleep loss is observed with the $Notch^{spl-1}$ gain-of-function mutants. Immunohistochemistry reveals that the *Notch* receptor is expressed in glia, whereas *Delta* is localized in neurons. Importantly the expression of the intracellular domain of *Notch*, a dominant activated form of the receptor, in glia is sufficient to prevent learning deficits after sleep deprivation. Together these results identify a novel neuronal-glia signalling pathway dependent on *Notch*. These data highlight the emerging role of neuron-glia interactions in regulating both sleep and learning impairments associated with sleep loss.

P3.075

Identification and characterization of new protein partners of serotonin transporter

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The plasma membrane serotonin transporter (SERT) plays a critical role in the regulation of serotonergic transmission by enabling serotonin reuptake into the cells. This transporter is of major pharmacological and clinical interest, particularly as it represents one of the primary targets of several widely prescribed antidepressants. It is now well established that SERT does not function as an isolated protein. SERT functional activity is regulated by a combination of multiple mechanisms including both post-translational modifications and association with intracellular proteins. During the last decade, several SERT-interacting proteins have been identified, principally by means of yeast two-hybrid screens. We have recently used a proteomic approach that enabled us to characterize a reciprocal modulation of SERT and neuronal NO Synthase (nNOS) activity mediated by their physical interaction. To get further insight into SERT-associated protein complex, we used high-resolution mass spectrometry to identify novel proteins interacting with SERT C-terminus, or whole SERT protein expressed in two different cell culture models. This shotgun analysis of SERT interactome led us to identify several new partners of SERT. These include Calcineurin, a calcium-dependent

serine/threonine phosphatase, ASCT2 (Alanine Serine Cysteine Transporter 2), a neutral amino acid transporter, VELI-3, a PDZ domain-containing protein which regulates plasma membrane distribution of several receptors and transporters, and a set of proteins related to the SNARE complex, possibly involved in SERT export to the plasma membrane. Moreover, we showed that both physical interaction of SERT with Calcineurin and Calcineurin phosphatase activity increase SERT plasma membrane expression and 5HT uptake via SERT. In addition, co-expression of VELI-3 with SERT likewise increases 5HT uptake, whereas co-expression of ASCT2 decreases SERT activity, possibly via modification of its glycosylation status. Collectively, these proteomic studies identify novel regulation mechanisms of SERT activity that might influence serotonergic transmission.

P3.076

Heterogeneity of the septum-hippocampal pathways: evidence for 4 distinct neurotransmitter phenotypes

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The dentate gyrus (DG) is the main entrance to the hippocampal formation for cortical and sub-cortical regions. In rat, extrinsic inputs to the DG originate from the suprammamillary nucleus (SuM), the medial septum diagonal band complex (MS/DB) and the entorhinal cortex. This system plays a crucial role in control of several hippocampal-dependent cognitive functions and emotional behavior. However, the anatomical-functional substrate by which such regulation occurs is still poorly understood. Recently we demonstrated that the SuM provides two distinct neurochemical projections to the DG. The first one originates from neurons in the lateral region of the SuM (SuML) and displays a unique dual phenotype for GABAergic and glutamatergic neurotransmission. Its axon terminals contain markers of GABAergic neurotransmission, including the synthesizing enzyme of GABA, GAD65, and the vesicular GABA transporter, VGAT, as well as a marker of glutamatergic neurotransmission, the vesicular glutamate transporter, VGLUT2. The second pathway originates from neurons in the most posterior and medial part of the SuM (SuMM) that innervates exclusively the ventral dentate gyrus and contains VGLUT2 only (Soussi et al., EJN 2010).
The current study was designed to dissect MS/BD-DG projections and their neurotransmitter phenotypes. With immunohistochemical and tracing methods, we demonstrate that MS/BD provides at least 4 distinct pathways to the dorsal DG. The first one corresponds to the previously described cholinergic projections. Its axon terminals labeled either for Chat or VAChT do not contain VGLUT2 or GAD65. A few of these boutons located mainly in the hilus of the DG contain GAD67. The second projection contains markers of the GABAergic neurotransmission only, including GAD67. The third pathway is glutamatergic. Its axon terminals, which contain VGLUT2 only, innervate the supragranular layer (SGL) of the DG. Finally, we observe a fourth projection that interestingly displays similar dual phenotype for the GABAergic and glutamatergic neurotransmissions and distribution in the SGL than afferents from the SuML. Its axon terminals co-express VGLUT2 and GAD65 or VGAT but not GAD67. This study highlights a complex connectivity between the septum and The DG.
Support: INSERM

P3.077

Biphasic action of axonal GABA-A receptors on presynaptic calcium influx

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Although ionotropic GABA receptors (GABAARs) have long been known to exist on the axons of many different cells their effect on axon excitability and synaptic transmission remains controversial. Here using high-speed Ca^{2+} imaging it is shown that they induce a biphasic effect in parallel fibers of the

cerebellar cortex. Multicellular measurements indicate a facilitation of action potential-evoked Ca^{2+} transients that is subsequently followed by depression. However, the receptor activation does not increase influx of Ca^{2+} into individual fibers but instead increases the probability of action potential generation. These results provide a description of the effect of presynaptic $GABA_AR$ activation and explain why reports of the effect of their activation have been so varied.

P3.078

Somato-dendritic group II metabotropic glutamate receptors contribute to the inhibition of hippocampal mossy fiber transmission

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Activation of group II mGluRs, which comprise mGluR2 and mGluR3, inhibits glutamate release at mossy fiber synapses in the hilus and CA3 area of the hippocampus. The mGluRIIs mediating the inhibition of glutamate release are considered to be localized on mossy fiber terminals (Shigemoto et al., 1997; Yokoi et al., 1996). Here we examined the possible mechanisms through which mGluRIIs block transmission at mossy fiber synapses.
First we confirmed, in hippocampal slice cultures from mouse, that application of the specific mGluRII agonist DCG-IV (2 µM) blocked antidromically evoked action currents in granule cells at minimal stimulation but not at high intensity of stimulation (n=7). This axonal response to DCG-IV was markedly reduced by dendrotoxin 1 (200 nM), which is a potent inhibitor of voltage-sensitive K^+ channels (n = 6), but not by tertiapin Q (0.5 µM) which blocks inward rectifying potassium channels (n=3).
Next we tested whether mGluRII activation modifies somato-dendritic responses recorded from patch-clamped granule cells. Interestingly we found that application of DCG-IV (2 µM) induced a pronounced outward current in granule cells (66.3 ± 7.4 pA), which persisted in presence of TTX (69.3 ± 7.3 pA, n = 5), and the NMDA antagonist D-AP5 (62.4 ± 19.7 pA, n = 4), and which was blocked by the mGluRII antagonist LY341495 (n = 4/4). This DCG-IV-induced outward current was associated with an increase in a potassium conductance, and was blocked by tertiapin Q (20 ± 2 pA, n = 5), but not by dendrotoxin 1 (54.6 ± 14.5 pA, n = 5). The response to DCG-IV was not altered in mGluR3 KO mice (69.8 ± 11 pA, n = 5), but was abolished in mGluR2 KO mice (1.75 ± 1.18 pA, n = 5).
These data indicate that activation of mGluRIIs with a somato-dendritic localization powerfully hyperpolarizes granule cells, an effect which will impede the generation of action potentials. This mechanism will contribute to the integration of somatic input in granule cells and will thereby modify the responses of networks targeted by the mossy fibers.

P3.079

Analysis of $GABA_B$ receptor phosphorylation: a proteomic approach

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G protein-coupled receptor responses and trafficking are regulated by a vast array of different effectors and post-translational modifications, one of the most important being receptor phosphorylation. Receptor phosphorylation can be a result of activation of various kinases, such as the G protein-coupled receptor kinase (GRK) family or second-messenger kinases, PKA and PKC.

The obligate heterodimeric $GABA_B$ receptor is subject to phosphorylation on both its $GABA_{B1}$ (GB1) and $GABA_{B2}$ (GB2) subunits by a number of different kinases, calmodulin kinase and AMP kinase, for example. Phosphorylation of the $GABA_B$ receptor has been recently demonstrated to be dependent on activation of the ionotropic glutamate NMDA receptor. However, there is a relative paucity of information relating to how GABA and other $GABA_B$ receptor ligands regulate the phosphorylation state of the $GABA_B$ receptor.
We employed a proteomic approach to ascertain the phosphorylation state of the $GABA_B$ receptor after pre-treatment with various $GABA_B$ receptor ligands. HEK-293 cells expressing the $GABA_B$ receptor were treated with $GABA_B$ ligands for 30 minutes, after which the cells were solubilised and the receptor was extracted and purified using immuno-affinity purification, then analysed through mass spectrometry. Through this approach we have found evidence that GABA regulates a string a phosphorylation sites in the C-terminal tail of the GB2 subunit, S^{770}, T^{772}, S^{775} and T^{777}, which after pre-treatment with GABA, all had the potential to be phosphorylated, with a double phosphorylation at S^{775} and T^{777}. The threonine at position 818 also appears to be phosphorylated, but in a constitutive manner.
It remains to be elucidated how these phosphorylated residues affect the signalling or trafficking of the $GABA_B$ receptor. This will be determined through structure-function studies using various functional outputs and analysis of receptor cycling.

P3.080
Excess of Tau binding to microtubules impairs axonal transport in vivo

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The Tau protein, enriched in axons, binds to and stabilizes microtubules. Its microtubule binding is negatively regulated by phosphorylation on many Ser/Thr sites. This regulation seems required for the correct function of neurons since an excess of Tau bound to microtubules has been reported to impair vesicular kinetics in cell culture and in vitro. However, the few in vivo studies looking for a deleterious role of Tau onto vesicular kinetics revealed no defects. Here, using several Tau isoforms that differentially bind to microtubules, we show that an excess of hypophosphorylable, microtubule-bound Tau impairs vesicular kinetics and neurohormone release in vivo.

P3.082
Critical role of endozepines in gliotransmission and brain glucose sensing

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Hypothalamic glucose sensing is involved in the control of feeding behavior and peripheral glucose homeostasis, and its dysfunction is believed to be an important component in the development of

obesity and diabetes. Glial cells have been shown to be part of a “glucose sensing unit”, but their mode of communication with neurons remains elusive. Diazepam-binding inhibitor (DBI) and its processing products, notably the octadecaneuropeptide (ODN), collectively named endozepines, are secreted by astroglia, and ODN, acting through a metabotropic receptor, has been shown to be a potent anorexigenic factor. In this study, we investigated the involvement of endozepines in brain glucose sensing. First, we showed that 16-h fasting markedly reduced DBI expression, and that intracerebroventricular administration of glucose in rats increases DBI mRNA levels in hypothalamic glial-like tanycytes, as well as pro-opiomelanocortin (POMC) mRNA levels. Then, we demonstrated that glucose stimulates endozepine secretion from hypothalamic explants. Next, feeding behavior experiments were conducted to test the involvement of endozepines in brain glucose sensing. We found that the feeding-suppressing effect of central or peripheral administration of glucose was blunted by co-injection of an ODN metabotropic receptor antagonist. Conversely, the hyperphagic response elicited by central glucoprivation (2-deoxyglucose administration) was suppressed by an ODN agonist. The anorexigenic effects of centrally-injected glucose or ODN agonist were suppressed by blockade of the melanocortin-3/4 receptors, suggesting that glucose sensing involves endozepinergic control on POMC neurons. Finally, we found that brain endozepines modulate blood glucose levels, suggesting their involvement in a feedback loop controlling whole-body glucose homeostasis. Collectively, these data indicate that endozepines, through activation of POMC neurons, are a critical relay in brain glucose sensing and potentially new targets in the treatment of metabolic disorders. *Supported by Inserm, Inserm-FRSQ exchange program and the Conseil Régional de Haute-Normandie*

P3.083

Evidence for P2X4 involvement in hippocampal remodelling after *Status epilepticus*

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Within the CNS, functions of P2X4 receptors (P2X4R) are still poorly understood, yet, their activation in neurons and microglia correlates with high or pathological neuronal activities. In this study, using P2X4R-deficient mice, we investigated the potential involvement of P2X4R in a model of kainate (KA)-induced *status epilepticus*.
In wild-type mice, KA injection induced an early and transient decrease of the potassium chloride transporter KCC2 that was not observed in P2X4R-deficient mice. We also found that *status epilepticus* was associated with a progressive P2X4R upregulation in the hippocampus, mostly localized in activated microglial cells. In P2X4-deficient mice, several features of microglial activation such as cell recruitment and upregulation of voltage-dependent potassium channels were impaired 48 hours post-KA injection. Consistent with the role of P2X4R in activity-dependent degenerative processes, CA1 area was partially protected from neuronal death in P2X4-deficient mice compared to wild type animals.
Our findings demonstrate that both neuronal and microglial P2X4R are brought into play during neuronal hyperexcitability and contribute to excitotoxic damages.

P3.084

CDK-mediated phosphorylation of the Kvβ2 auxiliary subunit regulates Kv1 channel axonal targeting

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Voltage-gated potassium Kv1 channels are localized to the axon where they control action potential propagation and neurotransmitter release. Kv1 axonal targeting requires the highly conserved Kv1 T1 tetramerization domain and the association of the Kvbeta auxiliary subunits. Recent studies have shown that the interaction of the N-terminal of Kvbeta2 to the plus-end microtubule binding protein 1 (EB1) is also crucial for Kv1 targeting. However, little is known about the mechanism regulating Kvbeta2-EB1 interaction. Based on previous studies showing that the interaction of EB1 with its other partners was highly regulated by phosphorylation, we first identified phosphorylation sites of the auxiliary subunit Kvbeta2. We find that the auxiliary Kvβ2 subunit of Kv1 channels purified from brain is phosphorylated on serine residues 9 and 31, and that cyclin-dependent kinase (CDK)-mediated phosphorylation at these sites negatively regulates the interaction of Kvβ2 with EB1. Endogenous CDKs, EB1 and Kvβ2 phosphorylated at serine 31 are colocalized in the axons of cultured hippocampal neurons, with enrichment at the axon initial segment (AIS). Acute inhibition of CDK activity leads to intracellular accumulation of EB1, Kvβ2, and Kv1 channel subunits within the AIS. Our studies reveal a new regulatory mechanism for the targeting of Kv1 complexes to the axonal membrane through the reversible CDK phosphorylation-dependent binding of Kvβ2 to EB1.

P3.085

Characterization of different subpopulations of astrocytes sorted by sedimentation field-flow fractionation

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Previous studies suggest that different astrocyte subpopulations played a key role during central nervous system repair and in the formation of the glial scar. After central nervous system damage, an extensive deposition of extracellular matrix produced by the activated astrocytes limits the extension of the lesion but impairs axone outgrowth and functional recovery.
In this study, we used sedimentation field-flow fractionation (SdFFF) to sort astrocyte subpopulations. Cells, prepared from rat newborn cortex, were submitted to SdFFF. After SdFFF elution, cells were cultured for one week, and analyzed by immunocytofluorescence using antibodies against specific markers: glial fibrillary acidic protein (GFAP), O4, β-tubuline III, and F4/80, specific respectively for astrocytes, oligodendrocytes, neurons, and microglial cells. Moreover, the expression of α-smooth muscle actin, the actin isoform expressed by myofibroblasts, the so-called activated fibroblasts involved in many repair mechanisms, was studied.
By SdFFF, three main fractions were isolated. For immunocytofluorescence analysis, F1, F2, and F3 were compared with the total eluted population (TP). TP, F1, F2, and F3, contained 74%, 96%, 12%, and 98% of GFAP expressing astrocytes respectively. The size of the astrocytes in F1 was important and cells were spreaded while astrocytes in F3 were small and showed a tendency to aggregate. Interestingly, in F3, astrocytes only express GFAP, while in F1, astrocytes express both GFAP and α-smooth muscle actin. Moreover, F3 showed higher migratory capacities compared with F1. In F2 and in the total population, α-smooth muscle actin expression was barely detected.
This study demonstrates that SdFFF enables the isolation of almost pure astrocyte subpopulations after one week in culture while, with the classical technique, about 84% of astrocytes are obtain after 3 weeks in culture. Then, SdFFF permits the preparation of efficient biological tools allowing the exploration of different astrocyte subpopulations involved in central nervous system repair.

P3.086

Ciliary neurotrophic factor, a candidate for endogeneous progenitor cell mobilization in the context of myelin repair

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Since the discovery of endogenous populations of neural stem progenitor cells (NSPCs) in the adult brain, their mobilisation appears as a promising strategy for myelin repair. Indeed, recent data showed their spontaneous but insufficient recruitment toward lesions in an attempt to replace injured cells. Two main cell types are involved in this process. Oligodendrocytes progenitors cells (OPCs) present throughout the brain and newly formed progenitors generated by neural stem cells in the subventricular zone (SVZ). One way to promote remyelination would be to enhance the recruitment of those cells. This implies to increase our knowledge of the molecular and cellular mecanisms involved in the directed migration of endogenous progenitors toward the demyelinated lesions. Our working hypothesis is that after brain insults, strongly re-expressed signals could represent candidate factors to increase endogenous progenitor's mobilization by controlling their directed migration. In this context, Ciliary Neurotrophic Factor (CNTF) appears as an interesting candidate. Using in vitro and in vivo approaches, we examine how this cytokine can influence NSPCs migratory behaviour.

P3.087

The role of the p53-family of transcription factors in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neuromuscular disease affecting adults in their fifties. It is characterised by the progressive degeneration and loss of upper and lower motor neurons as well as progressive skeletal muscular atrophy. A dozen genes are known to be able to lead to ALS, but the one encoding Cu/Zn Superoxide Dismutase 1 (SOD1) is the most frequent.
Previous work from our laboratory has shown an induction of p53 in motor neurons in a mouse model that replicates ALS. Furthermore, our laboratory analyzed muscular transcripts from this mouse model and from ALS patients, which revealed an induction of genes related to p53 and its family (p63, p73). The transcription factors of the p53 family are intimately linked to cellular mechanisms like cell death decisions, differentiation processes, cellular homeostasis and metabolism - mechanisms that are deregulated in many neurodegenerative diseases. Our work unveils a coherent p53 response:
- Individual assessment of p53-family members, regulators and target genes from transcripts highlighted p63 and other genes related to apoptosis, senescence and differentiation.
- Immunohistological observation showed an increase of p63 protein levels in degenerating mouse myofibers and p53 immunoreactivity in a subset of small, different muscular cells.
The p53 response may participate in the pathological events during ALS, either as consequence or even the cause of neuronal and muscular degeneration.
However, further transcript analyses indicate that the p53-family has no strong link to metabolic alterations in the ALS context. Thus, we see its role in the regulation of cell death and differentiation. To support the hypothesis of an activation of the p53 pathway, we pharmacologically induced cellular stresses in the C2C12 myoblasts. These stresses are also found in some neurodegenerative disorders and have been described as consequence of mutated SOD1 in ALS: endoplasmic reticulum stress, oxidative stress or DNA damage. Additionally, a drug sufficient to trigger neurodegenerative symptoms in mice was used. The drugs were all able to induce p53 stabilisation, supporting our initial hypothesis. We think that p63, alongside the p53 family, has a role in ALS and will investigate the consequences of its activation.

P3.088

The giant protein AHNAK, required for Schwann cell polarisation and function

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Schwann cell (SC) differentiation during development, and their proper function in intact mature peripheral nervous system and during regeneration from a nerve lesion, relies on their intricate interaction with axons on one hand, and basal lamina on the other. Perturbation of these interactions, e.g. due to lack or mutation of basal lamina components, or the corresponding SC receptors, leads to severe defects in axon sorting and myelination, causing peripheral neuropathies and muscular dystrophies. We had previously found the giant phosphoprotein AHNAK (700 kDa) to be strongly and constitutively expressed in myelinating and nonmyelinating SC, localized at the inner face of the plasma membrane. Work from several groups including our own suggested that AHNAK not only interacts with the cortical actin cytoskeleton, but may serve as enormous scaffolding protein binding several ligands at a time. In good correspondence with this hypothesis, we demonstrated that interfering with AHNAK expression in primary cultured SC via siRNA transfection induced a change in distribution and a downregulation of expression of the laminin receptor dystroglycan, followed by a change in SC morphology and their detachment from the laminin substrate. Here, we report that electron microscopic analysis of sciatic nerve from mutant mice deficient for ahnak, both young and aging, reveals reproducible defects suggestive of abnormal sorting and ensheathing of myelinated axons and particularly, nonmyelinated Remak fibers, comparable to findings reported for integrin-ko mice. Light microscopic analysis is performed on teased fibers from sciatic nerve to determine whether lack of AHNAK affects the distribution of receptors on axons or SC. Cultured primary SC derived from ahnak-ko mice, when compared to wildtype cells, exhibit an altered morphology, and reduced motility and proliferation rate. Comparison of protein maps from wildtype and ahnak-ko SC via two-dimensional gel electrophoresis, and co-immunoprecipitation experiments are performed to identify potential partner proteins of AHNAK. Our work should thus contribute to a better comprehension of the molecular mechanisms implicated in the physiological function of SC, and certain pathological disorders of the peripheral nervous system.

P3.089

***Clostridium perfringens* epsilon toxin binds to subsets of neurons and oligodendrocytes**

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Epsilon toxin (ET) produced by C. perfringens types B and D is a highly potent pore-forming toxin ranking among the 10 most poisonous substances. ET-intoxicated animals express very severe neurologic disorders and nerve tissue damages thought to result from formation of vasogenic brain edema and indirect neuronal excitotoxicity. Several reports have suggested that ET binds to glial cells such as astrocytes and oligodendrocytes, but not neurons. Using immunostaining techniques, we found that several brain structures were labeled by ET, the cerebellum being a prominent one. Analysis of the co-localization of ET staining with specific markers in the cerebellar slices revealed that ET binds to the cell body of neurons and oligodendrocytes, but not to astroglial cells or nerve endings. Identification of the granule cells as neuronal ET targets in the cerebellum was confirmed by the observation that ET (10^{-7}M but not 10^{-8}M) induced intracellular Ca^{2+} rise followed by cell death. Electrophysiological and pharmacological analysis of the ET effects on cerebellar slices revealed that

ET does not act directly on nerve terminals. When applied at 10^{-7}M, ET decreased trans-membrane resistance and depolarized the granule cells soma leading to the firing of the neuronal network and ensuing stimulation of quantal glutamate release. However ET had no detectable direct effect on inhibitory interneurons. The effect of ET on oligodendrocytes was assessed using primary cultures, and the 158N cell line, which is derived from oligodendrocytes. In these cells ET (10^{-9}, 10^{-8}, 10^{-7}M) induced intracellular Ca^{2+} rise indicating a susceptibility to ET higher in oligodendrocytes than in granule cells. Electrophysiological recordings performed on 158N cells revealed that ET enhanced BK channels opening frequency (this later effect is consistent with ET-induced Ca^{2+} intracellular rise).Overall, this works suggests that part of ET-induced damages observed in neuronal tissue is due to a direct effect of ET on subset of neurons. Since ET is pore-forming in renal cells, this may relate to the toxin's pore forming activity. The mode of action of ET on oligodendrocytes seems to strongly differ from that on neurons. The effect of ET on oligodendrocytes deserves further investigations.

P3.090

Covariation of voltage-dependence of A-type and H-type currents maximizes the dynamic range of rebound firing in dopaminergic neurons

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Recent studies in invertebrates have demonstrated that neurons with virtually identical firing patterns show considerable variability in the level of expression of some of their underlying conductances. Although this observation seems paradoxical at first, further studies demonstrated coregulated expression of ion channels or the amplitude of voltage-gated currents that may stabilize activity in spite of the variations of properties of individual channels.
We discovered considerable variability in the voltage-dependence of I_A and I_H (>20mV) in dopaminergic (DA) neurons of the substantia nigra pars compacta in rat, and show that these properties are strongly correlated across individual neurons and have a significant impact on the neuron's rebound properties. Non-saturating concentrations of specific blockers of I_H (ZD7288) and I_A (AmmTX3) induced opposite changes in rebound properties following both long hyperpolarizations and short synaptic-like hyperpolarizations, including the delay to the first action potential, demonstrating the strong opposing roles of I_A and I_H. Measuring I_A, I_H and the rebound properties in the same cells demonstrated that the I_H half-activation voltage (I_H V50) and I_A half-inactivation voltage (I_A V50) showed a strong positive correlation ($r= 0.60$, $p< 0.001$, $n=85$), and that I_H and I_A V50s were both significantly correlated with components of the rebound following long hyperpolarizations ($p< 0.001$, $n=80-88$).
In dynamic-clamp experiments, we used values obtained from voltage-clamp recordings to simulate I_A/I_H V50s within or outside the distribution observed for the biological currents to assess their functional impact on rebound properties. We demonstrate that, unlike the coregulation of channel expression, the coregulation of V50s tends to maximize the degree of variability of the rebound duration (>75%) while values taken outside the biological distribution tend to minimize variations in rebound duration (12%). While coregulation of channel expression seems to stabilize firing properties, our data suggest that coregulation of voltage-dependence might optimize information coding. In addition, this coregulation of V50s might be a target for rapid and robust modulation of rebound properties.

P3.091

Development of short term plasticity properties at the transient medial olivocochlear-inner hair cell (MOC-IHC) synapse

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From birth until the onset of hearing (postnatal day (P) 12), IHCs are transiently innervated by cholinergic medial olivocochlear (MOC) fibers. At this synapse, transmitter release is supported by both N- and P/Q-type voltage-gated calcium channels (VGCCs) (Zorrilla de San Martín et al., J. Neurosci 2010). The fast formation and retraction of the MOC-IHC synapse suggest there may also be associated changes in synaptic transmission throughout this period. Short term plasticity (STP) is a dynamic process that depends on the balance between facilitation and depression of synaptic responses caused by preceding activity. Our goal is to determine whether there are changes in STP at the MOC-IHC synapse during development and, if so, to understand the mechanisms underlying them. Synaptic activity was recorded in voltage-clamped IHCs from excised apical turns of the mouse cochlea at two developmental stages (P5-7 and P9-11) during electrical stimulation of the MOC fibers. Ten-pulse trains at 10, 20, 40 and 100 Hz applied to P5-7 MOC-IHC synapses led to 1.8±0.3; 1.7±0.2; 1.8±0.3 and 2±0.4-fold increase in synaptic efficacy, respectively, estimated as the ratio between the mean amplitude of the fifth and the first evoked synaptic current (S_5/S_1); n=7-10. The same protocols applied to P9-11 synapses led to a progressive decrease of the S_5/S_1 value (0.8±0.1; 0.7±0.1; 0.6±0.1; 0.4±0.1 for the 10, 20, 40 and 100 Hz trains, respectively; n=12-18). Depression upon high frequency stimulation at P9-11 was reversed to facilitation when reducing quantal output either by decreasing $[Ca^{2+}]_o$ or by blocking P/Q-type VGCCs with ω-Agatoxin IVA (200 nM). Our results show there is a developmental switch from facilitation to depression upon high frequency stimulation consistent with the increment in the probability of release. We are now studying whether these changes in synaptic transmission can be accounted for by differences in the coupling between calcium influx and transmitter release.

P3.092

Cellular image analysis: provide aid in the diagnosis of brain cancer

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This work fits into the context of medical imaging; it is to process microscopic color images in order to provide aid in the diagnosis of brain cancer. Our goal is the development and implementation of a chain of image analysis, capable of detecting a possible presence of pathological cells in a cell microscopy image. The role of this analysis is to identify nucleus and cytoplasm of brain cells in the microscopic image to be processed. We calculate the surface ratio of nucleus to the cytoplasm of each cell, and classify them into two categories (healthy cell or pathological cell). The identification of the constituents of the image pre-processed is done by operation of the image segmentation; this method is based on mathematical morphology and neural networks. It is the colour watershed controlled by a Multi layers Perceptron. Neural networks are involved in this method of image segmentation, to overcome the problem of variability of images to be processed, i.e. they contribute to the robustness of the proposed implementation.

P3.093

Deregulation of gene expression in peripheral blood mononuclear cells at different stages of the Parkinson's disease

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Peripheral blood mononuclear cells (PBMC) appear to be an interesting model to study Parkinson's disease (PD) since PBMC's infiltration within substantia nigra of *post-mortem* PD cases and animal models was noted and deregulation of dopaminergic pathways was identified in these cells. In order to get insight into early mechanisms involved in PD pathogenesis, we employed a transcriptomic approach in PBMC from PD patients at different stages of the disease and controls. Four groups were designed: a control group (subjects free of personal and familial neurological disorder), a pauci-symptomatic group (PD subjects without dopaminergic treatment at a *de novo* stage or carrying pathogenic mutation), a mild disorder group (PD subjects with Hoehn and Yahr scale < 3) and a severe disease group (PD subjects with Hoehn and Yahr scale ≥3). We compared gene expression patterns of PBMC using Agilent whole human genome expression micro-arrays (44K). Analyses of differential expression were performed with GeneSpring GX software. Genes with significant differences (fold change >1.5 and Welch *t*-test $p < 0.05$) were analyzed using Ingenuity Pathway Analysis software which identified significantly deregulated canonical pathways. In each stage, pathways already described in PD pathogenesis were identified. Of interest, a set of common genes were identified among the deregulated genes belonging to the 3 stages. In conclusion, these data showed the progression of the molecular pathways involved at the different stages of the disease. Moreover, the PBMC transcriptome study could lead to identify specific biological markers and future therapeutic targets.

P3.094

Switch from positive to neutral living environments increases vulnerability to cocaine

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Life experiences, especially during early stages of life, can dramatically determine vulnerability to diseases at adulthood. Early exposure to positive environmental conditions such as enriched environments (EE) has been shown to reduce the occurrence and the intensity of neurological and psychiatric disorders including addiction to drugs. However, it is not known whether exposure to EE during early stages of life would protect from addiction even when, at adulthood, individuals may find themselves in non-enriched conditions. Here, we show that switching mice at adulthood from EE to non-enriched standard environments (SE) not only eliminates EE-induced reduction of cocaine effects but even increases the rewarding effects of cocaine. This increased vulnerability is associated with emotional distress and with increased mRNA levels of corticotropin releasing factor (CRF) in the bed nucleus of the stria terminalis. The involvement of CRF in this increased sensitivity to cocaine's rewarding effects was confirmed by the fact that increases in cocaine-induced conditioned place preferences were completely blocked by chronic administration of the CRF receptor type 1 antagonist antalarmin. These results indicate that not only beneficial effects of early exposure to EE do not last over time, but also that depriving individuals of enrichment results in paradoxical increases in rewarding effects of cocaine. Therefore positive life conditions during early stages of life, if they are not maintained at adulthood, may have negative emotional consequences and increase the vulnerability to external aggressors such as drugs of abuse.

P3.095

Characterization of AMPK activity in models of Huntington's disease pathogenesis

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Huntington's disease (HD) is a neurodegenerative pathology induced by cell toxicity caused by polyglutamine (polyQ) expansion in the N-terminal end of huntingtin (htt). Using a *C. elegans* transgenics that express expanded polyQs in neurons, we identified a gene network that is centered onto FoxO and that protects neurons from expanded polyQs. screen for genes that modulate the early phases of expanded polyQ neurotoxicity. Among these genes, we identified AMPK, a well-known energy sensor involved in lifespan and health span extension in worms and mammals. AMPK is amenable to be regulated by different drugs. Some of these drugs, like metformin, are approved for human use, hence AMPK may be of therapeutic interest in HD. Therefore, we sought to characterise AMPK in the context of neuronal vulnerability caused by expanded htt in different models. First, we investigated the function of AMPK in *C. elegans*, and its potential interaction with well-established neuroprotective genes, by using mutants and drugs, such as metformin. We then validated our results in an in vitro model of HD: striatal cells from HdhQ111 knock-in mice, carrying the expanded (109Q) or wild type (7Q) alleles of htt. Finally we tested the potential of AMPK activation in mice, by over-expressing a gain-of-function allele of AMPK-gamma, which activates AMPK function. By doing epistatic analysis in worms, we show that AMPK is required for neuroprotection through the FOXO/*daf-16* signaling pathway. The depletion of AMPK enhances neuronal impairment, and activation by metformin induces neuroprotection, suggesting that this enzyme may be of therapeutic interest in HD. Similar findings were obtained in striatal cells from HdhQ111. Finally, preliminary results suggest that activation of AMPK protects from the neuropathological effects of N-terminal expanded htt in a lentivirius-based model of HD in mice. These results strongly suggest that AMPK is of therapeutic interest in HD and warrant further investigation of AMPK activity in HD neurons.

P3.096

Anti-absence activity of type-1 metabotropic glutamate receptors

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The mGlu1 metabotropic glutamate receptor modulates synaptic responses in thalamic relay neurons, and is therefore strategically positioned to control the development of spike-and-wave discharges (SWDs) in absence epilepsy. We examined the role of mGlu1 receptors in absence epilepsy using WAG/Rij rats, which develop spontaneous SWDs after 3 months of age. Symptomatic WAG/Rij rats, showed a reduced function of mGlu1 metabotropic glutamate receptors in the thalamus, as assessed by *in vivo* measurements of agonist- stimulated [^{3}H]inositolmonophosphate formation after i.c.v. injection of [^{3}H]myo-D-inositol, and i.p. injection of lithium chloride. Symptomatic WAG/Rij rats also showed lower levels of thalamic mGlu1a receptors with respect to age-matched controls, as detected by immunoblotting, immunohistochemistry, and *in situ* hybridization. Reduction of mGlu1a receptors in WAG/Rij rats was restricted to a thalamic region that excluded the ventroposterolateral nucleus.

Systemic treatment with the mGlu1 receptor enhancer SYN119 reduced spontaneous SWDs in WAG/Rij rats. Subcutaneous doses of 10 mg/kg of SYN119 only reduced the incidence of SWDs, whereas higher doses (30 mg/kg) also reduced the mean duration of SWDs. In contrast, treatment with the non-competitive mGlu1 receptor antagonist, JNJ16259685 (2.5 and 5 mg/kg) increased the frequence of SWDs. These results suggest that absence epilepsy might be associated with a reduction of mGlu1 receptors in the thalamus, and that mGlu1 receptor agonists/enhancers might be developed as novel ant-absence drugs. We are currently examining the response of mice lacking mGlu1 receptors to drug-induced SWDs and we are searching for functional polymorphism the *Grm1* gene (the gene encoding for the mGlu1 receptor) in children with absence epilepsy.

P3.097

Antidepressant-like behavioral effect of almorexant, a dual orexin receptor antagonist, in the unpredictable chronic mild stress in mice

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Major depressive disorder (MDD) is characterized by various behavioral and neurobiological features, including mood disturbances, loss of interest in pleasurable activities, sleep abnormalities, significant weight change, dysregulation of hypothalamic-pituitary-adrenal (HPA) axis. Interestingly, several studies have demonstrated that hypothalamic neuropeptides orexins are involved in the regulation of homeostatic and autonomic functions such as energy balance, sleep-wake cycle, food/drug reward and emotions. Animal and human studies suggest an involvement of orexinergic system in pathophysiology of MDD, but the putative involvement of orexinergic system in the depressive-like state is still unclear. Thus, we assessed the behavioral effects of almorexant (ACT-078573, 100 mg/kg/day, p.o.), a new antagonist of the two orexin receptors, alone or associated with selective serotonin reuptake inhibitor antidepressant (fluoxetine, 20 mg/kg/day, p.o.) chronically administrated to BALB/c mice subjected to 9-week unpredictable chronic mild stress (UCMS), a rodent model of MDD. UCMS induced physical and behavioral disturbances with deterioration in coat state, increase of immobility in the tail suspension test (TST), decrease in time spend in the open arms of the elevated plus maze (EPM), increase of agonistic behavior in the resident-intruder test (RI), and increased latency to eat in the Novelty-Suppressed Feeding test (NSF). Seven week treatment with fluoxetine reversed all the behavioral effects of UCMS, and interestingly, 7-week treatment with almorexant also reversed behavioral disturbances induced by UCMS, except in NSF. Similar effects were observed when almorexant and fluoxetine were injected in combination. No effects of treatments were observed in control mice, except in TST where fluoxetine, almorexant and combination of the two treatments reduced the time of immobility. These data suggest that orexinergic system may contribute to the pathophysiology of MDD, and highlight the putative involvement of orexins in behavioral disturbances observed in depression. Nevertheless, further research is needed to investigate more accurately the role of orexin in the modulation of brain regions involved in depressed-like states.

P3.098

The NMDA receptor antagonist ketamine suppresses appropriate context processing of target information in rats: implication for cognitive deficits in schizophrenia

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Context processing disturbances are one of the core cognitive deficits in schizophrenia. Notably, patients are unable to use contextual information (e.g., a task instruction or a sentential embedded meaning) to treat appropriately target information that can take various significations depending on the ongoing situation. Based on the pioneering work of Rescorla (1968) on the use by rats of contextual information to treat a target ambiguous Pavlovian conditioned stimulus (CS), we here report that the NMDA receptor antagonist ketamine, a potent psychodysleptic agent which induces psychosis in normal human subjects, dose-dependently suppressed such context processing in rats. Indeed, under ketamine treatment, rats stopped to use contextual information to treat appropriately a target tone CS. In addition, we also report that the deleterious effects of ketamine on such a contextual information processing was dose-dependently reversed by different classes of anti-psychotic agents, like the typical neuroleptic haloperidol and the atypical anti-psychotic olanzapine. Of interest, alike haloperidol, the dose-dependent, olanzapine-induced reversion of ketamine effects in this paradigm was linear, a pattern rarely observed with atypical anti-psychotic agents (e.g., clozapine, olanzapine, risperidone) in animal models of schizophrenia. The present results will be discussed in the framework of designing new animal models that mimic as closely as possible, cognitive deficits that are encountered during complex and misunderstood psychiatric diseases such as schizophrenia.

P3.099

The potential of the TNF superfamily member LIGHT in the regeneration of motor axons

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive muscle weakness, resulting in generalized paralysis. ALS is characterized by the selective loss of motoneurons in the brain and the spinal cord. Nerve terminals are the first affected during the pathological process and the axonal degeneration precedes clinical symptoms. Deciphering this pathological mechanism is crucial for the identification of potential therapeutic targets for ALS. Involvement of death receptors has been documented in ALS. Fas (CD95) death pathway has been proposed to contribute to the pathological process by actively and selectively triggering death of a proportion of motoneurons. Recent work from our laboratory discovered a novel motoneuron-restricted death pathway, which implicates the activation of LT-bR by LIGHT and that is triggered by the proinflammatory cytokine IFNg. Importantly, signs of activation of LIGHT/IFNg and Fas death pathway have been documented in ALS mice and sporadic patients.
Growing evidence suggests that both Fas ligand and LIGHT also modulate axon growth in neurons. Indeed, It has been shown that Fas contributes to axonal regeneration after spinal or sciatic nerve injury. LIGHT, through activation of HVEM, negatively regulates axon growth in developing sensory neurons. Therefore, the investigation of the functional duality of death receptors is crucial for the development of effective treatments of ALS.
We first focused our work on LIGHT and IFNg pathway. We show that recombinant IFNg and LIGHT through activation of LT-bR induces axon growth and branching in primary motoneurons. In addition, we demonstrate that functional recovery after sciatic nerve crush is delayed in *Light*-deficient mice, suggesting that LIGHT contributes to nerve regeneration. This regeneration coincides with an up regulation of LIGHT and IFNg in the sciatic nerve but not in the spinal cord. We are currently investigating how the pro-apoptotic versus pro-regenerative function is controlled, and whether the bivalent feature of death receptors might play a role in motoneuron pathology.

P3.100

Bromocriptine induced conditioned preference place in partial bilateral 6-OHDA denervated rats

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Parkinson's disease (PD) is characterized by a degeneration of mesencephalic dopaminergic neurons within both the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). The SNc cell loss is responsible for PD motor dysfunctions (bradykinesia, tremor and rigidity), while that of the VTA could promote PD behavioral disorders such as addiction to dopamine replacement treatment and behavioral addictions. Dopamine replacement therapies (L-Dopa, dopamine receptor agonists) are responsible for the appearance of these behavioral disorders. The VTA projection is the main dopamine source to the Nucleus accumbens (NAC). This projection constitutes an important neural substrate in reward process since it plays a positive reinforcement which can elicit hedonic reactions. The goal of our study is to illustrate the impact of dopamine receptor agonist (Bromocriptine, D2R) in the appearance of hedonistic behavior in a partial bilateral 6-OHDA-lesioned VTA using the conditioned place preference (CPP) paradigm. Our preliminary data revealed that Bromocriptine induced a high CPP score in rats with a severe VTA lesion.
Mots clés: Parkinson's disease, Addiction, Dopamine, VTA, CPP

P3.101

Facets of insight and impulsivity in schizophrenia

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Insight and impulsivity are particularly problematic in patients with schizophrenia. They increase the risk for the violence, substance abuse and suicide, but no studies have yet examined the relationship between insight and psychometric assessment of impulsivity in schizophrenia. We hypothesized that lack of insight would be associated with enhanced impulsivity. The aim of this study is to clarify the causal relationship between the level of insight and multi-facets nature of impulsivity in patients with schizophrenia.
Forty stable individuals with schizophrenia (matched by age and education) were administered the Structured Clinical Interview for DSM-IV (SCID), the positive and negative syndrome scale (PANSS), self-report Birchwood Insight Scale (BIS), Beck Cognitive Insight Scale (BCIS) and UPPS Impulsive Behavior scale.
Our results provide further evidence that patients with schizophrenia lack insight into their illness and they are more impulsive than healthy subjects, as indicated by their higher mean UPPS scores. We also found that lack of illness awareness were significantly correlated with lack of Premeditation ($rs = -0.327$; $p < 0,05$), lack of Perseverance ($rs = -0.392$; $p < 0,05$) and the levels of Urgency ($rs = -0.347$; $p < 0,05$). Additionally, BCIS total score, self-reflectiveness and self-certainty were positively correlated with Urgency respectively ($rs=0,477$; $p < 0,001$, $rs=0,468$, $p < 0,001$ and $rs=0,388$; $p < 0,05$). These results suggest a specific relationship between self-reported impulsivity and poor insight.
Keywords: *Insight; implusivity; schizophrenia.*

P3.102

Treatment response heterogeneity in children with ADHD during treatment with modified-release methylphenidate in the observational OBSEER study

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Introduction: OBSEER was a prospective, observational study examining effectiveness and safety outcomes over 3 months in patients (aged 6-17 years) with attention deficit hyperactivity disorder (ADHD) receiving once-daily modified-release methylphenidate (Equasym XL) under routine care. Children with ADHD vary in their response to drug treatment and follow different trajectories of symptom severity over time.
Aims: To investigate predictors for different treatment response trajectories.
Methods: Change in ADHD symptoms, as rated by parents and teachers on the German ADHD Symptom Checklist (FBB-ADHD), was examined post-hoc in patients who had measurements at all three study visits (baseline, 1-3 weeks and 6-12 weeks after first use of Equasym XL), using growth mixture modelling to detect trajectory groups. Sex, age, methylphenidate dosage before switch to treatment with Equasym XL, conduct problems, emotional symptoms and quality of life were considered as predictors of trajectory groups. Model selection was based on a formal statistical criterion (Bayesian information criterion) and clinical considerations.
Results: Models with up to 8 classes were computed for parent (*n* = 699) and teacher (*n* = 521) ratings. Under consideration of the covariates, a 4-class solution fitted the data best for both informants. Of these 4 classes, one class had low ADHD symptoms in the beginning and a stable course over the entire study (low-stable), the other three had high ADHD symptoms at study start. Among these high-symptom classes, one showed a stable course (high-stable), one had a strong decrease immediately after medication switch (high-baseline response), and one had a strong decrease during Equasym XL treatment (high-intervention response). All covariates except sex were significant in predicting trajectory classes.
Conclusions: Different treatment response trajectories can be detected and group membership can be predicted. These results help to inform practitioners which patients are most likely to benefit from treatment with Equasym XL.
Funding: Supported by Shire Development Inc.

P3.103

Effects of enriched environment on mnesic and neurobiological alterations in adolescent mice subjected to alcohol binge drinking-like conditions

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The extent of new alcohol consumption modes in teenagers like binge-drinking is quite alarming considering that adolescence is a critical time period for brain development and personality traits building, with high susceptibility to addictive drugs. Thus, alcohol use at adolescence has been associated with deficits in learning capacities and spatial memory performance, together with some alterations of episodic memory. However, long-term neurobiological effects of alcohol consumption at adolescence are still unclear, especially because life conditions seem to strongly influence vulnerability to alcohol addiction. Within this context, we wanted to investigate, in alcohol preferring mice (C57BL/6J), the consequences of a chronic intermittent alcohol exposure (CIAE) at adolescence on memory performances assessed in a model of visual episodic-like memory, the object recognition task (1 min-inter-trial interval), and on hippocampal expression of immediate early gene, c-fos, during the mnesic task. Doses of either 25% (v/v) ethanol (2 g/kg) in saline, or saline, were administered intraperitoneally (i.p.) every other day during 2 weeks to 30-day-old pups (P30) housed either in standard conditions or in an enriched environment for 3 h daily. Novel object recognition (NOR) test was performed 24 h after the last ethanol administration. Mean c-Fos expression per section was quantified in the dorsal and ventral dentate gyrus (DG) and CA3 parts of the hippocampus. CIAE led to a significant deficit in recognition memory (discrimination index d2:~-90%, $p < 0.05$), but did not affect locomotor activity. Moreover a significant increase in c-fos expression was observed in the dorsal part of the hippocampus (dentate gyrus ~+50%, $p < 0.05$ and CA3~+200%, $p < 0.05$) suggesting an enhanced activation of this region to the repeated alternation of alcoholisation/withdrawal periods. Whether or not these effects persist at adulthood remains to be determined. In any case, housing in enriched environment, particularly known for its beneficial effects on memory, prevented both early mnesic deficits and associated changes in hippocampal c-fos expression normally caused by alcohol treatment.

P3.104

Modification of *Mecp2* dosage alters axonal transport through the Huntingtin/Hap1 pathway

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The methyl-CpG binding protein 2 (*Mecp2*) gene is located on the X chromosome and encodes a protein implicated in the modulation of transcription of several target genes. Its expression is high in postmitotic neurons favoring cerebral development and synapse maintenance. A wide spectrum of MECP2-pathies results in an altered MECP2 dosage, ranging from deficiency (Rett Syndrome, RTT) to overexpression (Duplication Syndrome) in humans and neurological pathologies in mice. Pathogenic mechanisms are thought to involve the deregulation of specific genes such as *Bdnf* but may also involve defects in the general buffering of cell transcription. Here, we identify, in *Mecp2*-deficient mice (*Mecp2*$^{-/y}$), a set of transcripts specifically involved in the Bdnf transport along neurites. Huntingtin (*Htt*) and huntingtin-associated protein (*Hap1*) mRNAs and proteins are reduced in the brain of adult *Mecp2*-deficient mice. Consequently, we observed an abnormal Bdnf protein repartition in the brain of *Mecp2*-deficient animals suggesting a defective axonal transport *in vivo*. We showed, using fast-videomicroscopy that the velocity of Bdnf-containing vesicles is reduced in the axon of *Mecp2*-knocked down cortical neurons and this can be rescued by the re-expression of *Mecp2*. Overexpression of *Mecp2* was also detrimental for the Bdnf trafficking dynamics, which is in agreement with the observation that *Mecp2* overexpression causes a severe neurological phenotype. Defect in axonal transport is not restricted to Bdnf as transport of the amyloid precursor protein (App) that depends on Htt and Hap1 is also altered, ruling out a Bdnf-specific effect.
Our study reveals an unexpected link between Huntingtin, the protein mutated in Huntington's disease and Mecp2 that is associated with Rett syndrome and demonstrate that the Mecp2 protein plays a crucial role in the regulation of axonal vesicular trafficking.

P3.105

Influence of a delay between lesion and transplantation on neo-angiogenesis, cell proliferation and survival of cortical grafted embryonic neurons

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Damage to the adult motor cortex leads to severe deficits in motor function. One approach for overcoming the generally limited capacity of the mature central nervous system to regenerate axons in response to cell loss is the transplantation of embryonic neurons. The transplanted cells are dependent on the host circulation for the supply in oxygen and metabolic compounds. Moreover, the interaction between fetal and host endothelial cells is of major importance for the establishment of a functional vascularization.
Here, we examined the effect of a delay in transplantation after occurrence of a lesion on blood vessels evolution and grafted cells outcome. To this end we have combined the use of genetically modified mice over-expressing GFP under the control of βactin promoter as embryonic tissue donor and in vivo 2-photon microscopy in order to study the temporal and spatial patterns of blood vessels relatively to the dynamic of the graft axonal outgrowth. Cell proliferation and apoptosis in the graft was also determined at different time points by post-mortem immunostaining.

Our data show that the blood supply to the cortical tissue transplanted without a delay originated primarily from the regenerated host vessels. By contrast, the microvasculature of the cortical tissue transplanted into the lesion cavity with a delay of 1 week arise both from the transplanted tissue and sprouting of the host vessels. In addition, a delay between lesion and transplantation enhanced grafted cell proliferation, survival as well as the density of the projections developed by grafted neurons.
Taken together these results show that introducing a delay between the lesion and transplantation can significantly enhance graft vascularization, survival and the establishment of transplant-to-host projections. Enhancement of angiogenesis in the damaged cortical area may thus improve repair and recovery.

P3.106

Assessment of language regions plasticity in left and right focal epilepsy by functional magnetic resonance imaging

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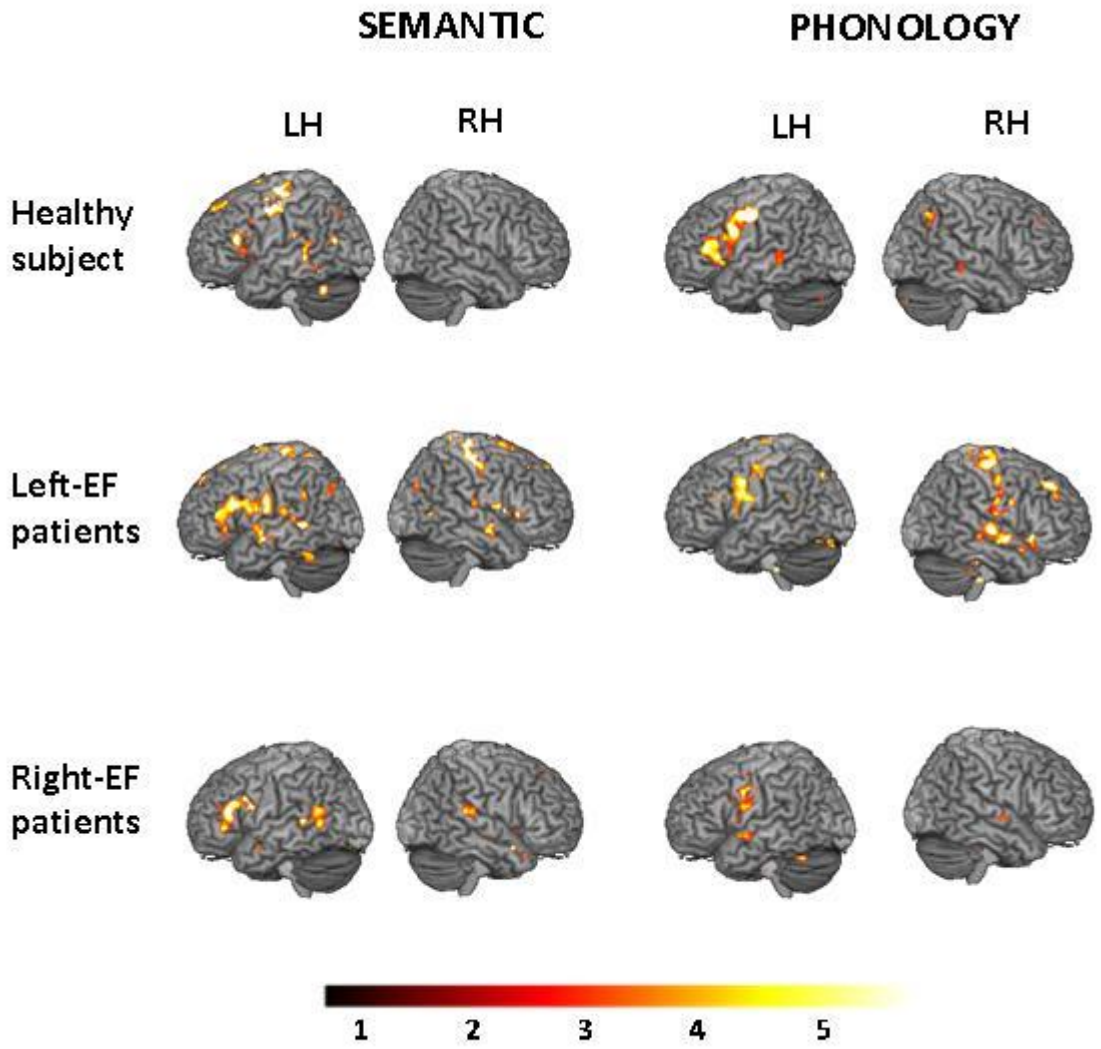
Introduction: This fMRI study explores in patients with focal epilepsy, the inter-hemispheric reorganization of language within inferior frontal (IF) and superior temporal (ST) gyri, according to three factors:
(a) hemispheric location of the epileptic focus, EF (left, right);
(b) the quantification method for assessing hemispheric specialization (lateralization indices calculation, LIC; statistical method, SM and
(c) language task (phonological, PH; semantic, SEM).
Methods: Six left and six right epileptic patients, as well as six right-handed controls, performed a PH and a SEM task. A pseudo-randomized event-related paradigm was used in two runs (SEM, PH), in a 3T MR scanner. After applying classical spatial pre-processing, two random-effect group analyses were performed with individual contrast images. Two symmetrical (left-right) regions of interest (ROI; IFG, STH) were delineated. Two quantification methods for the hemispheric specialization have been used: LIC (Abbott et al., 2010) and SM - ANOVA on parameter estimates (% of MR signal).
Results: Patients were lateralized significantly atypically then controls (Fig. 1). According to EF location, left group exhibited greater right-hemisphere involvement than right group. In patients, LIC (Fig. 2) showed the following pattern of lateralization for both PH and SEM: left predominance of IF for both EF groups; bilateralization of ST in EF-left and left-predominance in EF-right. The SM (Fig. 3) showed: IF lateralization for PH, SEM was similar to that obtained with LIC; ST lateralization for SEM was similar to that obtained with LIC; ST lateralization for PH showed a more complex pattern, illustrated in Fig.3.



[Figure1]

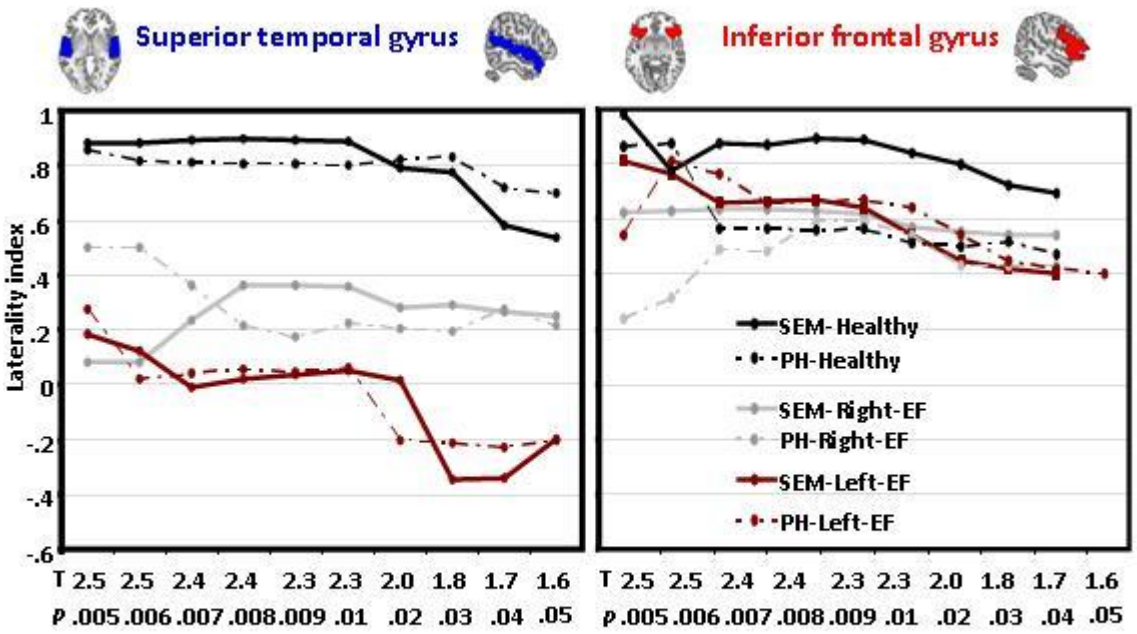


Figure 2: distribution of LI plotted as a function of T or p threshold values in Healthy and patients (left-EF, right-EF), for each task and for each ROI (IFG, STG).

[Figure2]

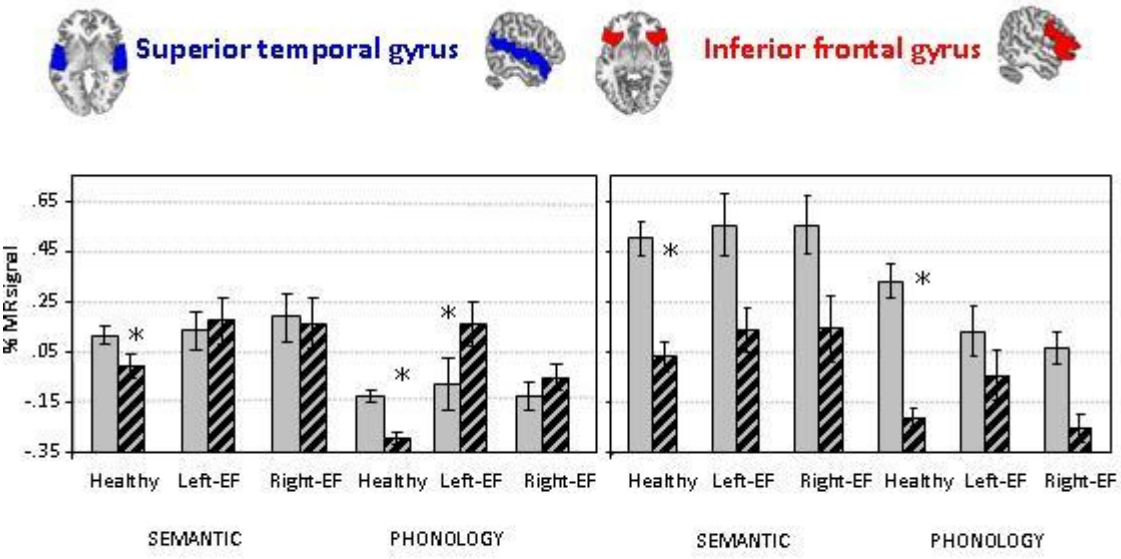


Figure 3: % of MR signals intensity variation in IFG and STG according to Hemisphere (left-LH-, right-RH-) and Participant status (Healthy, left-EF, right-EF) for the two language tasks.

[Figure3]

Conclusion: Language representation is more atypical (bilateralized) in epileptic patients than in controls. The hemispheric location of EF does not have show significant effect on specialization. Our results do not show that one quantification method is more suitable than the other one. Supplementary participants and analyses should be included and performed respectively, in order to increase the robustness of these findings.

P3.107

Investigation of seizure-generating zones in cortical malformations: multisite recordings on brain slices in a rat model of subcortical band heterotopia

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Malformations of Cortical Development are important causes of mental retardation and account for 20-40% of drug-resistant epilepsy in childhood. Although the link between cortical malformations and clinical manifestations is well established, mechanisms leading to the epileptic condition are still incompletely understood.
Mutations in the doublecortin (DCX) gene are responsible for X-linked lissencephaly and Subcortical Band Heterotopia (SBH). SBH is caused by improper neuronal migration, with DCX-deficient neurons accumulating at abnormal positions, forming a band of ectopic neurons in the white matter. The majority of patients with SBH have mild to moderate mental retardation and intractable epilepsy, but clinical studies have been unable so far to precisely identify seizures generator(s).
A rat model for SBH can efficiently be generated by knocking-down Dcx expression with in utero RNAi, causing migratory arrest of Dcx-deficient neurons and SBH formation (Bai et al., 2003). This model (the Dcx-KD rat) accurately reproduces the main genetic and anatomic features of the human disease, and is also associated with aberrant network activities (Ackman et al., 2009) and spontaneous epileptic manifestations (Lapray et al., 2010). The zone responsible for seizure generation in this relevant animal model is however still unknown. Here, we investigated this important issue by using extracellular multisite recordings from microelectrode arrays on cortical slices prepared from Dcx-KD and control rats.

P3.108

Impact of inhaled nitric oxide on the white matter injury induced by prenatal hypoxia and postnatal hyperoxia in neonatal rats

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White matter injury (WMI) remains the common cause of cognitive deficits and cerebral palsy in premature infants. Oxidative stress has been supported as a contributing factor to the pathogenesis of WMI. Despite major advances in the mechanisms leading to WMI, neuroprotective strategies are still limited. Inhaled nitric oxide (iNO) is widely used in treatment for respiratory diseases in newborns. Recent studies suggested that iNO also has a remote effect on the developing brain by promoting myelination in neonatal rodent brain. In the present study, we fist described a model of white matter injury induced by postnatal hyperoxia following gestational hypoxia in the neonatal rat brain, which is highly relevant for clinical practices in NICU. We next investigated the impact of iNO on these WM lesions.
Pregnant rats were exposed either to hypoxia (FiO_2=10%) or normoxia (FiO_2=21%) for embryonic days (E) 5 to E21. One day before delivery, they were randomly assigned to hyperoxia (FiO_2=80%) or normoxia for 8 days (E21 to postnatal day (P) 7). Rat pups in experimental groups were exposed to iNO 5ppm from E21 to P7. Brains were evaluated at P3 and P10 using immunohistochemistry and RT-PCR analysis.

Rat pups subjected to gestational hypoxia or to postnatal hyperoxia exhibited an increase of microglial activation, cell death in white matter. These pups showed a myelination deficit associated with a reduced density of both immature and mature oligodendrocytes (OLs) and a reduction of transcription factors leading to differentiation and maturation of OLs such as Nkx2.2, P27kip1, and Sox10. All these white matter changes were potentiated in pups exposed to postnatal hyperoxia following gestational hypoxia. iNO attenuated hypoxia and/or hyperoxia induced WMI by reducing microglial activation and cell death, promoting cell proliferation, preventing reduced myelination and enhancing OLs maturation. These findings demonstrated that combination of postnatal hyperoxia and prenatal hypoxia results in a more severe brain injury and indicated caution when applying high oxygen concentration during neonatal resuscitation. Our data demonstrated the neuroprotective role of low doses of iNO in neonatal rats.

P3.109

A new subcortical heterotopia gene critical for neurogenesis and migration

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The proliferation, migration and differentiation of neurons are processes critical for cortical development. A number of cytoskeletal proteins (e.g. DCX, LIS1, TUBA1A) have been found to be mutated in severe cortical malformations, such as type 1 lissencephaly and subcortical band heterotopia (SBH), associated with intellectual disability and epilepsy. Here, we used homozygosity mapping and transcriptome studies to identify the mutant gene in the *HeCo* mouse model. *HeCo* mice arose spontaneously and exhibit a heterotopic band of cells in the isocortex. This abnormal band contains proliferating cells as well as pyramidal cells and interneurons. Mice suffer from epilepsy and cognitive deficits, thus resembling SBH in human. Transcriptome studies revealed the perturbed expression of a gene, which maps to the genomic region identified by homozygosity mapping. In *HeCo* mice, a retrotransposon insertion in an intron disrupts the full length gene and creates aberrant chimeric transcripts. Screening the human ortholog in heterotopia patients identified compound heterozygous mutations in three affected individuals in one family, with a severe form of globular heterotopia. We show that this gene is expressed in proliferating, migrating and differentiating neurons during corticogenesis in a high lateral, low-medial gradient. These studies thus identify a new heterotopia gene, pinpoint a new biochemical pathway involved in cortical development and contribute to our understanding of pathophysiological mechanisms leading to subcortical heterotopia.

P3.110

Differential involvement of basal ganglia structures during motor seizures in a primate model

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Focal motor seizures are characterized by an excessive synchrony among a neuronal population located in the motor cortex, leading to controlateral motor signs. The involvement of the whole basal ganglia (BG) network during focal motor seizures remains largely unknown. To understand the relationship between neocortical paroxysmal focal discharges and BG, we studied the modifications of their neuronal activity during seizures.
This study was performed on two *Macaca fascicularis*. A cannula was attached to the skull to inject penicillin on demand within the arm motor cortex territory. We simultaneously recorded single unit activity of BG and epidural EEG. For each neuron, we calculated the mean firing rate (FR) and evaluated the type of firing pattern (rhythmic oscillatory or irregular), during interictal periods and at the onset and the offset of the seizure. The statistical reliability of differences between these different periods was assessed by non parametric tests.
The mean FR of neurons of the input stages of BG was significantly higher during seizures as compared to interictal periods (STN: 29.4±19.8 Hz vs.18.9±12.3 Hz; GPe: 74.6±49.5 vs 47.6±31.4; putamen: 11.6±7.7 Hz vs. 5.9±4.9 Hz). The proportion of oscillatory neurons significantly increased during the seizure, compared to the interictal period, and reached 71% and 77%, in STN and GPe respectively, at the end of the seizure. The rhythmic activity of these neurons was synchronized with the ictal EEG spikes. In the putamen, oscillatory neurons increased significantly (53%) during seizure as compared to interictal state but remained stable during the course of the seizure as compared to STN-GPe neurons. Furthermore, oscillatory frequency of neurons of the putamen stayed significantly above that of cortical ictal spikes. Interestingly, no significant modification of the firing was found during the seizure neither in the GPi nor in the SNr cells.
In conclusion, the STN-GPe loop strongly follows the ictal EEG pattern while the putamen, even if involved, behave independantly during motor seizure. Surprisingly, SNr and GPi are not involved during the seizure. BG behave differently during motor seizure and that features must be taken into account when considering neuromodulation of BG to abort seizure.

P3.111

Neurotoxicity of metabolites originating from the organophosphate herbicide glufosinate ammonium: a comparative study

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Glufosinate ammonium (GLA) is an organophosphorous amino acid, structurally related to glutamate. GLA is the acting part of a broad-spectrum herbicide used all around the world. It is a competitive and irreversible inhibitor of glutamine synthetase (GlnS) a vital enzyme in plants.
An increase in the use of GLA-containing herbicides could be expected because of:
i) the introduction of genetically engineered crops of agro-economical interest tolerant to GLA, in several countries;
ii) the arrival of weeds naturally resistant to glyphosate, the currently most used herbicide.
This would confer to GLA-containing compounds the role of substitute herbicide.
Several studies have shown that GLA is neurotoxic in mammals. GLA is able to inhibit brain GlnS. This astroglial enzyme play a key role in the Glu/Gln/GABA cycle and brain glutamate homeostasis. In a previous study we showed that intraperitoneal (i.p.) injection of GLA, 75 mg/kg, induces in mice seizures we characterized by behavioural and electroencephalographic means. Recently, we showed effects of GLA on mouse brain after chronic treatments, at doses comparable to the "Acceptable Daily Intake" for Human. We found that such treatment induces memory impairments, brain structural modifications, astroglyosis and brain GlnS activation.
The transgenes, inserted in GLA-resistant crops encode an N-acetyltransferase able to give N-acetyl-glufosinate (NAG) from GLA. NAG is no more an inhibitor of plant GlnS. NAG is the main metabolite found in GLA-tolerant crops. In addition, five hydroxy-methylphosphinyl (hmp) organic acids were also characterized: 4-(hmp)-2-oxo-butanoic acid (PPTO); 4-(hmp)-2-hydroxy-butanoic acid (PPTOH); 4-(hmp)-butanoic acid (MPB); 3-(hmp)-propionic acid (MPP) and 2-(hmp)-acetic acid (MPA). These metabolites could be present in animal or human diet *via* ingestion of foods prepared from GLA-

resistant crops. Excepted for MPP, nothing is known about the potential neurotoxicity of these metabolites.
In the present work several metabolites, (PPTO, PPTOH, MPP, MPA, NAG) were synthesized to treat mice under acute conditions. Their effects on CNS were investigated by behavioural observations, electroencephalographic recording and measuring GlnS activity in three different brain areas.

P3.112

Protein Phosphatase-1 repression by cocaine is mediated by methyl-CpG binding protein-2 (MeCP2) in adult rat brain

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The catalytic subunit of protein phosphatase-1, PP1β, is highly expressed in the brain and its intervention in synaptic and structural plasticity is well documented. PP1 has also been shown to be implicated in long term depression and in learning and memory mechanisms. Since chronic intake of drugs of abuse is known to induce brain plasticity and implies memory formation and stabilization, PP1 is likely to participate in the long term effects of drugs of abuse. Our previous results have shown, using microarrays, that *PP1β* is differentially regulated by repeated injections of cocaine to rats. We have also demonstrated that cocaine induces the expression of the methyl-binding protein MeCP2 together with epigenetic modifications and provided the first evidence for changes in brain DNA methylation triggered by a pharmacological agent. Whether the regulation of *PP1*β expression by cocaine can be mediated by DNA methylation or by MeCP2 was therefore investigated.
Treatment of PC12 cells with the demethylating agent 5-aza-2'-deoxycytidine resulted in a strong increase in the level of *PP1*β RNA, whereas that of *PP1*α remained unaffected, indicating that *PP1*β is specifically regulated by a DNA methylation-dependent mechanism. Using chromatin immunoprecipitation (ChIP) with an antibody directed against MeCP2, we demonstrated that MeCP2 binds to the 5' region of *PP1*β gene in these cells. The binding was also observed in rat striatum in which it was found to be increased in response to chronic cocaine treatment, an increase that likely results from changes in *PP1*β DNA methylation and/or from high cocaine-induced MeCP2 levels, as previously reported. Consistent with the function of MeCP2 as a transcriptional repressor, a decrease in the expression of PP1β protein was observed after a chronic cocaine treatment using immunohistochemistry. The decrease was found in the striatum and in the prefrontal cortex.
In summary, our data show that PP1β expression in adult brain is modulated by chronic cocaine treatment and by a mechanism involving DNA methylation. The binding of MeCP2 to *PP1*β gene highlights an additional MeCP2-target gene and further supports the role of epigenetic factors in the long term effects of cocaine.

P3.113

Lentiviral-mediated silencing of PSD-95 diminishes L-DOPA-induced dyskinesia in experimental parkinsonism

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L-dopa-induced dyskinesia (LID) is a common debilitating complication of dopamine (DA) replacement in Parkinson´s disease (PD). Recent evidence suggests that LID may be associated to an alteration of DA and glutamate receptors interactions and downstream-signaling cascades. Despite our recent progress regarding the preferential recruitment and anchorage of D1 receptor (D1R), NR2A NMDA or GluR2/3 AMPA subunits at membrane in LID experimental model, the molecular mechanisms mediating such (lack of) trafficking, anchorage, and function remain elusive. Interestingly, the synaptic scaffolding protein PSD95 thought to play a role in stabilizing glutamate receptors in the postsynaptic density, interacts with D1R and regulates its trafficking and function. Furthermore, expression and subcellular distribution of PSD95 are knowingly altered in rat model of LID.
To demonstrate whether the modulation of PSD95 levels might interfer with LID severity, we investigated the consequences of overexpressing / downregulating PSD95 expression using lentivirus (LV) constructs in experimental model of LID. 6-OHDA-lesioned rats were injected into the striatum with LV carrying shPSD95, PSD95 or controls (shPSD95+PSD95 or nonsense). After a recovery of 3 weeks, rats were treated with L-dopa (10 days, 6 mg/kg, ip) and the frequency of abnormal involuntary movements (AIMs) was scored. In a second experiment LVs were injected after animals were rendered dyskinetic by chronic L-dopa treatment, and, following a 2-week of recovery, L-dopa treatment was resumed. Knockdown of the PSD95 suppressed AIMs induced by L-dopa. In a third experiment shPSD95-LVs were injected in the gold standard model of LID, the MPTP-lesioned macaque model rendered dyskinetic by chronic L-dopa treatment. Knockdown of the PSD95 suppressed LID compared with control group.
Our results suggest that downregulation of PSD95 not only interfers with the development of dyskinesia but also reverses already established dyskinesia, thus identifying PSD95 as a novel therapeutic target and support feasibility of gene therapy approach for controlling dyskinesia.

P3.114

Continuous high frequency Subthalamic nucleus DBS controls ongoing focal motor seizures in a primate model

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Seizures arising from primary motor cortex are often pharmacoresistent, and disabling with frequent controlateral motor jerks often leading to functional hemiparesis and cognitive decline. In these cases, surgery is not appropriate as post operative motor deficits occur due to location of epileptic focus in eloquent area. Basal ganglia are implicated in seizure generation and propagation. Micro electrode recordings performed in past studies indicated that input structures of basal ganglia like as GPe,putamen,STN are strongly modified during seizures. Pilot studies in humans showed the possible effect of chronic DBS applied to STN to treat pharmacoresistant motor seizures. Here, we aimed at studying the therapeutic effect of electrical inhibition of the STN with high frequency stimulation.
We generated and characterized a stable, predictable model of focal motor epilepsy by intracortical injection of penicillin in 2 primates and documented it's pharmacoresistence. We then stereotactically implanted DBS electrodes in the Subthalamic nucleus (STN) and embedded stimulator at the back of the animals. Subthreshold stimulations were applied,at 130 Hz,. Stimulator was turned ON when penicillin was injected. Sham stimulation at 0 volt was used as a control situation, each monkey being its own control. The time course, number and duration of seizures occurring during epochs of 1 h were compared during ON and sham stim periods. Each experimental session lasted 6 to 8 hours,.
Results: A total of 1270 seizures were induced during 31 sessions in 2 primates. The occurrence of first seizure was significantly delayed as compared to sham situation. Total time spent in focal seizures was significantly reduced by ≥60% on an average ($p \leq 0.05$) after STN stimulation, due to a significant decrease in the number of seizures especially so during the first 3 hours after stimulation.

The duration of individual seizures reduced moderately. There was a trend of a decrease in interictal spikes during ON stimulation. Bipolar and monopolar stimulation modes were equally effective.
This study provides original data in primates showing the potential therapeutic effect of chronic HFS-STN DBS to treat focal motor seizures which could be translated to human disease condition.

P3.115

Serotonin 5-HT_4 receptors contribute to the preferential vulnerability of female to eating disorders under stress

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Anorexia nervosa is one of the leading causes of death in young women in industrialized countries. The 5-HT_4 receptor (5-HTR_4) knock-out (KO) male mouse is hyposensitive to stress-induced anorexia. Here, we found that 5-HTR_4 KO female mice were insensitive to stress-induced anorexia. An acute intraperitoneal injection of RS39604, a 5-HTR_4 antagonist (0,5 mg/kg), also suppressed stress-induced anorexia in wild type (WT) mice. We then sought to determine whether additional modifications could be associated with this gender difference in responses to stress. Since type II diabetes is currently associated with anorexia, we analyzed the levels of two anorectic agents, insuline and leptine. In baseline conditions, the levels of pancreatic insulin, but not in plasma, was decreased only in 5-HTR_4 KO female mice (-50%). Following stress the levels of pancreatic insulin decreased in wild-type female but not in 5-HTR_4 KO female mice. The absence of 5-HTR_4 in KO male mice did not affect the levels of insulin in any of experimental conditions. No changes in glycemia were detected with the exception of slight increase in mutant female mice in basal and stressful conditions. Other gender differences included lowered leptinemia in both unrestrained and restrained 5-HTR_4 KO male mice. This study indicates that 5-HTR_4 are involved in the outcomes of stress and may probably participate in triggering the preferential vulnerability of women to eating disorders.

P3.116

Pharmacogenetic response to tianeptine in major depressive episode associated with 5HTT/SLC6A4, 5HTR2A and THP2 genes

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Introduction: Major depressive episode, aka depression, is the most frequent psychiatric disorder, affecting 5-10% of the worldwide population with a sex ratio of 2 women for 1 man. Antidepressant drugs are available for treatment of this disease but more than 30% of patients failed to respond to this therapy. Treatment response to antidepressant drugs is modulated by genetic factors. The tianeptine molecule does not inhibit serotonin reuptake as usual antidepressant treatments. Possible targets to this treatment are the serotonin transporter (5HTT/SLC6A4), the serotonin receptor 2A (5HT2A) and, the neuronal-specific enzyme that controls brain serotonin synthesis, the tryptophan hydroxylase-2 (TPH2).
The aim of this work was to study the pharmacogenetic response to this specific tianeptine treatment in MDE. In this goal, the genotyping of polymorphisms in candidate genes 5HTT/SLC6A4, 5HTR2A

and THP2 was performed in outpatients treated with tianeptine for a major depressive episode (MDE) to search for an association to a positive treatment response.
Methods: A total of 3500 outpatients were treated with tianeptine for a MDE during 4 to 8 weeks. The criteria for a MDE were examined by the clinicians according to the DSM-IV diagnosis and the duration of each symptom was recorded during the inclusion, and at 4 to 8 weeks of treatment. The Hospital Anxiety and Depression Scale (HAD) was evaluated at the two visits. DNA was extract from saliva sample and screening of single nucleotide polymorphisms (SNPs) was carried out by Taqman assays.
Results: All clinical and genotype data were collected and complet for 1855 tianeptine-treated patients. The SNP rs6354 in SLC6A4 gene was significantly associated with response to tianeptine (p=0.009; Odds ratio=1.26; 95% confident interval=1.06-1.50). Two SNPs in 5HT2A gene and one in TPH2 gene were also associated to treatment response (rs7322347 p=0.03, rs7997012 p=0.04 and rs7955501 p=0.04).
Conclusions: We found a pharmacogenetic association between variants of serotonin genes and the response to tianeptine in major depressive episode.

P3.117

Trace amine-associated receptor 1 (TAAR1) modulates monoaminergic neurotransmission and controls monoamine-driven behaviors, as revealed by the first potent and selective TAAR1 agonist, RO5166017

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Trace amines (TAs), such as p-tyramine, beta-phenylethylamine and tryptamine, are metabolites of amino acids with structural similarities to classical biogenic amines. TAs are found at low concentrations in the brain and have long been viewed as “false neurotransmitters”. In 2001, discovery of the trace amine-associated receptor 1 (TAAR1), a dedicated seven-transmembrane domain receptor binding TAs with high affinity, suggested specific roles for TAs. Given the well documented link of TAs to neuropsychiatric disorders such as depression, schizophrenia and addiction, TAAR1 has quickly become a promising drug target. Studies in TAAR1 knock-out mice have supported this hypothesis, revealing that TAAR1 is an important modulator of the monoaminergic system. The mutants are hypersensitive to the effects of amphetamine, with enhanced locomotor activity and increased striatal release of dopamine, serotonin and noradrenaline.
Further deciphering of TAAR1 biological functions now requires selective ligands. Using the first antagonist, EPPTB, we recently demonstrated in dopaminergic neurons of the ventral tegmental area that TAAR1 is functionally associated to the dopamine receptor D2, and that it tonically activates inwardly rectifying K+ channels to reduce basal firing frequency. Now, we report on the first selective TAAR1 agonist, RO5166017, showing high affinity and potent functional activity at TAAR1 stably expressed in HEK293 cells, high selectivity versus other targets and favorable pharmacokinetic properties. In mouse brain slices RO5166017 inhibits the firing frequency of dopaminergic and serotonergic neurons in regions where TAAR1 is expressed, i.e. the ventral tegmental area and dorsal raphe nucleus, respectively. Similar to the antipsychotic olanzapine, RO5166017 prevented cocaine-induced hyperlocomotion in wild-type but not *Taar1* knock-out mice, suggesting specific TAAR1-mediated antipsychotic-like effects. These data reveal that TAAR1 plays neurophysiological roles and further document its close interaction with the monoaminergic systems. Selective TAAR1 agonists may represent novel types of treatments in neuropsychiatric disorders.

P3.118

Role of the striatal direct pathway in normal and pathological behaviors

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The striatum is the main input structure of the basal ganglia network, a set of subcortical interconnected nuclei, involved in cognitive and motor processes. The dorsal striatum is implicated in

motor function and learning whereas the ventral striatum is essential for motivation and drug reinforcement. GABAergic medium sized spiny neurons (MSNs), which constitute 95% of striatal neurons, form two pathways: striatonigral MSNs project to the internal part of the globus pallidus and substantia nigra pars reticulata (inhibitory direct pathway) and coexpress dopamine receptors 1 and substance P whereas striatopallidal MSNs project to the external part of the globus pallidus (excitatory indirect pathway) and coexpress dopamine receptors 2 and enkephalin. The specific role of these two pathways in striatal functions remains poorly understood because the two populations of MSNs are morphologically and electrophysiologically indistinguishable. To identify the function of the direct pathway in basal ganglia-related behaviors, we designed transgenic mice in which we used bacterial artificial chromosomes to selectively express the human diphtheria toxin (DT) receptor in striatonigral MSNs to allow their inducible specific ablation. We are currently controlling the selectivity of cell loss by immunohistochemistry and *in situ* hybridization after local intrastriatal administration of DT. We showed that the mice expressed specifically the transgene in striatonigral MSNs and that striatal injection of DT leads to the ablation of striatonigral MSNs. The next step consists in testing the transgenic mice in two behavioral paradigms in which the striatonigral MSNs are supposed to play a crucial role: a protocol of drug reinforcement and of L-DOPA-induced dyskinesia, a very invalidating side effect due to the chronic treatment of parkinsonian patients with L-DOPA.
This research is supported by the CNRS, Université de la Méditerranée, Fédération pour la Recherche sur le Cerveau and Fondation de France.

P3.119

Early life stress - and maternal diet - induced programming of vulnerability to natural reward: influence of sex differences

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Aversive events during the early life induce persistent epigenetic modifications that lead to behavioral and neurobiological impairments in the individual. In rats, offspring of pregnant dams submitted to prenatal restraint stress (PRS rats; 45 min/day during the last 10 days of gestation) display depressive-like disorders and increased vulnerability to drugs of abuse. Interestingly, PRS acts in a sex-dependent manner with males and females displaying two distinct behavioral and neurochemical profiles. First, we wanted to assess in PRS animals (PND 23-24 and 5/6 mo. of age) the vulnerability to social and food-reinforcing stimulus. PRS and non-stressed (controls) rats were tested in conditioned place preference for social/ high palatable (HP) food stimulus (4 and 8 days of training, respectively). PRS male rats were more sensitive to social and food-reinforcing stimulus thus confirming enhanced vulnerability to addiction-like profile; interestingly, PRS induced an opposite effect in females. Then, we evaluated the interplay of PRS with prenatal supplementation with folic acid (FA). Actually, FA given as a supplement during pregnancy has been credited with a beneficial role in preventing a range of birth defects, and low folate status has been associated to depressive phenotype in later life. FA was supplement in pellets (5 mg/kg) throughout pregnancy and lactation period. We found that maternal behavior was reduced by gestational stress and that FA-supplementation exacerbated this profile. In addition, maternal consumption of a FA-supplement diet during pregnancy and lactation acted synergistically with PRS, thereby programming an increased drive for the consumption of palatable foods in PRS male rats only. Our results indicate that early life stress has a strong impact in vulnerability to natural reward and highlight the importance of sex differences in determining such vulnerability.
Keywords: Prenatal restraint stress, programming, conditioned place preference, folic acid, high palatable food

P3.120

Cellular mechanisms of α-synuclein-mediated neurotoxicity

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α-Synuclein (α-Syn) is a small phosphoprotein of unknown function that plays a central role in Parkinson´s disease. Here, we studied the behavioral and cellular consequences of human α-Syn expression in *Drosophila* neurons. Pan-neuronal expression of either native α-Syn or its mutant pathogenic form α-SynA30P triggers progressive locomotor impairments and strongly decreases brain dopamine levels. No noticeable loss of dopaminergic neurons was observed though these cells present striking abnormalities, such as swelling of mitochondria and of axonal varicosities. Ectopic dopamine synthesis in serotoninergic neurons makes these cells susceptible to α-Syn toxicity, potentially explaining dopaminergic neuron vulnerability. Further, we observed that pan-neuronal expression of α-SynA30P but not native α-Syn induces a pre-apoptotic state in dopaminergic neurons and decreases *Drosophila* resistance to oxidative stress. Native α-Syn, in contrast, shows neuroprotective effects against both α-SynA30P and oxidative stress in the *Drosophila* model.

P3.121

Atypical gaze preference for non-social situations in autism spectrum disorders

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Autism Spectrum Disorders (ASD) are a group of five closely-related neurobiological disorders (Autistic Disorder, Asperger's Disorder, Pervasive Developmental Disorder Not Otherwise Specified, Childhood Disintegrative Disorder, and Rett's Disorder) characterized by a triadic symptomatology : altered social interactions, impairment in verbal and non verbal communication and repetitive and stereotyped behavior, interest and activitiy (Phetrasuwan et al. 2009). Impairment in biological motion perception (Blake et al. 2003 ; Hubert et al. 2007 ; Freitag et al. 2008) associated to an altered mirror neuron system are questioned as deleterious factors for social behavior development in ASD. In healthy adults, cerebral activation (Lotze et al, 2006 ; Hirai and Kakigi, 2009) and cognitive processing (Neri, Luu & Levi, 2006) are different whether human motion portrays emotion or communication. We propose to test overt visual-spatial attention to human motion in social and non-social situations in ASD patients and healthy population.
Using a non invasive eye tracking methodology, we recorded visual exploration of 25 patients with ASD compared to 70 healthy participants, aged from 4 to 43 years. Both groups were presented point-light animations (Johansson, 1973) depicting two human agents in social interaction (games, sharing emotion, non verbal communication) or performing non-social motion (non expressive and non communicative). Both kinds of animations were simultaneously displayed.
Results showed that ASD group explored social ($p < .001$) and non-social stimuli ($p < .01$) less than control group. Moreover ASD group explored social stimuli less than non-social stimuli ($p < .01$) and age had a significant effect on exploration of social and non-social stimuli in ASD and control groups.

These results suggest a lack in overt visual-spatial attention to social human motion in ASD patients. Moreover attention to social human motion increase with age in healthy population and ASD patients. The effect of mental development and social abilities are discussed.

P3.122

Cognitive dysfunction in a transgenic rat model of Huntington's disease using an spatial operant reversal task (SORT)

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Huntington's disease (HD) is an autosomal dominant disorder caused by an expanded CAG trinucleotide repeat. HD is dominated by chorea and other involuntary movements but it is becoming clearer that HD patients have cognitive deficits starting prior to motor dysfunction. The HD 51 CAG transgenic rat (von Hörsten et al, 2003), exhibits progressive cognitive and motor deficits that resemble human HD. Previous research has shown that motor deficits begin when these rats are 12 months of age, but there are no reports of cognitive dysfunction occurring prior to this. The present study used a spatial operant reversal task (SORT) to assess early motor, motivation, and memory function. Nine month old male, homozygotic and wild type rats were maintained at 85% of their baseline weight and underwent three testing phases in the operant chamber. The first phase lasted nine days and used a progressive fixed ratio schedule (FR), starting at FR2 and continuing to FR512, with the left lever, but not the right lever, being reinforced. The second phase was the spatial reversal portion and was identical to the first, but with only the right lever presses being reinforced. The third phase consisted of FR32, with the correct lever being determined pseudo-randomly. On these tasks homozygote HD rats made significantly more spatial reversal errors on days 1, 8 and 9 (FR2, FR256, and FR512) and significantly more errors on days 1, 2, 4, and 5 on the pseudo-random portion of the study than did wild-types. No significant differences were found between the HD rat and wild type brains when measuring the area of the cortex, striatum and the lateral ventricles, using cytochrome oxidase labeling. Also, no significant differences were found in the number of NeuN positive cells in the cortex and striatum using unbiased stereology. These results indicate that this operant model successfully detects early cognitive dysfunction, prior to motor deficits, in the HD 51 CAG transgenic rat, and suggest that the SORT could provide a useful screen for therapeutic studies. The cognitive deficits observed in this operant model of HD are not due to overt cell loss or large structural changes, but may be due to some underlying mechanism of cell dysfunction or disrupted cortical-striatal pathways.

P3.123

Do Parkin and alpha-Synuclein interact in a common physiopathological pathway leading to Parkinson's disease?

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Parkinson's disease (PD) is a common neurodegenerative disorder with severe motor symptoms due to the progressive degeneration of the dopaminergic nigrostriatal pathway. Lewy bodies (LB), intracellular inclusions mainly composed by alpha-Synuclein (alpha-Syn), are an additional neuropathological hallmark.
Mutations in *alpha-syn* (A53T, A30P, and E46K; multiplications) lead to abnormal accumulation of the protein and cause autosomal dominant forms of PD. Mutations in *parkin* lead to loss of protein function and to autosomal recessive early-onset PD. Several post-translational modifications of alpha-Syn (phosphorylation on S129, ubiquitylation, truncation) have been identified but their roles in protein aggregation and disease are still unclear. A consistent set of data suggest that alpha-Syn and Parkin act in a common physiopathological pathway. In particular, overexpression of Parkin has neuroprotective effects in drosophila and rodent models of synucleinopathy.
We studied the effects of Parkin deficiency in transgenic mice overexpressing the A30P variant of human alpha-Syn (hA30P-alpha-Syn), which develop an age-dependent neurodegenerative motor phenotype. We analyzed motor impairment by a longitudinal behavioural follow-up and pathological accumulations of alpha-Syn species by immunohistochemistry and electron microscopy. Surprisingly, Parkin deficiency delayed disease manifestation a dose-dependent manner in hA30P-alpha-Syn mice. Symptomatic mice presented neuronal deposits of S129-phosphorylated and ubiquitylated alpha-Syn throughout the brain stem and spinal cord. By using a newly developed antibody against alpha-Syn truncated at D135 (Elan Pharmaceuticals), we demonstrated the presence of D135-truncated alpha-Syn species co-localizing with S129-phosphorylated alpha-Syn only in diseased mice; these species were also detected in LB in brains of PD patients. Parkin deficiency did not alter the pattern of accumulation of these proteins or the characteristics of the deposits in end-stage animals.
This work supports the hypothesis that Parkin and alpha-Syn interact in early stages of neuronal dysfunction, although the underlying molecular mechanisms remain to be clarified.

P3.124

Behavioural, biochemical and electrophysiological consequences of lead exposure in the rat

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Lead exposure is known to affect the central nervous system, particularly during brain development. As little is known about lead exposure in adult stage, the present study was aimed to investigate its effects on motor and non-motor behaviors, on monoamine levels, and on the neuronal activity of subthalamic nucleus (STN), a basal ganglia structure playing a key role in the pathophysiology of Parkinson's disease. Sprague-Dawley male rats were exposed to lead acetate (20mg/kg/day, i.p.) during 3 weeks. Control animals received sodium acetate (20mg/kg/day, i.p.) injections during the same period of time. A battery of motor and non-motor behavioral tests was used. “Open field” actimeter and rotarod were used to quantify the locomotor activity and motor coordination respectively. Sucrose preference test was used to evaluate depression-like behaviour and elevated plus maze for anxiety. At the end of behavioral tests, animals were used to record STN neuronal activity using extracellular recordings. Our results show that lead-exposure induced motor disabilities expressed by a reduction in locomotor activity and deficits in motor coordination. Furthermore, lead induced anxiety without “depressive-like” behavior. Electrophysiological results show that lead affected the discharge pattern of STN neurons with an increase in the number of bursty cells without affecting the firing rate. Taken together, our results suggest that the bursty pattern of STN neurons observed after lead-exposure may be at the origin of motor and non-motor disabilities manifested by the animals.

P3.125

Involvement of T-type calcium channels in tonic seizures revealed using maximal electroshock seizure in mice

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The role of T-type calcium channels in generalized epilepsy has been identified using pharmacology and genetic animal models in rats and mice. In the case of absence epilepsy, changes in both Cav3.1 and Cav3.2 expression levels and properties have been identified. On the contrary, little is known regarding the role of T-type calcium channels in induced generalized epilepsy models. Towards this aim, maximal electroshock seizure (MES) was used to determine whether T-type calcium channels are involved in tonic-clonic seizures in mice. The MES model consists of a corneal application of an electrical impulse (50Hz, 38mA, 0.5s). In mice, MES induces tonic-clonic seizures that are inhibited by anticonvulsivant drugs, such as carbamazepine. On one hand, using Cav3.1 knockout (KO) mice, we found that Cav3.1 KO mice develop less tonic seizures than wild type (wt) mice. This suggests that Cav3.1 is potentially involved in the development of tonic seizures. Similarly, we have identified that oral administration of a selective T-type calcium channel inhibitor, TTA-A2, induced a strong and significant decrease of the percentage of mice showing tonic hind limb seizure induced by MES. Notably, we did not observe any protective effect of TTA-A2 on clonic seizures induced either by MES or by the injection of 60mg/kg or 80mg/kg of the GABAergic antagonist pentylentetrazol (PTZ). Importantly, the protective effect of TTA-A2 is markedly reduced in Cav3.1 KO mice. Altogether, these data are in support of a role of T-type calcium channels in the development of tonic seizures and suggest that the protective/anticonvulsivant properties of TTA-A2 on tonic seizures would be mediated mainly by the Cav3.1 isoform.

P3.126

Effect of anti-Nogo-A antibody treatment in hand dexterity recovery following unilateral hemisection: electrophysiological study in non-human primates

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Anti-Nogo-A antibody treatment has shown in both rat and non-human primate to improve recovery of hand dexterity following spinal hemisection. Such behavioral improvement was correlated to new sprouting of corticospinal (CS) axons caudal and rostral to the lesion. Nevertheless, the functional role of such new CS sprouting in the recovery process needed to be assessed. Separately the BDNF has shown to improve axons growth and to be implicating in inhibition of the neurite outgrowth inhibition, so potentially to improve the recovery after a spinal cord injury. In recent work we assessed the effect of combined treatment of anti-Nogo-A antibody and BDNF after a spinal cord lesion in adult macaque monkeys using transcranial electrical stimulation (TES). The obtained results were correlated to behavioral recovery of the hand dexterity. The behavioral and TES data was analyzed in 4 adult monkeys that were submitted to unilateral cervical spinal lesion (C7/C8). Two monkeys were treated intrathecally with anti-Nogo-A antibody and BDNF, whereas a control antibody was infused in the other monkeys.

The TES results showed that there were no significant differences between treated and untreated monkeys. These results were correlated with the recovery of the hand dexterity that didn't show any beneficial effects of this combined treatment compared to the control group.
Therefore, following these results our ongoing study will investigate the functional role of these new projections only in anti-Nogo-A antibody treated monkeys, using sophisticated methods: stimulus triggered averaging of EMG activity from chronically recorded forelimb muscles in monkeys before and after lesion. In this project, we will focus principally on the primary motor cortex.

P3.127

Sex specific effect of prenatal restraint stress on circadian pattern of activity in rats

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Prenatal restraint stress (PRS) in the rat is a well-documented model of early stress known to induce depression-like behavior. Interestingly, most the enduring changes induced by PRS are sex-dependent, with a depression-like phenotype predominating in females, and an anxiety-like phenotypes in males. PRS reduced hippocampal neurogenesis, CREB phosphorylation and mGlu5 receptor expression only in male rats. There are exceptions, however. For example, a prolonged HPA axis response to stress and a reduced expression of mGlu2/3 receptors in the hippocampus are seen in both male and female PRS rats. Here, we examined the relationships between PRS, gender and the circadian system, by monitoring the running wheel behavior in male and female adult PRS rats, first under a regular light-dark (LD) cycle, and then after an abrupt 6-h advance shift in the LD cycle. We also measured the hippocampal expression of mGlu receptors and the hypothalamic CRH content in males and females at 8.00 a.m. and at 8.00 p.m. The locomotor activity pattern of both male and female PRS rats was erratic and more fragmented compared to controls. PRS increased and decreased total locomotor activity in males and females, respectively. PRS induced a significant phase advance in the circadian activity rhythm only in male rats, and increased the time required to resynchronize the activity rhythm after an abrupt phase advance of the LD cycle to a larger extent in females than in males. At the beginning of the light phase, PRS induced a strong increase of the hypothalamic CRH levels in males but not in females. At the beginning of the dark phase, PRS increased the hypothalamic CRH levels in male rats but reduced CRH levels in female rats at the opposite PRS induced a decrease of the hypothalamic CRH levels in females rats. Finally, PRS reduced hippocampal levels of both mGlu5 and mGlu2/3 receptors at 8 a.m. and at 8 p.m. in males, and only levels of mGlu2/3 receptors in females. Those observations highlight the importance of the circadian system in the gender-specific outcome of PRS.

P3.128

Pharmacological approach to eliminate mutant GFAP aggregates in cellular models of Alexander disease

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Alexander disease (AxD) is a rare and fatal leukodystrophy caused by mutations in the gene encoding glial fibrillary acidic protein (GFAP). The pathological hallmark of this dominant disease is the formation of cytoplasmic inclusions within astrocytes known as Rosenthal fibers (RFs), consisting of wild-type and mutant GFAP co-aggregated with stress proteins. The presence of mutant GFAP and the increase in level of the protein are involved in RF formation. The aim of our work is to explore the modulation of GFAP aggregation via the protein refolding (chaperone molecules) and degradation (autophagy) machineries.
We used two *in vitro* models -murine astrocytes and human astrocytoma-derived cells (U373MG)- transiently expressing mutated GFAP (^{mut}GFAP), in which the aggregates exhibited the characteristics of RFs to test therapeutic approaches. Treatments with different molecules induced an increased expression of chaperones and autophagy pathway. These effects were associated with a significant decrease of the number of ^{mut}GFAP aggregates-bearing cells, probably due to the prevention of the aggregation process and/or elimination of ^{mut}GFAP inclusions *in vitro*. All the molecules we tested *in vitro* are easily usable *in vivo* and the next step of our study is to deliver these drugs to our ^{mut}GFAP knock-in mice.
This work is supported by ELA Research Foundation, Jerome Lejeune Foundation, and INSERM.

P3.129

Specific requirement for zebrafish ataxin-7 in differentiation of SCA7 vulnerable neurons: photoreceptors and cerebellar Purkinje and granule cells

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Expansion of a polyglutamine (polyQ) tract in the N-terminal region of ataxin-7 underlies spinocerebellar ataxia type 7 (SCA7), a dominant late onset neurodegenerative disorder, which is characterized by progressive and specific loss of photoreceptors (PR) and cerebellar Purkinje (PC) and granule cells (GC). The molecular basis of this neuronal vulnerability that contrasts with the broad expression of the causative protein remains among the most enigmatic features of this disease. To get insights into the function of wild-type ataxin-7, we cloned the zebrafish SCA7 orthologue, investigated its pattern of expression and analyzed the phenotypes induced following morpholino oligonucleotide (MO)-mediated depletion of the protein.
Zebrafish ataxin-7 transcription takes place throughout development from the one cell-stage onward. In adult brain, high levels of transcript accumulation were observed in several neuronal populations including CG, but not PC. Next, we showed that severe depletion of the protein induced embryonic lethality with the few surviving embryos showing gross developmental abnormalities. Interestingly, moderate ataxin-7 depletion did not markedly affect development, but induced severe differentiation defects of PR combined with a strong reduction in the number of PC and CG, thus demonstrating an essential and specific requirement for ataxin-7 in the differentiation of these three neuronal populations.
Altogether, our data show that while severe ataxin-7 depletion impairs embryonic development in zebrafish embryos, partial ataxin-7 loss-of-function specifically impairs the differentiation of all three neuronal populations, which are vulnerable to neurodegeneration in SCA7 patients. Because ataxin-7 is involved in histone acetylation and transcription regulation, our results also suggest that ataxin-7 plays an essential role for proper transcription of genes required for full differentiation of PR, PC and GC. In turn, because a large number of genes involved in cell differentiation also play role in the maintenance of differentiated state, our results also suggest that ataxin-7 loss-of-function could also play role in the physiopathology of the disease.

P3.130
Mocos, a new candidate gene in autism

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Molecular studies on Autistic Spectrum Disorders (ASD) have found misexpression of genes involved in cortical organization, synapse formation, neurotransmission and neuromodulation (Gepner and Féron, 2009). But what are the initial events leading to these anomalies? In order to answer this question, we studied primordial cells, i.e. undifferentiated stem cells. We focused our attention on nasal olfactory stem cells that are easily accessible in living adults (Delorme et al, 2010). Using pangenomic microarrays, we compared the transcriptome of undifferentiated nasal olfactory stem cells from 11 adults with mild to very severe ASD and 11 age- and gender-matched healthy individuals. We confirmed that the Wnt/TGF pathway was altered in most patients. We also observed, for the first time, a downregulated expression of Molybdenum Cofactor Sulfurase (Mocos), in 8 out of 11 patients. This result was validated by qPCR. We are now assessing human olfactory stem cells when they are differentiated into neurons. We expect to observe cellular anomalies in newly formed neurons when stem cells originate from ASD patients or when control stem cells are treated with a shRNA specific for Mocos.
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Delorme et al (2010) The human nose harbours a niche of olfactory ecto-mesenchymal stem cells displaying neurogenic and osteogenic properties. Stem Cells Dev. 19(6): 853-66

P3.131
Modeling and gene silencing in Spinocerebellar ataxia type 7

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Spinocerebellar ataxia type 7 (SCA7) is a dominantly inherited neurodegenerative disorder. The mutation has been identified as a CAG trinucleotide repeat expansion in the coding region of the *SCA7* gene which encodes the ataxin-7 protein (ATXN7). In the present work we engineered lentiviral vectors encoding either truncated wild-type and truncated mutant human ataxin-7 to develop an SCA7 *in vivo* model. In this study, we demonstrate that overexpression in the cerebellum of adult mice of mutant but not wild-type human ataxin-7 is associated with the formation of ubiquitinated ataxin-7 aggregates, loss of the calbindin and parvalbumin markers, disruption of the neurofilaments and microtubule-associated protein type 2, and strong activation of astrocytes, suggesting neuronal dysfunction.
Gene silencing by RNA interference (RNAi) is a process that suppresses the expression of a gene at the RNA level. RNAi holds promise as a potential therapy to treat dominantly inherited human diseases, many of which are currently untreatable, such as SCA7. Thus, miRNA-based approaches may provide more appropriate biological tools for expressing inhibitory RNAs in the brain, the implications of which are crucial to the development of RNAi for therapeutic applications. In the present work, we also engineered lentiviral vectors encoding a microRNAs (miRNA) cassette targeting human ATXN7. The efficacy of these miRNAs was assessed by co-transfecting COS-7 and 293T cells with plasmids expressing truncated wild-type or truncated mutant human ATXN7 (trATXN7-10Q or

trATXN7-100Q) with the miRNAs vectors and analyzed by immunofluorescence, western-blot and RT-PCR.
We are currently testing these miRNAs in the lentiviral-based mouse model of SCA7 in order to check if we obtain a reduction in the SCA7 neuropathological readouts of neurotoxicity.

P3.132

Oscillatory entrainment of subthalamic nucleus neurons and behavioural consequences in rodents and primates

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Oscillatory activity in local field potential (LFP) recordings in the beta band (15-30Hz) has been reported in the subthalamic nucleus (STN) of parkinsonian patients. From these studies, a direct causal link between these oscillations and the manifestation of motor symptoms has been proposed, although this hypothesis has not yet been proven and even questioned by several recent theoretical and experimental approaches. To assess this hypothesis, we investigated the functional role of STN LFP oscillations in the experimental domain of Parkinson´s disease (PD). We used LFP signals showing significant power in the beta frequency range (23Hz), as a stimulus both *in vitro* and *in vivo*. We first demonstrated in rat brain slices that STN neuronal activity was driven by the LFP stimulation. We then applied beta stimulation to the STN of 16 rats while monitoring locomotor activity. Beta stimulation was also applied to the STN of two monkeys performing arm movements. Stimulus-induced clinical effects such as hypokinesia, rigidity, tremor, dyskinesias, myoclonias and dystonias were appraised in both rats and monkeys. Stimulation of the STN at 23 Hz induced no significant change in locomotor activity and rigidity in either rodents or primates. Stimulus-induced clinical symptoms were seen only in a small fraction of the rat population. Both monkeys displayed stimulus-induced myoclonias, dystonias and tremor. The induced tremor had the same frequency as the stimulation (23 Hz), however, and thus cannot be assimilated to the typical parkinsonian rest and postural tremor. In addition to showing the first LFP induced behaviour in both rats and primates, our study demonstrates the absence of a causal link between beta oscillations and parkinsonian motor symptoms.

P3.133

Suffering of dopaminergic nigral neurons after cholinergic denervation in macaques

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Parkinson's disease is characterized by the loss of dopaminergic neurons, but non-dopaminergic lesions also exist. In particular, a decrease in the number of cholinergic neurons of the pedunculopontine nucleus (PPN) has been reported. These cholinergic neurons display a massive ascending projection to the basal ganglia, and in particular to the dopaminergic neurons of the substantia nigra at the origin of the nigrostriatal pathway. Literature data indicate that stimulating cholinergic neurons result in a release of dopamine in striatum. Our hypothesis is that degeneration of cholinergic neurons as that which occurs in Parkinson's disease induces a decrease in excitation of dopaminergic neurons, leading to the suffering of these neurons.
A cytotoxic agent specific to cholinergic neurons (diphteric toxin conjugated to urotensin) has been stereotaxically injected bilaterally in the PPN of 3 macaques (Macaca fascicularis). After a survival

delay of 5 weeks, monkeys have been sacrificed and their brains remove and cut into serial sections. Cholinergic and dopaminergic neurons have been labeled using NADPH-diaphorase reaction and immunohistochemistry of the tyrosine hydroxylase (TH), respectively. Neurons were then quantified stereologically. The cytotoxic injection induced a mean loss of 54% of NADPH-diaphorase neurons of the PPN, but no loss of TH neurons. The presence of HLA-DR cells and of fluorojade-positive neurons in the substantia nigra are signs of microglial activation. Moreover, dopaminergic cell bodies displayed morphological modifications, characterized by a decrease in their size all the more that the cholinergic lesion induced was severe.
Our data show that dopaminergic neurons suffer from morphological modification when their cholinergic, excitatory innervation is lost, a process that could be involved in the loss of dopaminergic neurons in Parkinson's disease.

P3.134

Treatment with HO1 inductor gets to deleterious ROS production in a cerebral neuroinflammation model

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Neuroinflammation process is involved in most of neurodegenerative diseases. The inducible heme oxygenase 1 (HO-1) turns heme into biliverdin, carbon monoxide and Fe^{2+}. Authors have reported its anti inflammatory properties in different rodent models. In this study, we aimed to assess the effect of HO-1 induction on microglia activation in an *in vivo* model of excitotoxic neuroinflammation in rat.
A preliminary study by Western Blot analysis was performed to check out the impact of hemin i.p. treatment on brain HO-1 protein level in rat treated by 50 mg/kg of hemin *vs* control. Twenty-two male Wistar rats were exposed to intrastriatale injection (bregma +0,7 mm) of 150 nmol of quinolinic acid (QA) or vehicle (PBS) at D0. Animals received either an inductor of HO-1 (hemin, 50 mg/kg [H50]; i.p.; n=11) or vehicle (DMSO; i.p.; n=11) every day from D0-24h to D0+48h. Rats were sacrificed at D0+72h, their brains fixed and removed for immunochemistry examination of the Cd11b as index of microglial activation.
Chronic treatment with hemin significantly induced cerebral HO-1 expression (+38 ± 2%, $p< 0.05$). Tissue processing for immunochemistry showed a significantly different loss of tissue in ipsilateral hemisphere of QA/H50 (51 ± 9%) rats *vs* QA animals (17 ± 6.5%). Cerebral damage was limited to the striatum in QA whereas it affected both striatum and cortical regions in QA/H50 rats. The Cd11b level was significantly ($p< 0.05$) increased in further cortex areas (bregma +3.1 mm) of ipsilateral hemisphere in both QA (+71 ± 5%) and QA/H50 (+541 ± 3%) rats *vs* control. Moreover, Cd11b level was significantly ($p< 0.05$) higher in QA/H50 *vs* QA animals.
HO-1 cerebral induction has been reported for its potential neuroprotective role in various rodent models. However, this enzyme appeared to worsen brain injury in our excitotoxic model. Destruction of brain structures in the cerebral hemisphere exposed to striatal injection of QA and microglial activity were both massively increased in animals receiving HO-1 inducer hemin. Such tissue destruction could be linked to ROS production but this remains to be proven. Therefore, we are currently measure the HO-1 expression and ROS levels in cerebral structures in our neuroinflammation rat model exposed to chronic hemin treatment.

P3.135

Effect of a newly designed metabotropic glutamate receptor 4 agonist with antiparkinsonian properties on glutamate and gaba activity in the Globus Pallidus

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Parkinson's disease is due to the progressive degeneration of substantia nigra pars compacta dopaminergic neurons. This dopamine loss leads to an imbalance in basal ganglia network activity with a subthalamic nucleus glutamatergic overactivation. Strategies have been developed to identify a target whose modulation could lower subthalamic nucleus activity. Metabotropic glutamate receptor 4 (mGlu4) appears to be a relevant target because it is expressed in the basal ganglia as an autoreceptor on glutamatergic and heteroreceptor on GABAergic nerve endings. Indeed, several agonists of this receptor have antiparkinsonian effects in either pharmacological or lesional models of the disease. Nevertheless the mechanisms involved in their antiparkinsonian action have still to be understood. To achieve this goal, we have used intracerebral *in vivo* microdialysis to monitor glutamate and GABA extracellular concentrations in the globus pallidus (GP) of either sham or unilaterally 6-OHDA lesioned rats in the substantia nigra pars compacta . Indeed, GP is the primary output structure of the striatum which receives both GABA striatopallidal and glutamate subthalamopallidal inputs. Consequences on GABA and Glutamate extracellular concentrations of both systemic and local (through reverse dialysis) administration of a newly designed compound, namely LSP1-2111, were studied. Preliminary data in sham animals show that this compound induces a decrease in glutamate extracellular concentration in a concentration range of 500 to 1000 µM, whereas it has no clear cut effect on GABA extracellular concentration. In contrast, LSP1-2111 at a dose of 1000 µM decreases both GABA and Glutamate extracellular concentrations in 6-OHDA lesioned rats. Antiparkinsonian effects of LSP1-2111 could thus be mediated by the modulation of both GABAergic and glutamatergic activites in the GP. These results point to a critical role of the GP in Parkinsonian conditions and reveal a specific action of mGlu4 receptors in this structure to reduce motor symptoms of the disease. Funded by ANR and Era-Net program.

P3.136

Pathological changes, altered *liver X* target gene expression and autophagy in retinae of Niemann-Pick type C mice

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Niemann-Pick type C (NPC) disease is a rare, ultimately lethal autosomal recessive disorder which causes neurodegeneration and hepatosplenomegaly. The disorder is caused by mutations in the *npc1* or *npc2* gene, which encode proteins that mediate the exit of cholesterol from the endosomal lysosomal system (EL). Npc1-deficient cells show accumulation of unesterified cholesterol and other lipids in the EL. Recent studies have shown that Npc1 deficiency induces the expression of genes that mediate cholesterol release from cells (Wang et al., 2007) and modifies the autophagy pathway in specific types of neurons (Claudepierre et al., 2010). Both changes bear the potential to develop new therapeutic approaches to NPC disease as well as other neurodegenerative diseases like Alzheimer´s. Therefore, we investigated these changes in more detail using the retina of Npc1-deficient mice as model.
As a first step, we studied how disturbances of cholesterol transfer in the EL system changes age-related protein expression in retinae of NPC1-/- mice in comparison to wildtype mice at 4, 6, 8 and 10 weeks of age. We focused on *liver X* target genes (LXR) involved in cholesterol transport (ApoA1, ABCA1, ABCG1 and ApoE) and on autophagic markers (ATG5, Beclin 1 and LC3). In parallel, we studied the onset of pathological changes in retina of Npc1-deficient mice, namely the accumulation of lipofuscin particles in the retinal pigment epithelium (RPE). To analyse the conditions for autophagy-induction in Npc1-deficient neurons, we established a cell-culture model using pharmacologic induction of the Npc1 phenotype. Altogether our study aims to reveal the impact of autophagy and LXR-mediated expression on neuronal cell death in neurodegenerative diseases and should help to find new therapeutic approaches for their treatment.

P3.137

Bee venom as a new agent for the symptomatic treatment of Parkinson's disease: behavioral and electrophysiological evidence from rat models

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Parkinson's disease (PD) is a neurodegenerative disorder resulting from the loss of the substantia nigra pars compacta (SNc) dopaminergic (DA) neurons innervating the basal ganglia, a subcortical network involved in the control of movements, and is characterized by three main motor symptoms: akinesia, rigidity and resting tremor. The current therapies are either very efficient at the beginning of the treatment but ultimately lead to debilitating side effects (L-DOPA therapy) or are restricted to eligible patients (deep brain stimulation). Thus, it remains important to develop new therapies.
A recent clinical observation (by Dr A. Hartmann) reported that the motor disabilities of a parkinsonian patient were consistently alleviated following bee venom injections within a desensitization protocol for allergy. The present study aimed at confirming the beneficial symptomatic effects of bee venom in rat models of PD and at deciphering the underlying mechanisms of action. Using a pharmacological model (catalepsy induced by systemic injection of haloperidol) and a lesional model (unilateral SNc injection of 6-OHDA), we demonstrated that systemic bee venom injection alleviates the motor deficit. Then, using an *in vivo* electrophysiological approach in anaesthetized rat, we analyzed the effects induced by bee venom injection on neuronal activity in the basal ganglia output structure substantia nigra pars reticulata (SNr) following acute interruption of DA transmission. Systemic injection of DA receptor antagonists (haloperidol or raclopride+SCH 23390) induced a bias in the influence exerted by the direct inhibitory and the indirect excitatory striato-nigral circuits as evidenced by the responses evoked in SNr neurons by stimulation of the motor cortex. Bee venom injection prevented this bias. These results suggest that bee venom restores the functional properties of the basal ganglia circuits underlying the disinhibitory process by which this system contributes to motor activity. Current analyses are focused on the impact of bee venom on the firing pattern of SNr neurons which, after dopamine antagonist injection, switched from a tonic and regular mode to an irregular one with bursts of spikes and pauses.Supported by CNRS, Fondation de France and Univ de la Méditerranée.

P3.138

Ghrelin prevents cognitive, emotional and neurochemical alterations in a mouse model of alzheimer's disease

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Increased brain deposition of amyloid β protein (Aβ) and cognitive deficits are classical signs of Alzheimer's disease (AD) that have been widely associated to oxidative stress and cholinergic neurotransmission alterations. On the other hand, recent evidences indicate that Ghrelin (Ghr), a 28 amino acid peptide hormone produced from the stomach and hypothalamus that promotes positive energy balance, presents neuroprotective and memory-enhancing properties that may be useful in AD. In the current study, we assessed the molecular mechanisms, mainly the modifications in oxidative stress parameters and acetylcholinesterase (AchE) activity, whereby the pretreatment with Ghr prevents the $A\beta_{1-40}$-induced depressive-like behavior and cognitive impairments in mice. Swiss adult mice (3 months old) received a single intracerebroventricular (i.c.v.) administration of Ghr (3 nmol/µl) or PBS (1 µl) 15 min before the i.c.v. infusion of $A\beta_{1-40}$ (400 pmol/mice) or PBS (1 µl) and 10 and 14 days later they were evaluated, respectively, in the tail suspension and step-down inhibitory

avoidance tasks. Independent groups of animals, submitted to the same treatments, were sacrificed 24 h after $A\beta_{1-40}$ infusion and cortical and hippocampal AchE activity and oxidative stress parameters were analyzed. The pretreatment with Ghr prevented the development depressive-like behavior and spatial learning and memory impairments in $A\beta_{1-40}$-infused mice. Moreover, $A\beta_{1-40}$ reduced significantly the cortical activities of the antioxidant enzymes glutathione peroxidase, glutathione reductase and catalase while promoted a marked increase in the cortical AchE activity and hippocampal lipid peroxidation. Of high importance, the pretreatment with Ghr prevented all these neurochemical changes induced by $A\beta_{1-40}$ administration in mice. Altogether, our findings demonstrate that Ghr can ameliorate Aβ-induced cognitive and emotional impairments associated with oxidative stress and cholinergic neurotransmission alterations in mice. Therefore, Ghr may represent a promising therapeutic agent for the treatment of AD

P3.139

Early programming of anxiety in infancy by maternal stress

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Stress during gestation is associated with an increased incidence of developmental and neuropsychiatric disorders in adulthood. In rat, restraint stress during pregnancy (PRS) leads to several behavioural changes, such as enhanced anxiety-like behaviour in the adult offspring. Little is known about the effect of PRS in pups and infant rats. In the present study, rats subjected to PRS emitted significantly more ultrasonic vocalizations (USVs) in response to isolation at postnatal day 10 (PND10) as compared to controls. In addition, PRS rats did not show the phenomenon of “maternal potentiation”, i.e. the increase in USVs in response to a brief maternal reunion after isolation, normally seen at PND10 and PND14. The suppression of the USV in isolated pups during a male odour exposure was exclusively seen at PND10 in control rats, whereas it was also observed at PND14 in PRS rats. After weaning (PND22), PRS rats continued to show anxiety-like behaviour in the open field and in the elevated plus maze. A reduced expression of mGlu1, mGlu2/3, and mGlu5 metabotropic glutamate receptors in the hippocampus has been associated with the anxious-depressive phenotype of adult rats. Hippocampal levels of mGlu1 and mGlu5 receptors were already reduced in infant PRS rats at PND10, whereas expression of mGlu2/3 receptors declined only at PND22. Plasma leptin levels were also reduced in PRS pups at PND14. Taken together these data offer a clear-cut demonstration that PRS causes anxiety-like behaviour in the early developmental phase and raise the attractive possibility that early interventions interfering with the epigenetic programming may correct the pathological phenotype of adult PRS rats.

P3.140

Modifications of peripheral blood mononuclear cells in a rotenone experimental analogue of Parkinson's disease

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Alterations of the immune system have been widely documented in many neurodegenerative disorders and are thought to occur among earliest events in their pathogenesis. In particular, modifications of peripheral lymphocytes subsets and extravasation of $CD4^+$ T lymphocytes within the cerebral parenchyma are documented in Parkinson's disease (PD); blood brain barrier alterations have also been reported *in vivo* in early PD patients as well as in animal models of declared Parkinsonism induced by various environmental toxins, thus providing a rationale for investigating these aspects in PD at early stages of the disease process. The inhibition of mitochondrial complex I by compounds such as MPTP, rotenoids or acetogenins generates interesting experimental analogues of Parkinsonism. In this study, we used cumulative low doses of the organic pesticide rotenone in the rat. At higher doses, this compound has been shown to replicate most features of PD: motor alterations, dopaminergic cell death and intracellular inclusions similar to Lewy bodies. Blood samples were collected on heparin tubes before and after 1, 2, 3 and 4 weeks of exposure to 0.5mg/kg/day rotenone. Peripheral blood mononuclear cells (PBMC) were isolated on a ficoll gradient. Specific lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$) were determined at each time point using flow cytometry. In parallel, total RNA was extracted from PBMC and hybridized on Agilent whole rat genome oligo microarray (44K) in order to investigate rotenone-induced expression changes in this peripheral compartment. In addition, the activity of mitochondrial complexes was assayed at several time points. At the end of rotenone exposure, animals were perfused with paraformaldehyde and brains were collected for immunohistochemical analyses. Here, we report alterations in the gene expression profile in PBMC during rotenone exposure. Interestingly, metabolic pathways that were the most altered by rotenone are related to lymphocyte activation although we could not evidence modifications between lymphocytes subsets at the time points investigated. In conclusion, our work establishes the basis for further investigations aiming at delineating altered parameters in these early steps of the pathological process.

P3.141

Age-induced prefrontal over-activation and working memory impairments in mice are suppressed by the cholinergic agonist S 38232

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We previously showed that ageing induced working-memory (WM) impairments in a sequential alternation (SA) task in mice. Aims of the present study are i) to analyse by immunohistochemistry the brain areas sustaining SA performance in young and aged mice and ii) to compare in aged mice the memory-enhancing effects of S 38232 (an agonist of β2 cholinergic receptors) to donepezil (a cholinesterase inhibitor).
WM of C57Bl6 mice aged of either 4-6 or 18-20 months was studied in the T-maze SA-task. Mice were submitted to sequential delayed alternation and then perfused and processed for immediate early gene phospho-CREB (pCREB) analyses used as a marker of neuronal activity in several brain's regions. Given the results, a mechanistic approach was implemented in aged mice to investigate the effects of infusions of Rp-AMPc (an inhibitor of PKA involved in CREB phosphorylation) in prelimbic cortex (PL) on SA performance. In addition, the memory-enhancing effects of S 38232 and donepezil were compared in aged mice. Both compounds were injected ip 30 min prior to behavioral testing.
Immunocytological analyses showed that pCREB was similarly increased in the CA1 region of the hippocampus in young and aged mice. In contrast, pCREB was increased specifically in PL of aged mice as compared to young ones. The causal role of the over-activation of the PL in the SA deficits of aged mice was established, since local PL infusions of Rp-AMPc restored memory performance in aged mice. In addition, both donepezil and S 38232 increased performance of aged mice.
Immunocytological results showed that donepezil increased pCREB in the CA1 whereas in contrast, S 38232 attenuates the increase observed in PL of aged mice.
Overall, these data show: 1) that the increase of pCREB activity in the prelimbic cortex of aged mice contributes to their WM deficit ; 2) that the β2-AchR agonist S 38232, by down-regulating the over-

activation of the PL in aged mice restore WM performance ; 3) that donepezil increases performance of aged mice by increasing hippocampal rather than prelimbic cortex activity. In conclusion, these data reveal the different involvement of HPC and PL on WM performance in young adult and aged mice respectively and the implication of the cholinergic transmission in these processes.

P3.142
Postural control and sensory integration in Parkinson's Disease

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Impairment of postural control is a common consequence of Parkinson´s disease (PD). Increasing evidences demonstrate that the pathophysiology of postural disorders in PD includes deficits in proprioceptive processing and integration. However, the nature of these deficits has not been thoroughly examined. We propose to establish a link between proprioceptive impairments and postural deficits in PD using two different experimental approaches manipulating proprioceptive information. In the first one, the subjects stood on a platform that tilted slowly with oscillatory angular movements in the frontal or sagittal planes. The amplitude and frequency of these movements were kept below the semicircular canal perception threshold. Subjects were asked to maintain vertical body posture with and without vision. The orientations of body segments were analysed. In the second one, the postural control was tested using tendon-vibration method, which is known to generate illusory movement sensations and postural reactions. Vibrations were applied to ankle muscles. The subject´s whole-body motor responses were analyzed from Centre of Pressure displacements.In the first experiment, the parkinsonian patients (PP) were unable to maintain the vertical trunk orientation without vision. Their performances with vision improved, without fully reaching the level of control subjects (CS). In the second experiment, the postural reactions of the PP were similar to those of the CS at the beginning of the perturbation and increased drastically at the end of the perturbation's period as compared to those of CS and could induce fall.These results will bring new concepts on the sensorimotor postural control, on the physiopathology of posture, equilibrium and falls in PD and on the role of basal ganglia pathways in proprioception integration. Nevertheless, in order to assess precisely the role played by sensorimotor integration deficit in postural impairments in PD, further studies establishing the links between clinical features and abnormalities are now required
Acknowledgement: This work was supported by “France Parkinson” and “Musique en tête” Associations.

P3.143
Is Alzeimer's a synaptomatrix disease?

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The synaptomatrix (Vautrin, Neurochem Int. 2010, 57:85-96) is defined as the transcellular assemblage of pre-, post-, and inter-synaptic molecules directly involved in the transmission of fast synaptic signals such as postsynaptic receptor/channels, SV2 and synaptotagmin presynaptic 'vesicle proteins'. The use-dependent remodelling of the synaptomatrix providing the synapse its functional plasticity necessary for memory is supported by exo- endocytotic pre- and postsynaptic vesicular trafficking. (The presynaptic vesicular traffic was wrongly systematically assigned to the sole quantal release likely to be directly driven by the Ca^{2+} transient.) Memory loss being the first symptom in Alzheimer's disease (AD) suggests an implication of the synaptomatrix turnover. For decades scientists and pharmaceutical companies have focused on ways to target the amyloid plaques and

neurofibrillary tangles thought to play the prime role in causing Alzheimer´s disease (AD). However, among the pathologic hallmarks of AD neurodegeneration, only synaptic loss in AD patients brain closely correlates with the degree of dementia *in vivo*. Sherrington defined the synapse as a "*functional contact*". The most likely functions of amyloid precursor protein (APP) is trophic activity and cell adhesion which is consistent with APP trafficking and proteolytic processing being necessary for structural synaptic plasticity. Thus, APP like presynaptic vesicle proteins traffic through PKC-dependent secretory and recycling pathways (Sun and Alkon, Pharmacol Ther. 2010, 127:66-77). Yet, a rare AD therapeutic strategy (now in clinical trial phase II) is based on compensating for the early deficient PKC activity in AD patients. This is consistent with AD being a dysfunction of use-dependent and PKC-dependent presynaptic vesicular traffic regulating dynamically the synaptic contact components including the synaptomatrix.

P3.144

***sir-2.1*/SIRT1 and *daf-16*/FOXO promote a normal pre-synaptic structure in dysfunctional *C. elegans* neurons expressing expanded polyglutamines**

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Sirtuins, FOXO or the Sir2-FOXO pathway have been shown to protect diseased neurons in several simple models of neurodegenerative disease, suggesting the activation of pro-longevity factors is neuroprotective and providing a basis to develop disease-modifying strategies. We previously showed that, in *C. elegans* transgenics expressing N-terminal huntingtin (htt) in touch receptor neurons, increased *sir-2.1*/SIRT1 dosage strongly protects from neuron dysfunction, an effect mediated by *daf-16*/FOXO. Here, we explored whether this activity of Sir2-FOXO may involve the regulation of synaptic structure. To this end, we have generated *C. elegans* transgenics that co-express N-terminal htt and a synaptic vesicle reporter in touch cells, which are believed to be glutamatergic neurons. We used these animals to analyse the chemical synapses formed by the touch cells at the head (ALM cells) as they are easy to visualize. Our data suggest that expanded polyQ expression significantly reduces the response to touch in these animals, an effect accompanied by a reduction in the number of GFP clusters/synapse. This latter alteration might correspond to a hyperactive synapse, as suggested by loss-of-function of genes that support synaptic activity. Gene perturbation experiments suggest that *sir-2.1*/SIRT1 and *daf-16*/FOXO promote a normal pre-synaptic structure. These effects may involve the putative DAF-16 target *unc-32*, which encodes a subunit of V0 V-ATPase, a complex important to synaptic vesicle activity in neurons. Additionally, we observed that SIRT1 inhibitors such as EX-527 aggravate expanded polyQ toxicity at the synaptic and behavioural levels whereas compounds such as lithium are protective at both level. Our results suggest that raising the activity of Sir2-FOXO protects the synapse of an expanded polyQ neuron and they highlight the potential of using *C. elegans* as an *in vivo* system to screen candidate genes and compounds at the synaptic level.
Work supported by Inserm, FRM, ANR and CHDI
Vazquez and Offner contributed equally.

P3.145

Cerebellar abnormalities following hypoxia alone compared to ischemic-hypoxic forebrain injury in the developing rat brain

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Premature birth is a significant risk factor for adverse motor, cognitive, and behavioral outcomes in survivors . In the constellation of long-term neurodevelopmental deficits, some are potentially referable to cerebellar injury in particular impaired motor functions as hypotonia, fine motor incoordination, ataxia, and impaired motor sequencing. Cerebellar atrophy may occur as a result of a primary acquired injury, such as hemorrhage or infarction. The incidence of cerebellar lesions is of 17% in very preterm infants born before 30 gestational weeks (28 post-ovulatory weeks). A recent study showed that unilateral injury confined to the preterm cerebral hemisphere was associated with a significantly decreased volume of the contralateral cerebellar hemisphere. These data suggest a significant crossed trophic effect between the developing cerebral and cerebellar structures among preterm infants. To test this hypothesis two-day-old (P2) rat pups were subjected to either permanent ligation of the right carotid artery followed by 2.5 hrs hypoxia inducing a telencephalic hypoxic-ischemic lesion or a global hypoxia alone. Cellular and regional injury in the cerebellum was studied at 1, 2 and 19 days using immunohistology. Following hypoxia and ischemia-hypoxia, all neuronal populations of the cerebellum were damaged in a subset of Purkinje cells. The decrease in number of interneurons, thickness of molecular and granular layers was significant following hypoxia. Diffuse myelination damage with loss of preoligodendrocytes was more severe following hypoxia than ischemia-hypoxia. Global hypoxia in rat at P2 produces extensive damage of many cell types of different areas of the cerebellum and the addition of unilateral forebrain ischemia does not increase the severity of these changes. Our data provide insight into the mechanisms of the changes observed in the cerebellum of premature newborns. In our model, the early hypoxia at P2 comparable to very preterm infants (around 28 weeks of gestation) may correspond to cerebellar damage rather involving the anterior sensorimotor cerebellar than the cognitive/emotional posterior cerebellum. More studies are necessary to evaluate the spatiotemporal cerebellar damage following hypoxia and/or ischemia in the preterm brain.

P3.146

Longitudinal PET follow-up of nigrostriatal innervation in the MPTP-monkey model of Parkinson´s disease: correlation with clinical symptoms before and after graft of neural precursors

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Parkinson disease is a neurodegenerative pathology characterized by the progressive death of the DA neurons of the substantia nigra. Positon Emission Tomography (PET) studies have established, using different radiotracers, that dopaminergic depletion is of the order of 60% prior to symptoms becoming apparent, suggesting powerful compensatory or adaptive processes. The specific toxin MPTP produces symptoms in humans and animals similar to those observed in PD patients, is responsible for similar DA cell loss, and is thus considered as a realistic pharmacological model of PD with the additional advantage of allowing the investigation of events during the presymptomatic period, i.e. prior to the onset of clinical symptoms. In the MPTP-monkey model spontaneous recovery can be observed subsequent to intoxication being suspended. What is not presently known is if this recovery reflects a compensatory mechanism that is intrinsic or extrinsic to the DA system. These issues are important because they provide information about the early phases of the disease when decisions about the therapeutic course of action have to be made. Using longitudinal DA monitoring with PET the present study seeks to compare the DA lesion at different stages of intoxication. We aim to determine and compare the status of the DA lesion between full blown symptomatic cases and MPTP monkeys that show spontaneous recovery from clinical symptoms using DA transporter binding potential.

Our results show that the compensatory mechanisms that lead to motor recovery in MPTP-monkeys occurs despite a lesion that remains around 70% of the nigrostriatal DA system, and thus depend on extrinsic DA mechanism. Further, we show that pushing the lesion to about 90% induces stable parkinsonian symptoms.
These results are coherent with the large body of evidence showing that compensation involves systems other than DA. These compensatory mechanisms are efficient as long as about 20% of SNpc neurons, at the origin of the nigrostriatal pathway, are conserved.
We are currently exploring the therapeutic potential of grafts of neurons derived from ES cells to reverse symptoms in the MPTP monkey using in vivo follow-up by PET of ^{11}C-PE2I binding to DA transporters and ^{18}F-DOPA uptake.

P3.147

Hippocampo-septal neurons: a new neuronal target for β-amyloid peptide in the rat dorsal hippocampus

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The septo-hippocampal (SH) pathway plays a crucial role in memory formation. In Alzheimer's disease, amyloid beta peptide (Aβ) accumulation is associated with hippocampal network dysfunction. In the rat, intrahippocampal injections of Aβ induce aberrant inhibitory SH network activity in vivo and impairment of memory processing. In the present study, we track down the origin of these alterations in anaesthetized rats. After hippocampal Aβ treatment, a selective loss of long range projecting neurons to the medial septum (MS) which contain calbindin (CB) or calbindin and somatostatin (CB/SOM) were observed. Other local GABAergic neuronal subpopulations containing CCK, NPY or parvalbumin, were not altered. Thus, the present study identifies hippocampo-septal neurons containing CB or CB/SOM as specific targets for Aβ deposits. The selective impairment of this population of neurons appears to be responsible for the previously observed decrease in the bursting activity of MS neurons. In this regard, in Aβ-treated but not in control rats, 55% of the slow-firing MS neurons recovered rhythmic bursting activity following glutamate application. Thus, Aβ accumulation in the hippocampus can trigger modification of the SH pathway via an alteration of a specific neuronal population. Identifying this specific target contributes to the understanding of the mechanisms underlying the deleterious effects of the Aβ peptides, commonly accepted as one of the main agents in Alzheimer's disease.

P3.148

Cannabinoids in neuroinflammation and neurogenesis: from normal ageing to Alzheimer's disease models

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Our recent work clearly demonstrated the benefits from low chronic infusion of the synthetic cannabinoid WIN-55,212-2 (WIN) in reducing the effect of normal ageing on memory function, neuroinflammation and neurogenesis. As of today, there is no available cure for Alzheimer's disease (AD) and it seems that a preventive approach may be adequate and efficient to delay the appearance

of the disease. Our project thus aims to evaluate the potential benefits of preventive treatment with WIN *in vivo* and to determine its precise mechanisms *in vitro.*
HEK cells expressing the APPswe mutation were used to determine the influence of WIN on the amyloid peptide (Aβ) and cytokine production, in presence or not of inflammation induced by TNF-α. 5xFAD mice were used to determine WIN effects on neuroinflammation, neurogenesis, Aβ production and memory performances.
In HEK cells overexpressing APPswe, WIN seems to modulate inflammation as well as to influence the balance between sAPPα and Aβ production. 5xFAD mice characterization of inflammatory markers, neurogenic processes and behavioural impairments have been conducted to evaluate properly the effects of WIN on the onset of AD in this animal model.
Cannabinoids, because of their anti-inflammatory and neurogenic properties, may be good candidates for a new preventive therapeutical approach aiming at delaying the onset of AD in patients and thus potentially diminishing the appearence of new cases.

P3.149
Sodium imaging: a novel tool for optical probing of neural circuits

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The Na^+ concentration gradient across the plasma membrane drives key processes in the nervous system such as action potential generation, forward and back-propagations, excitatory synaptic transmission and neurotransmitter reuptake membrane. It is also essential in maintaining cellular homeostasis. Optical measurements of Na^+ dynamics with fluorescent indicators could thus be a powerful approach to asses the function of neural networks. Thus far however, cellular events behind neuronal activity have been monitored mainly indirectly by Ca^{2+} imaging owing to the comparatively small amplitude of Na^+ changes occurring during physiological responses and to the lack of satisfactory fluorescent Na^+ indicators. In order to improve Na^+ imaging we tested several candidate synthetic Na^+ dyes. We identified one compound, Asante Natrium Green that appeared to fulfill our requirements including
(i) homogeneous and stable cytosolic loading,
(ii) excitation in the visible spectrum as well as with 2-photon,
(iii) absence of significant cell toxicity,
(iv) linear dependency of fluorescence on Na^+ concentration in the range of usual physiological responses,
(v) high efficiency of Na^+ detection allowing measurement of small Na^+ events in brain tissue,
(vi) kinetic parameters allowing the recording of fast sodium transients,
(vii) effective bulk-loading of the cell-permeant form in tissue permitting imaging of multicellular ensembles.
In conclusion, Asante Natrium Green brings essential improvements to Na^+ imaging, especially for deep tissue measurements.

P3.150
Visual threshold evaluation in human and non-human primates

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The cross-modal integration can be defined as the capability to integrate information from different modalities at the same time, with the aim at enhancing perception and also better adapting to our environment. Several studies in human and non-human primates showed that, in a sensory-motor task close to the sensory thresholds, cross-modal integration leads to a significant increase of the percentage of correct responses and a decrease of the reaction time. These advantages are assimilated to facilitatory effects. One of the essential parameter to assess this effect of multisensory integration lies in the search of the sensory thresholds. Indeed, the reliability of the estimated

thresholds depends not only on the used stimulus (here visual and auditory cue), but also on the used methodology. For the auditory stimuli (noise or tones), the thresholds were determined by an automated procedure based on intensity steps of 1 dB. The choice of the visual cue (static, moving, shape) was however less evident. In the perspective to find the most relevant visual thresholds, we tested a series of protocols performed in human and non-human primates to determine the most appropriate visual cue. In this study, we have developed a method to evaluate the visual thresholds based on automated behavioural procedure with positive reinforcement, using a green flash generated by a light-emitting diode (LED). Indeed, the LED allows a precise modulation of its luminous intensity by the modification of its frequency (Hz). In this case, the precision of thresholds evaluation was obtained by steps of 1 Hz. The preliminary results obtained on 6 human subjects' show that the lowest mean visual threshold obtained in one subject was at 24.3 Hz (range 24.3-28 Hz).
In conclusion, the LED is a reliable method for the evaluation of visual thresholds. The average threshold determined on several subjects will serve as a reference to set the visual intensity in dB, for subsequent precise control of visual and auditory intensities in the cross-modal task.

P3.151

Nutritional omega-3 deficiency and emotional behaviors: a role of CB1R?

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Mood and anxiety disorders, the most prevalent neuropsychiatric disorders, are a global problem and cause vast suffering worldwide. Core symptoms are loss of interest/pleasure, depressed mood and altered appetite. Recent studies revealed the key role of dietary omega-3 PUFA in mood disorders as they suggest that a low dietary omega-3 PUFA intake increases the risk to develop depression. However, the pathways through which the consumption of unbalanced omega-3 PUFA diet leads to alterations of mood are still not clear. Recent studies have shown that in rodents, emotional behavior is modulated by the endocannabinoid system which is a major regulator of emotional process and food intake. Moreover, we recently discovered that n-3 PUFA deprived diet induces a dramatic reduction in the cannabinoid receptor 1 functioning (CB1R) (Lafourcade, Larrieu et al., 2011). In this work, we examined the potential role of dietary n-3 PUFAs on emotional behavior and on CB1R agonist behavioral effect. To do so, we used C57BL/6J male mice fed with a n-3 PUFA deprived diet during one generation. Brain lipids were evaluated in the brain. In addition, we used behavioral tests to assess the emotional domains (social interaction, open-field test, elevated plus maze, forced swimming test).
Interestingly, we found that mice maintained on omega-3 deprived diet for one generation display a decrease of the n-3 PUFA docosahexaenoic acid (DHA) in the brain that is accompanied by an increase of depressive-like behavior and a disturbance of social behavior. Interestingly, an ip administration of a cannabinoid agonist markedly modified emotional behavior in mice eating standard or omega-3 balanced chow, and these effects were fully reversed by a co-administration of a specific CB1R antagonist. In contrast, CB1R agonist had no behavioral effect in n-3 deprived mice, further confirming that CB1R is inactivated in omega 3 deficient mice. In conclusion, our results highlight a crucial role of n-3 PUFA on emotional behavior and CB1R behavioral activity. Lastly, we will determine if the altered emotional behavior and CB1R dysfunction can be rescued by a dietary DHA supplementation for further information on the role of CB1R in emotional behavior disturbance of deprived omega 3 mice.

P3.152

Central and sensory mechanisms underlying mammalian locomotor-respiratory coupling

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The central nervous system contains neural networks that can generate rhythmic motor patterns underlying different vital behaviors. In certain circumstances, these rhythmogenic neuronal assemblies must interact to harmonize their activity pattern in order to satisfy organismal requirements. By combining electrophysiological, calcium imaging and lesion approaches on *in vitro* brainstem-spinal cord preparations from the neonatal rat, our study focuses on elucidating the cellular and synaptic mechanisms involved in the functional coupling of locomotor and respiratory functions.
During overground locomotion, a 1:1 coupling between stepping and breathing patterns occurs in neonatal rat. In hindlimb-attached semi-isolated preparations, entrainment of fictive respiratory rhythmicity recorded from phrenic nerves was systematically elicited by repetitive flexion-extension movements applied to a hindlimb, suggesting that the recruitment of limb proprioceptive afferents plays an important role in locomotor-respiratory coupling. Furthermore, in agreement with the spinal localization of the locomotor rhythm-generating networks, we found that the electrical stimulation of cervical or lumbar proprioceptive afferents was more effective in entraining fictive respiration than thoracic afferent stimulation. Moreover, a combination of pharmacological, lesion and calcium imaging experiments showed that the respiratory entrainment imposed by the cyclic electrical stimulation of cervical or lumbar proprioceptive inputs is mediated by spinal pathways that ascend to the pontine parabrachial nucleus in the lower brainstem. Finally our preliminary results showed that the activation of spinal locomotory circuitry by stimulation of sacral sensory afferents causes concomitant acceleration of respiratory rhythmicity and a rhythmic locomotory-timed modulation of the excitability of spinal respiratory neurons.

P3.153

Electrophysiological, molecular and morphological characterization of hilar hippocampal interneurons in mice

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New neurons are produced throughout life in the adult mammalian brain, in particular in the hippocampus, a key structure for learning and memory processes. These new neurons participate to the formation and retrieval of some forms of memories. Their premature death, observed in several mouse models of Alzheimer's disease could result from a deficit in their maturation, involving a dysfunction in GABAergic modulation via hilar hippocampal interneurons. Understanding this dysfunction requires the identification of hilar populations of interneurons that connect adult born granule cells.
To characterize hilar neuronal diversity, we used single-cell RT-PCR after patch-clamp recordings on dentate gyrus slices of two months old C57Bl6 mice. This technique enabled us to determine, for each recorded neuron, its electrophysiological, molecular and morphological properties. After a non-supervised cluster analysis based on electrophysiological parameters, two main classes of neurons could be identified. The major molecular characteristics of neurons (n=152) in the first cluster were the high occurrence of VGluT1, CR (Calretinin) and CCK (Cholecystokinin). Furthermore, these cells present typical active electrophysiological properties, like a high initial action potential amplitude followed by a strong adaptation of this amplitude during the firing. The second cluster of cells (n=153)

could be divided in four sub-populations: (1) neurons co-expressing VGluT1 and CR (n=11) ; (2) putative basket cells (n=25), distinguishable by their low membrane resistance, high action potential discharge frequency and the expression of GAD, PV (parvalbumin) and CCK ; (3) GABAergic interneurons (n=80) strongly expressing NPY (Neuropeptide Y) and finally (4) GABAergic interneurons (n=36) co-expressing NPY and SOM (somatostatin). To complete this characterization we are currently identifying the morphological properties of each cluster of cells by revealing the neuronal tracer used during our recordings.
This multiparametric neuronal characterization could help to identify the neuronal populations regulating adult born granule cells maturation and survival, in normal and pathological conditions such as Alzheimer's disease. Work supported by France Alzheimer, CNRS, Université Toulouse III.

P3.154
Synaptic mechanisms of olfactory gamma oscillations in the awake mouse

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Field potential oscillations are ubiquitous in the brain, and particularly in sensory systems. In the olfactory bulb (OB), the first central relay of the olfactory system, fast odour-evoked oscillations in the gamma band (40-100Hz) have been linked to odor coding and processing. These fast network oscillations regulate the timing of output neuron action potentials and are thought to provide the support for spike-timing dependent representation of odor information. Because of the variety of circuit interaction capable of generating oscillations and because of the many processing strategies oscillations may emerge from, it is useful to precisely understand the mechanism underlying oscillations in the OB.
In the OB, the output neurons, namely the mitral cells, interact with a large and homogenous population of axonless interneurons, the granule cells, to form a distributed inhibitory circuit. Interestingly, dendrodendritic reciprocal synaptic transmission between dendrites of mitral and granule cells remains the dominant mode of neuronal interaction in the OB. *In vitro* experiments have shown that OB gamma oscillations clearly rely on this dendrodendritic GABAergic circuit. Here, we show the existence of two discrete frequency channels within the broadband gamma oscillations in freely moving mice. Pharmacological local infusion combined to in vivo LFP and single-unit recordings suggest that low (40-70 Hz) and high (70-100 Hz) gamma oscillations may depend on distinct network mechanisms. Ongoing experiments using genetically modified mice will help us deciphering the involvement of different synaptic interactions in this network synchronization process. Lastly, the separation of gamma oscillations into discrete frequency channels will highlight the possibility of multiplexing coding strategies for odor representation in the olfactory bulb.

P3.155
Anti-saccade adaptation

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Saccade accuracy is known to be maintained thanks to adaptive mechanisms which reduce the final saccadic error. These mechanisms have been mostly studied for reactive saccades triggered by sudden presentation of a visual target (see for review Pélisson et al 2010). Fewer studies have

addressed the effectiveness of different paradigms to adapt voluntary saccades in healthy volunteers. While the transfer of reactive saccades (RS) adaptation to anti-saccades (AS) has been tested in different laboratories (Collins et al 2008, Cotti et al 2009, Panouillères et al 2009), the possibility of inducing an adaptation of AS has never been reported. In this study, we have tested the possibility to adaptively increase (forward paradigm) the amplitude of AS. Eight healthy subjects were submitted to 4 different AS tasks, a main adaptation task and 3 control tasks, performed in different sessions separated by 7 days. In all tasks, a visual target was presented at a random location (6, 9 or 12°) in the right visual field and subjects had to shift their eyes toward the mirror location in the left field (AS). In the adaptation task, adaptation of the leftward AS was elicited by presenting a feedback target at the time of AS termination (0 msec). This target was systematically displaced relative to the initial target mirror location by 10% in the direction of the AS. In control tasks, the time or the position of presentation of the feedback target was changed. We tested the amplitude of both AS and RS produced in the two different directions (left and right) immediately before and after each task. Repeated measures ANOVAs showed in the adaptation task, a significant enhancement of the amplitude of the left AS ($p< 0.01$) and the left RS ($p=0.03$) in post-test comparatively to pre-test. The same analyses performed in the control tasks failed to reveal any significant effect, indicating that these findings were specifically related to the adaptation protocol. In conclusion, this study demonstrates the possibility of inducing adaptation of AS, opening a new way to probe the saccadic plasticity mechanisms. In addition, this protocol based on forward paradigm could be used to help rehabilitation of visual exploration deficits (e.g. hemianopia) by an automatic increase of saccade amplitude

P3.156

How nasal airflow shapes spatio-temporal representation of odors at the olfactory bulb level: an optical imaging study

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Olfactory sense and respiration are intimately related: odorant molecules reach receptor cells in the olfactory epithelium in a periodic way with inspiration and several studies have demonstrated that olfactory bulb (OB) activity is largely shaped by respiration. It should be keep in mind that anesthetized rat breaths slowly and very regularly whereas behaving rat adapts its sniffing behavior (amplitude, frequency) according to numerous parameters (odor, task, experience). Recent Ca^{2+} imaging studies has evidenced that nasal airflow parameters could modulated the spatio-temporal representation of the incoming message from neuroreceptors at the glomerular layer level.
The aim of our study was to analyze how nasal airflow shapes the spatio-temporal distribution of odor evoked activity in the rat OB using voltage-sensitive dye imaging (VSDI). VSD signals originate mainly from post-synaptic activity and thus can measure odor-evoked activation of bulbar circuitry. Thus, compared to previous studies based on presynaptic activity measurements, VSD signals take into account the modulation of incoming activity by local interneurons.
Experiments were performed on urethane anesthetized rats. The OB was stained with voltage-sensitive dye RH 1838 and image series of the dorsal OB were acquired with a CCD camera at 160 Hz. We used a double canulation protocol in order to make nasal airflow sampling independent from animal respiration. Thanks to this technique, we were able to test different parameters of the nasal airflow as the frequency (from 1 Hz to 10 Hz) or the strength of inhalation flow.
Optical signals appeared as increase in fluorescence during odor presentation superimposed with a phasic component appearing after each inspiration. For a given flow rate, signal amplitude remained stable across frequency. Glomerular responses remained locked to the nasal respiration cycle even at high frequency sniffing and the magnitude of this modulation depended on the inspiration strength. Moreover, respiratory modulation is not homogeneous across all activated glomeruli. These results confirm that intranasal air dynamics also plays a critical role in shaping odor representation at the bulbar circuitry level.
This work was supported by an ANR grant (#ANR-07-NEURO-030).

P3.157

Impairment of sleep recovery after sleep deprivation in a murine model of Trisomy 21 (Down Syndrome) that over-expresses the Amyloid Precursor Protein (hAPP)

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Trisomy 21 (T21 or DS) is the most frequent cause of mental retardation and is associated with sleep abnormalities[1]and a risk for Alzheimer disease (AD). We developed a murine model (Ts1Cje/*hAPP*) derived from partial trisomic mice (Ts1Cje) which in addition carries the *hAPP* gene under its own promoter. Sleep recordings were performed in 2 month-old male transgenic (Tg) and wild-type (WT) mice during 48h under baseline conditions as well as after a sleep deprivation of 6h duration. Both groups exhibit the same patterns of wakefulness, slow wave sleep (SWS) and rapid eye movement sleep (REMS) at baseline. However, during the recovery period after 6 hours of sleep deprivation, Tg mice showed qualitative impairment of SWS as evidenced by a lower slow wave activity (in the 0.5-5 Hz band of the Electro-Encephalogram) than WT mice. This indicates that Tg mice lack the classical sleep recovery normally present after sleep deprivation
These data confirm the role of the APP over-expression in sleep as suggested by the results obtained in partial trisomic mice (Ts65Dn versus TS1Cje)[2] and in a previous model of transgenic mice expressing the *hAPP* gene[3]. The present results suggest that the hAPP protein impairs the recovery function of sleep. Such alteration might be related to abnormalities in the APP metabolism observed during prolonged wakefulness in transgenic APP mice[4] modeling Alzheimer disease, which would prevent notably the restoration of cognitive functions through recovery sleep.
Our DS murine model might contribute to a better understanding of the Down syndrome notably the sleep regulation aspects, and might lead to novel therapeutic pathways for improving sleep and related recovery functions in DS persons.

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P3.158

Elucidating functional dichotomy in the external globus pallidus: a major population of pallidal neurons specifically innervate striatum

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The direct influence of the globus pallidus (GPe) extends beyond its classic target, the subthalamic nucleus (STN), to every nucleus of the basal ganglia (BG). In Parkinsonian animals, two main types of GP neuron (GP-TI and GP-TA, representing 70% and 20% of GPe cells, respectively) can be recognised by their distinct and inversely-related firing rates and patterns. This functional dichotomy could actively support the excessive beta oscillations that arise in STN-GP network and other BG nuclei in Parkinsonism. Here we define some key molecular and structural properties of electrophysiologically characterised GP-TI and GP-TA neurons that underlie their integration within, and influence on, BG circuits. Marked differences in the firing rates/patterns of identified GP-TI and GP-TA neurons were mirrored by dichotomous molecular profiles and axonal connectivities. Both cell types are GABAergic; most GP-TI neurons express parvalbumin whereas most GP-TA neurons do

not. GP-TI neurons have extensive local axon collaterals and they also innervate one or more 'downstream' BG nuclei, including the STN, substantia nigra and entopeduncular nucleus. A minority of GP-TI neurons also sparsely innervate striatum. In contrast, GP-TA neurons have limited local axon collaterals and do not innervate downstream BG nuclei. Rather, the projection axons of GP-TA neurons are specifically directed to the striatum, where they densely ramify, with an individual cell giving rise to ~10,000 boutons. Targets of this projection include the dendritic shafts of striatal projection neurons, and the somata and proximal dendrites of several types of striatal interneuron. Thus, not only do GP-TA neurons challenge the dogma that all GPe neurons innervate STN, but also, at the individual cell level, they provide the largest known source of GABAergic innervation of the striatum. We conclude that, together, GP-TI and GP-TA neurons can orchestrate both normal and abnormal activities throughout the entire BG.

P3.159

Behavioral, neurochemical and pharmacological characterization of central neuropathic pain caused by spinal cord transection at thoracic level in rats

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In humans, spinal cord lesions induce not only major locomotor and neurovegetative deficits, but also, in about half of patients, severe neuropathic pain. In order to unveil possible molecular targets for innovative treatments against central neuropathic pain, we developed a rat model of spinal cord transection, and investigated its physiopathological characteristics.
The spinal cord of adult male Sprague-Dawley rats was transected at the thoracic level (T8-T9) under deep isoflurane anaesthesia. Sham animals underwent the same surgery except spinal cord transection. Thereafter, nocifensive responses to mechanical stimuli were assessed using von Frey filaments applied within the cutaneous territory around the incision. The same test was used to assess possible anti-neuropathic pain effects of drugs. qRT-PCR measurements of mRNAs encoding microglial and neuronal markers (OX42, ATF3) and proinflammatory cytokines (IL-1b, IL-6) were performed to monitor neuroinflammatory processes in the spinal cord and dorsal root ganglia (T6-T8, T9-T11).
Strong mechanical allodynia was observed in thoracic cord transected- but not sham- rats. This effect developed rapidly and persisted for at least 4 weeks after transection. In parallel, mRNAs encoding microglial/neuronal markers and proinflammatory cytokines dramatically increased (x50-150) in spinal tissues and/or dorsal root ganglia two days after transection, and were still up regulated (x2-5) three weeks later. Under acute treatment conditions, morphine (3-10 mg/kg ip) markedly reduced mechanical allodynia but baclofen (3 mg/kg ip) and amitriptyline (10 mg/kg ip) were inactive. Because chronic (0.25 mg/kg sc daily for three weeks) but not acute administration of the 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT significantly reduced allodynia in transected rats, further investigations with baclofen and amitriptyline under chronic treatment conditions have to be performed before any definitive conclusion can be drawn regarding their effectiveness.
Our data support the idea that spinal cord transection is a relevant model to investigate physiopathological mechanisms underlying central neuropathic pain, and to assess the potential interest of novel analgesic strategies.
Supported by ANR (06-Neuro-038), IRME, Institut UPSA de la Douleur.

P3.160

5-HT1A receptors direct the plasticity of layer 5 pyramidal neurons in the mouse prefrontal cortex

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Several psychiatric disorders such as major depression and anxiety as well as schizophrenia are associated with a dysfunction or a reduction of 5-HT1A receptors (5-HT1AR) in the prefrontal cortex (PFC). Postsynaptic 5-HT1ARs are located on GABAergic interneurons and pyramidal neurons. However, no extensive investigation has examined the modulatory role of postsynaptic 5-HT receptors on the excitability of layer 5 pyramidal neurons (L5PNs) which elaborate cortical output signals. The excitability of L5PNs is highly regulated by a balance between excitation (E) and inhibition (I) resulting from the activity of recurrent cortical networks. In this study, we investigate the role of 5-HT1ARs in the mouse PFC through a comparison of 5-HT-induced modulations of the excitability and plasticity of L5PNs between control (129/Sv) and KO 5-HT1AR mice. We performed electrophysiological recordings in L5PNs of composite responses evoked by electrical stimulation of layers 2-3. The E-I balance was determined by algorithmic decomposition of the global conductance change.
As expected, the E-I balance of control L5PNs exhibited the typical 20-80% ratio. This balance was significantly modified to 23-77% in KO 5-HT1A mice, suggesting that 5-HT1ARs intervene in the regulation of the E-I balance. We previously showed that the balance is highly regulated by homeostatic plasticity processes within the cortical network. We thus assessed such processes by application of a high frequency of stimulation (HFS) protocol in layer 2-3, which resulted either in a Long-Term Potentiation (LTP) of both E and I, a Long-Term Depression (LTD) of E and I or no plasticity. The proportion of L5PNs displaying LTP, LTD or no plasticity was different between control and KO 5-HT1AR mice. The lack of 5-HT1ARs also prevents the homeostatic control of the E-I balance when LTP was induced by HFS protocol.
Our results show that the activation of the 5-HT1ARs acts as an important switch in L5PNs of the PFC to orientate the plasticity of L5PNs towards LTP, LTD or no plastcity. The current study therefore highlights the fine role of 5-HT1ARs at the level of dendritic spines of L5PNs to tune their plasticity and consequently their possible pivotal role in depressive disorders as targets of new antipsychotics.

P3.161

Mono- and polysynaptic feed-forward inputs to mitral cells from olfactory sensory neurons

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Olfactory sensory neurons (OSNs) expressing the same odorant receptor converge in specific glomeruli where they transmit olfactory information to mitral cells. Surprisingly, synaptic mechanisms underlying mitral cell activation are still controversial. Using patch-clamp recordings in mouse olfactory bulb slices, we demonstrate that stimulation of OSNs produces a biphasic postsynaptic excitatory response in mitral cells. The response was initiated by a fast and graded monosynaptic input from OSNs and followed by a slower component of feed-forward excitation, involving dendro-dendritic interactions between external tufted, tufted and other mitral cells. The mitral cell response occasionally lacked the fast OSN input when few afferent fibers were stimulated. We also show that OSN stimulation triggers a strong and slow feed-forward inhibition that shapes the feed-forward excitation but leaves unaffected the monosynaptic component. These results confirm the existence of direct OSN to mitral cells synapses but also emphasize the prominence of intra-glomerular feed-forward pathways in the mitral cell response.

P3.162

Serotonin2C inverse agonists enhance purposeless oral movements and alter basal ganglia c-Fos gene expression in a region-dependent manner in rats

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Numerous studies suggest that full inverse agonists at serotonin2C (5-HT_{2C}) receptors affect brain function differently compared to neutral antagonists but this has not yet been firmly established in behaving animals. We combined immunohistochemistry and behavioral analysis in rats to investigate the effect of the new 5-HT_{2C} inverse agonist S32006 on c-Fos expression in the basal ganglia, and on purposeless oral movements, a sensitive, basal ganglia-mediated response to 5-HT_{2C} receptors in rodents. Intraperitoneal administration of S32006 (1-20 mg/kg) dose-dependently enhanced oral bouts with a maximal effect at 10 mg/kg. The neutral 5-HT_{2C} antagonist SB243213 (1 mg/kg) was inactive alone and abolished the increase in oral bouts induced both by S32006 (10 mg/kg) and by another well-characterized 5-HT_{2C} inverse agonist SB206553 (10 mg/kg). The induction by S32006 of oral bouts was not altered by selective depletion of endogenous 5-HT levels with 5,7-dihydroxytryptamine. In line with its pharmacological profile *in vitro*, the ability of S32006 to stimulate oral bouts may involve silencing of 5-HT_{2C} receptor constitutive activity through its inverse agonist properties. This action of S32006 at 5-HT_{2C} receptors was associated with changes in cellular activity in basal ganglia. Thus, S32006 enhanced c-Fos expression in the nucleus accumbens (NAc) at doses of 1-10 mg/kg, and also dose-dependently elevated c-Fos expression in medial parts of the striatum, subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr). Likewise, SB206553 (10 mg/kg) enhanced c-Fos expression in the STN, SNr and striatum (except the dorsomedial part) as well as the entopeduncular nucleus (EPN). SB243213 (1 mg/kg), which enhanced c-Fos expression in the STN and striatum only, dampened the c-Fos expression induced by SB206553 in the striatum, STN, EPN and SNr. The distinct pattern of c-Fos expression elicited by 5-HT_{2C} inverse agonists *vs* a neutral antagonist suggests a differential modulation of 5-HT_{2C} receptor-dependent transmission in basal ganglia. 5-HT_{2C} full inverse agonists likely elicit oral activity in rodents by interfering with constitutive activity at basal ganglia populations of 5-HT_{2C} receptors.

P3.163

Unconventional exocytosis at hair cell ribbon synapses - *vesicle fusion without neuronal SNAREs?*

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Exocytosis of synaptic vesicle critically relies on the SNAREs proteins (SNAP-25, syntaxin 1 and synaptobrevin 1 or 2), as shown by the severe arrest of synaptic release upon SNARE cleavage by clostridial neurotoxins or by genetic ablation. We report that exocytosis in mammalian auditory sensory cells (inner hair cells, IHCs) is resistant to clostridial neurotoxins attack. In addition, stimulation-secretion coupling was neither impaired in IHCs from lethal-wasting mouse mutant, characterized by a loss-of-function of synaptobrevin 1, nor from the synaptobrevin 2/3 and SNAP-25 null mice. We found mRNA but no synaptically localized protein of neuronal SNAREs in IHCs. Thus, auditory sensory cells exocytosis is unconventional and may operate independently of neuronal SNAREs.

P3.164

New vistas on the dynamic cortical association field in the cat primary visual cortex

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Long-range horizontal connections in the cat area 17 mediate the propagation of visual information at low speed (0.1-0.3 m/s), far beyond the classical extent of cortical discharge fields (MDF) (Bringuier et al, Science, 1999). We present here a quantitative study of the silent "surround" of the receptive field (RF) of neurons recorded intracellularly in area 17 of the anesthetized cat, based on elementary visual responses to sparse two-stroke apparent motion (AM) noise of Gabor patches (GP).
The full field exploration protocol was devised to explore all possible motion axis and eccentricities relative to the recorded MDF center. We analyzed the impact of 4 geometrical parameters defined in relation either with the motion axis or in a RF-centered framework: 1) the ISO- or CROSS-orientation of the paired GP relative to the motion axis; 2) the axial direction (centrifugal (CF) vs centripetal (CP)); 3) the eccentricity from the MDF center; and 4) the motion axis angle relative to the RF preferred orientation (and main axis). The intracellular responses observed for the dynamic AM sequence were compared with the linear prediction computed from the presentations of single static GPs in neighboring positions.
Our results show that : 1) optimized GP, flashed in the «silent surround», elicited a strong subthreshold depolarization that propagated up 10-15° of visual angle at slow speed (0.1- 0.3 m/s); 2) AM stimulus sequences facilitated or revealed *de novo* such peripheral responses; 3) Facilitation was most prominent in the ISO- and CP- conditions. 4) For 2/3 of the cells, response latencies were shortened in the CP- condition. 5) Only 1/3 of the cells behaved linearly, whereas AM produced significant facilitation and depression respectively in 50 % and 16 % of cells.
We conclude that a contingent of V1 cells integrate centripetal motion signals arising from the far periphery at saccadic speed, with facilitation for ISO- (collinear) configurations. These results are consistent with the hypothesis of a dynamic Association Field mediated by horizontal connectivity (Frégnac et al., 2010).
Funded by CNRS, ANR V1-Complex - 2010, EU FP7 FET Open (Brain i Nets) and FP7 FET (BrainScales).

P3.165

Simultaneous two-photon imaging of oxygen and blood flow in deep cerebral vessels

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Uncovering principles that regulate energy metabolism in the brain requires mapping of partial pressure of oxygen (PO_2) and blood flow with high spatial and temporal resolution. Using two-photon phosphorescence lifetime microscopy (2PLM) and the oxygen probe PtP-C343, we show that PO_2 can be accurately measured in the brain at depths up to 300 µm with micron-scale resolution. In addition, 2PLM allowed for the first time simultaneous measurements of PO_2 and blood flow in capillaries with less than one-second temporal resolution.
Using this approach, we detected erythrocyte-associated transients (EATs) in oxygen in the rat olfactory bulb and demonstrated the existence of diffusion-based arterio-venous shunts. Sensory stimulation evoked a functional hyperaemia accompanied by an increase in PO_2 in capillaries and by a

biphasic PO_2 response in the neuropil, consisting of an “initial dip” and a rebound. The differential distribution of PO_2 in olfactory bulb superficial layers is currently investigated.
2PLM of PO_2 opens new avenues for studies of brain metabolism and blood flow regulation.

P3.166

Activation of protein kinase C in the medullary dorsal horn produces mechanical hypersensitivity through superficial laminae nociceptive-specific NK1 receptor-negative neurons

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Dynamic and static mechanical allodynias are widespread and intractable symptoms of neuropathic pain for which effective therapy is still missing. During tactile allodynia, activation of sensory fibers which normally detect touch elicits pain. We recently showed that, in rats, a dynamic mechanical allodynia can be induced by selectively segmental glycinergic disinhibition and is due to the activation of a polysynaptic neuronal network in the superficial dorsal horn (DH) involving interneurons expressing the gamma isoform of protein kinase C (PKCγ). However, the role of these PKCγ interneurons in mechanical allodynia remains unclear. We assessed the effect of directly activating PKCγ in the medullary DH (MDH) by intracisternally (i.c.) injecting phorbol 12,13-dibutyrate (PDBu) in rats. We show that (1) PDBu induces both dynamic and static mechanical allodynias. (2) After i.c. injection of PDBu, innocuous mechanical stimulation results in a strong extracellular-signal regulated kinase phosphorylation (pERK1/2) in superficial MDH: lamina I, outer lamina II, inner lamina II and outer lamina III. In contrast to what happens following intradermal capsaicin injections, under PDBu, innocuous mechanical stimuli induce ERK1/2 phosphorylation only in neurons that do not express NK1 receptors. (3) Selective inhibition of PKCγ (by i.c. KIG31-1) prevents both ERK1/2 phophorylation and allodynia. The present findings thus reveal the involvement of a selective DH circuit in both static and dynamic mechanical allodynia, which operates through PKCγ interneurons and superficial laminae nociceptive-specific neurons that do not bear NK1 receptor. They suggest that pharmacological inhibition of PKCγ might provide a new tool for alleviating allodynia in clinical settings.

P3.167

Impact of Beehive Products on the Cardiovascular Neurophysiology Expands Novel Horizons in Apitherapy

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Apitherapy is the medical use of honey bee products. This can include the use of honey, bee venom, propolis, royal jelly, bee pollen and beeswax. Most claims of apitherapy have not been proved to the scientific standards of evidence-based medicine and are anecdotal in nature. A wide variety of conditions and diseases have been suggested as candidates for apitherapy, the most well-known being bee venom therapy for autoimmune diseases and multiple sclerosis. Paradoxically, however, recent studies evaluating the cardiovascular effects of honey bee products have revealed very promising results; apitherapy is still out of focus from the neuropharmacological point of view. Certainly, with the advent of new technologies and great advances in cardiovascular research, more properly conducted clinical trials are needed to objectively substantiate the efficacies of honey bee products. As will become evident, results of these trials are likely to alter radically how cardiovascular diseases will be treated in the future. In an effort to provide extensive information about the cardiovascular bioactivity of honey bee products, and to the best of our knowledge, this review

overviews, for the first time; (1) effect of honey bee products on the cardiac muscle activity via exploring its influence on the electrocardiographic parameters of isolated hearts, (2) precise mechanism of action of honey bee products on the cardiac cells via applying autonomic antagonists and ion channels antagonists, (3) influence of honey bee products on the cardiovascular neurophysiology of intact animals via monitoring the electrocardiogram and blood pressure, (4) cardioprotective efficacy of honey bee products which may diminish susceptibility and vulnerability of the heart to several cases of cardiac disorders leading to heart's malperformance, (5) therapeutic perspectives of honey bee products to help in the treatment of some cardiac and vascular diseases, (6) biochemistry of honey bee products using modern analytical techniques to clarify its cardioactive constituents and its relationship with the mode of action on the myocardium as well as the possible medical applications of honey bee products on the cardiovascular pathophysiology, toward opening of a novel gateway to cardiovascular apitherapy.

P3.168

fMRI visualization of odor-evoked transient activation in the rat piriform cortex

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Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) has recently been applied to the rodent central nervous system. In the olfactory system, reproducible and specific odor-induced spatial activity patterns have been demonstrated in the olfactory bulb. These results support previous optical imaging studies showing that odors elicit activity within glomerular layer domains. Despite these promising results, recording of BOLD signal in small animals remains a challenge particularly in deep brain structures. Indeed, most studies have used surface receiving coils to achieve a favorable signal-to-noise ratio compared to volume coils; however, the signal uniformity of these coils is restricted, leading to a superior sensitivity that is limited to dorsal brain structures located close to the coil, such as the olfactory bulb.
The aim of our study was to record BOLD odor evoked responses in the rat piriform cortex (PC) (the main area of olfactory bulb projections), which stretched on the ventro-lateral part of the rat brain. In this study, we took advantage of the use of a new phase-array surface coil. This 4 channel array was made of 4 receivers aligned along the coronal axis. This new coil provides improved signal uniformity in the whole brain and thus an increased contrast-to-noise ratio in deep brain structures. BOLD responses to different odors were acquired in the PC of anesthetized rats using a 7 T spectrometer. When compared with a previous study using a single receiver coil, the probability to record BOLD signal in the PC was largely increased with the phase-array coil. BOLD responses were mainly recorded in the anterior part of the PC. According to previous electrophysiological and anatomical studies, PC responses to odors were not spatially organized but extended in a large part of the anterior cortex. These results were the first report of BOLD responses in a deep rat brain structure and demonstrated the advantage of using multi-receivers coils. Data confirmed that BOLD fMRI is suitable for studying spatio-temporal activity associated with odor stimulation in olfactory system, particularly in the olfactory cortex which is less accessible to optical imaging studies. This work was supported by an ANR grant (#ANR-07-NEURO-030).

P3.169

Manipulating hippocampal progenitors to stimulate the production of new neurons in the adult mouse brain

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Hippocampal neurogenesis persists throughout life in the mammalian brain. Lifelong, self-renewable progenitors that locate in the hippocampal dentate gyrus give rise to granule neurons that become structurally and functionally integrated into the hippocampal circuitry, where they are believed to contribute to learning and memory processes. With aging, the rate of hippocampal neurogenesis decreases dramatically, suggesting that it might contribute to the cognitive decline that accompanies senescence. It is thus tempting to examine whether this reservoir of endogenous neural stem cells capable of proliferating can be genetically manipulated to enhance neurogenesis and improve cognitive aging.
Using retroviral transgene delivery, we over-expressed NeuroD1, a key regulator in neuronal differentiation, in hippocampal progenitors that locate in the dentate gyrus of adult mice. We observed that virtually all NeuroD1-transduced progenitors differentiated into cells expressing molecular and morphological phenotype of mature neurons. Importantly, we found that NeuroD1 overexpression accelerates maturation and adoption of the neuronal features. Using whole-cell patch-clamp recordings, we showed that NeuroD1-transduced cells display electrophysiological properties of functional mature granule neurons. Indeed, they were able to elicit action potentials and exhibited functional glutamatergic and GABAergic currents evoked by electrical stimulations. We conclude that neural progenitors within the hippocampal niche can be genetically instructed to boost neuronal production in the young adult brain in vivo. Future work shall determine whether such strategy can be used to overcome age-related decline of adult hippocampal neurogenesis and improve cognition.
Work supported by Fondation pour la Recherche sur le Cerveau, France Alzheimer, CNRS, Université Toulouse III.

P3.170

A functional link between the cerebellum and the hippocampus relevant to spatial navigation

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Path integration requires the online estimation of a navigator position using self-motion cues. This process is necessary to the acquisition of an internal representation of the environment in the absence of external cues. The hippocampal formation plays a key role in the development of spatial representations. The cerebellum is likely to mediate context-dependent optimization of navigation trajectories. Recent experimental evidences suggest that the cerebellum and the hippocampal formation are electrophysiologically coupled. We put forth the hypothesis that the cerebellum may contribute to the processing of self-motion cues, and therefore interact with the hippocampus for the elaboration of spatial representations. We adopt a multidisciplinary behavioural and electrophysiological approach to study the spatial navigation capabilities of transgenic mice (L7-PKCI), which have impaired PKC dependant long-term synaptic depression (LTD) at cerebellar parallel fiber-Purkinje cell (PF-PC) synapses. Behavioural data show that in a path integration version of the Morris water maze, L7-PKCI animals present a drift in their trajectory toward a platform in the dark. Electrophysiological recordings of hippocampal place cells in L7-PKCI were performed as they explored a circular arena. Our data revealed an alteration of hippocampal place cell properties in the

case of either an absence of external cues or a conflict between external and self-motion cues. This suggests that cerebellar PKC dependant mechanisms might be important for the processing of self-motion signals essential to the shaping of hippocampal spatial representation and for path integration process.

P3.171

Concurrent mental task affects the tonic discharge pattern of wrist extensor motor units associated with voluntary isometric contraction

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This study was aimed to determine whether the performance of a mental task affects motoneuron activity by using a dual-task paradigm. The tonic discharge pattern of wrist extensor motor units was analyzed in healthy subjects while they maintained a steady wrist extension force and concurrently performed a mental arithmetic (MA) task. The effects of counting backwards by seven performed either aloud or silently were tested. There is also a control condition in which no MA task was required. The data based on trial-by-trial analysis of the characteristics of motor unit discharge pattern showed that a shortening of the mean inter-spike interval (ISI) and a decrease in ISI variability occurred when MA task was superimposed to the motor task.. Aloud and silent MA affected equally the rate and variability of motoneuron discharge. Increases in surface EMG activity and in force level were consistent with the modulation of the motor unit discharge rate.
The present study has shown that a mental non-motor task affects the rate and the variability of the tonic discharge of single motor units during voluntary isometric contractions providing evidence that cognitive functions may influence the state of the motor system at all the level of its organization, including the motoneuron. The results clearly demonstrate that performing MA increases activation of wrist extensor motor units during the maintaining of a stable extension force. It is suggested that increase in muscle spindle afferent activity, resulting from fusimotor drive activation by MA, may have contributed to the increase in synaptic inputs to motoneurons during the mental task performance, likely together with enhancement in the descending drive. The present study provides new insight into the interactions between cognitive and motor task, which could have clinical consequences. Indeed, the dual-task paradigm, in which a MA task is superimposed to the motor task, is extensively used in Physical and Rehabilitation Medicine in assessment of motor disabilities and in rehabilitation in motor pathologies.

P3.172

Functional calibration of retinal implants using intrinsic optical imaging of the rat V1

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Stimulating the retinal network is seriously considered as a tool to restore visual function in a certain number of human retinal pathologies (ARMD and other retinopathies). Despite the fact that human clinical trials has already begun, there is to date no functional investigations of the efficiency of such

implant. To test the retinal implant, we proposed to analyze the retinal electrical evoked activity at the level of the population in the primary visual cortex. The rationale of this choice is to test the effective activity propagating in the visual system in response to the retinal stimulation. In this study, we first benchmarked the basic features of visual evoked activity in V1 of anesthetized rats (position, size, intensity) by using intrinsic optical imaging signals. In a second part, we then used these benchmarks to interpret the activity evoked by electrical retinal stimulation. As implants, we used sub-retinal matrices of 1mm diameter covering 15-20° of visual angle and supporting 9 electrodes surrounded by annular references (CEA-LETI, Grenoble) which are driven by the BIO-MEA system (BIOLOGIC). Using visual stimulation, retinotopic organization, retino-cortical magnification factor, stimulus intensity response function and dependence to stimulus size were first characterized. In a second step, we successfully developed acute retinal implantation to evoke electrically-induced cortical responses. We found that, contrary to visual stimulation, manipulation of a single parameter of the electrical patterns, such as intensity, leads to modulations of cortical patterns in several dimensions. This may imply the existence of a non-linear recruitment of the retinal network with potential diffusion of the electrical current. These observations are important for the development of functional retinal implants and optimal features of electrical patterns. Together with optogenetics and cortical brain machine interfaces, electrical stimulation of the retinal substrate achieved by retinal implants is one key-path towards a partial restored vision. We think that providing embedded functional tests of the implant is a necessary step for the progress in this field.

P3.173

Increased audiovisual integration in cochlear-implanted patients: an ICA analysis of PET data

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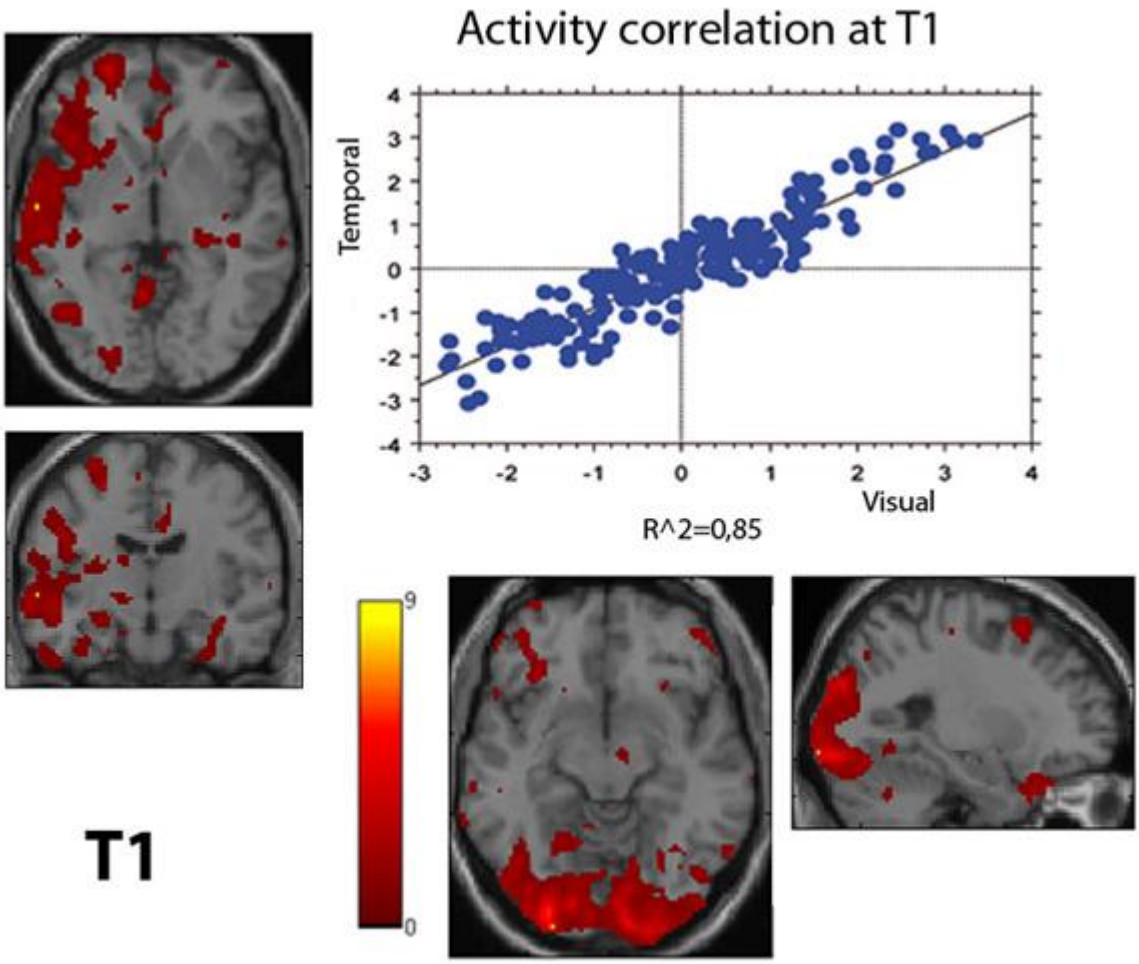
Our psychophysical data demonstrated that cochlear-implanted deaf patients (CIP) present a much higher level of visuo-auditory integration in word recognition compared to normal hearing subjects (NHS). Further, our recent study of PET activity in CIP revealed that at rest the visual cortex and the posterior temporal areas, which are implicated in audiovisual integration, elicited more activity in the experienced CIP compared to NHS. To verify whether active audiovisual integration is expressed by supra-normal level of activity in CIP, we explored the pattern of brain activity of patients in a visual, auditory and audiovisual word perception task. Three groups of subjects were analysed: shortly after implantation (T0), the same patients more than 6 months post-implantation (T1), and normal hearing controls. We used independent components analysis (ICA) of PET data to explore occipito-temporal brain activity. In between-group analysis, patients at T1 had greater activity in the left middle temporal cortex as compared with T0 and NH. In within group analysis, patients at T0 had a task-related component in the visual cortex; patients at T1 had two task-related components: in the left middle posterior temporal cortex and the in the visual cortex.
The middle posterior temporal area is known for its implication in audiovisual integration; in our study the time course of temporal and visual activity at T1 was highly correlated (Figure 1), it was also correlated with behavioural scores in the PET camera.



[Figure 1.]

Higher activity of these areas at T1 reflects the compensatory strategy in CI patients based on visuo-auditory synergy to compensate for the impoverished auditory input delivered by cochlear implants. Our data confirms the importance of audiovisual integration in experienced cochlear implanted subjects by establishing the neural underpinnings for this integration.

P3.174

On the reduced influence of long distractors on saccade metrics: evidence for inhibitory lateral interactions

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In most models of saccade generation, the computation of saccade metrics relies on excitatory and inhibitory lateral interactions within the motor map of the Superior Colliculus. These are responsible for averaging oculomotor responses, the responses that arise when the saccade target stimulus is presented along with a spatially proximal distractor stimulus in the periphery; due to short-distance excitatory interactions between the initially active peaks, the eyes' landing position is deviated toward the distractor. In this framework, the amount of distractor-related deviation is assumed to be a function of the level of activity associated with the distractor as opposed to the target. An increase of the size of the distractor should thus strengthen the distractor weight, and in turn increase the spread of neuronal

activity induced by the visual input, thereby leading to a more pronounced deviation of the eyes from the target. At the same time, a wider neuronal activity pattern at the distractor location may also induce more inhibitory interactions, thus leading to the opposite prediction, i.e., a less pronounced deviation of the eyes towards the distractor.
Here, we assessed the role of inhibitory interactions in saccade generation by using a saccade-target task in which a to-be-looked-at peripheral target was presented with or without a distractor. To test the paradoxical prediction that larger distractors can induce less deviation than smaller ones, we varied the distractor length in its vertical dimension, while holding constant the distance between distractor and target. Results confirmed that when a distractor was presented along with a target stimulus, the saccade's landing position shifted toward the distractor. The important fact was that the deviation increased with distractor length, but only up to a critical length; above this length, the effect reversed that is longer distractors led to progressively smaller deviations. These results suggest that inhibitory interactions play a critical role in determining saccade metrics; they can decrease the weight of a distractor in the spatial integration of distractor and target locations, at least when the neuronal activity pattern induced by the distractor is wide enough.

P3.175

Time course of spinal cell proliferation and differenciation after C4-C5 hemisection in the adult rat: influence of an acute administration of GM-CSF

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Inflammation within the first week after spinal cord injury (SCI) is known to play a key role in prognosis of functional recovery. However, recent studies highlight the dual aspect of inflammation, *i.e.* deleterious or beneficial for recovery after CNS damage. This study was aimed at determine in both the time course of neural and microglial cell interactions, in terms of proliferation and differentiation after SCI, and the influence of a proinflammatory cytokine, the Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), on microglial activation, glial scar formation, and the functional recovery.
A C4-C5 medio-lateral spinal hemisection was performed in 3 month old Wistar male rats. The animals were injected with BrdU on the 1st, 3rd,8th and 21th days postlesion and sacrificed one month after the injury. The GM-CSF was administrated one hour after the SCI (20 µg, *i.p.*) in a single rat group injected with BrdU 3 days after the SCI. The histological study consisted in a stereological quantitative analysis of the glial scar volume and estimation of the newly generated neural and microglial cells. In order to evaluate the impairment and recovery of their functional habilities, the rats were subjected once a week over one month to a sensory behavioral task (tape removal) and a spontaneous locomotor rating scale (modified BBB Scale, see Martinez et al., 2009).
Our findings point out the strong cell proliferation within and around the lesion site that contrasted with a weak BrdU immunoreactivity (-IR) in the spared CNS regions. These newly generated cells differentiated mainly in microglia (OX-42/BrdU-IR or Iba-1/BrdU-IR) and astrocytes (GFAP/BrdU-IR), while only rare neurons (NeuN/BrdU-IR) were identified. In the rats subjected to GM-CSF administration the increase in the number of migroglial cells was potentiated compared with the SCI group, while the astrocytic proliferation as well as the resulting glial scar were significantly decreased. The preliminary behavioural investigations indicated a correlative improvement in sensory and motor performances. Supplementary behavioural and histological analysis are in progress. These findings suggest a positive influence of the early inflammation in SCI.

P3.176

Trial-by-trial correlation between reaction time and EEG oscillatory activity in a forewarned simple reaction time task

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Reaction time (RT) corresponds to the time a subject takes to produce a measurable behavioral response following presentation of a sensory stimulus. This behavioral measure is commonly used to study cognitivo-motor processes like attention, decision making or motor preparation. In a given experimental paradigm requiring the same behavioral response across a number of seemingly identical trials, RT presents a wide variability. This variability indicate some variability in the underlying neuronal processes. In this study, we investigated if some features of non-invasive surface electroencephalographic (EEG) recordings correlate with trial-by-trial changes in RT. To address this issue, we used a forewarned simple reaction-time task in which the subjects had to press a left or right-hand button as fast as possible after the presentation of an imperative GO signal. Two seconds before the GO onset, a first cue was illuminated to indicate which button to press. To prevent subjects from anticipating the GO onset, 30% of NOGO were randomly presented. EEG signals are recorded from 64 electrodes mounted on an elastic cap. The amplitude and phase modulations of EEG oscillations are calculated on a trial-by-trial basis over the 2 s preceding the GO onset with a time-frequency transform that convolves the signal with a wavelet function. We focus particularly on the α (8−14 Hz) and β (15−35 Hz) bands known to be modulated by attention and motor preparation processes. We use a decoding approach with binary classifiers to detect the slowest and fastest reaction times before the GO onset. The preliminary results suggest that the phase of the α cycle at the GO signal has an influence on single-trial RT. RTs tend to be shorter for positive phases of the α cycle. Furthermore, we observe a negative correlation between both α and β amplitude and the RTs, over the fronto-central areas. These results support the notion that RT is modulated by the amplitude of α and β oscillations during movement planning. In addition, it suggests that the phase of the α cycle modulates excitability of the motor pathways.

P3.177

Entrainment of specific neuronal populations in the medial septum by light-activated channelrhodopsin2 in the mouse in vivo

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Septal neurons are believed to play an important role in the generation of the theta rhythms in the hippocampal system. However, the exact functional contributions of the cholinergic, GABAergic and other neurons are debated. Optogenetic strategies allow the selective stimulation of distinct neuronal populations in the medial septum, by targeting the expression of the light-activated cation channel channelrhodpsin2 (ChR2) to specific neuron types.
We used transgenic mice expressing ChR2 under the control of the cholineacetyltransferase (ChAT) promoter, the parvalbumin (PV) promoter (by infection with an AAV vector bearing the ChR2, or cross-breeding with Ai27 mice), and the Thy1 promoter.
Neurons in the medial septum could be reliably entrained by delivering blue light locally, using either square light pulses or sine waveforms. Local light stimulation was achieved by an etched optic fiber attached to a silicon probe, in anesthetized animals. Light-evoked spikes occurred within 10-35 ms of pulse onset, at a very high success rate that was reduced by increasing the stimulation frequency. The baseline firing rate of light-activated cells in ChAT-ChR2 was below 1Hz. In contrast, activated

neurons in the Thy1-ChR2 exhibited high firing activity. In addition to direct, short-latency activation, a longer lasting (> 100 ms) suppression was also detected upon stimulation of Thy1-ChR2 neurons. The magnitude of suppression was proportional to the stimulus intensity. Juxtacellular recordings combined with immunohistochemistry showed that non-ChR2 expressing cells could also be secondarily entrained and displayed a variety of response patterns.
When light stimulation was applied in the septum, the effect on field activity in the hippocampus (in anesthetized and freely moving mice) was drastically different depending on the neuronal population stimulated. In ChAT-ChR2 mice, a small deflection was seen in all hippocampal layers. In PV-ChR2 mice, the evoked potential was larger, displayed a marked source/sink pattern accross the hippocampal layers and was able to alter the LFP frequency. In Thy1-ChR2 mice, the evoked potential was very large, displayed a different source/sink pattern and could take over the LFP, up to inducing ictaform activity at higher light intensities.

P3.178

Reconstruction of the field excitatory post-synaptic potentials in the dentate gyrus from amperometric biosensor signal

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Introduction: Local field potentials (LFP) information can be obtained from amperometric neurochemical recordings. However, conversion from the amperometric high frequency components (HFC) to conventional LFP is a challenging task since the electrode impedance is difficult to determine and the electrical properties of microelectrodes change with the frequency.
Objective: To find and test a feasible and reproducible method to reconstruct field excitatory post-synaptic potentials (fEPSPs) in the dentate gyrus of the hippocampal formation from amperometric HFC.
Materials and methods: The electrode properties were modelled as an equivalent circuit consisting of 3 resistances and 2 capacitances. A single voltage pulse allowed estimation of the resistances and capacitances values. After determination of the electrode impedance, FFT and Inverse FFT were used to convert the amperometric signal to LFPs. Platinum electrodes and biosensors responses for different voltage pulses at different holding potentials were tested in saline or in PBS. Reconstructions of the evoked potentials elicited in the dentate gyrus of rats (n=3) and mice (n=2) by stimulation of the perforant pathway were compared to electrophysiological recordings obtained subsequently in the same preparations. Long term potentiation (LTP) was induced in rats by high frequency stimulation of the perforant pathway and demonstrated by the reconstructed fEPSPs.
Results: The estimated values of the resistances and capacitances of the equivalent circuit for different platinum electrodes and biosensors were found to be quite independent on the amplitude of the voltages steps (0.5-2 mV) and DC values (0-500 mV). Our method allowed perfect overlapping between reconstructed fEPSPs and true voltage recordings. Reconstructed fEPSPs showed typical inversion of the responses on the depth profile. Analysis of the slope of the rising phase of the fEPSPs showed potentiation of the synaptic efficacy in rats after high frequency stimulation.
Conclusions: Our results showed that for specific electrophysiological purposes a relatively simple electrode model can work satisfactorily , allowing the reconstruction of the fEPSPs in the dentate gyrus in order to demonstrate LTP during acquisition of neurochemical recordings.

P3.179

Spinal mGlu4 activation inhibits hyperalgesia in animal models of chronic pain by a modulation of the glutamatergic neurotransmission

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Glutamate plays a key role in the modulation of nociceptive processing. This excitatory amino acid exerts its action through two distinct types of receptors, the ionotropic and the metabotropic glutamate receptors (mGluRs). Eight mGluRs have been identified and divided in three groups. Here, we focused on group-III mGluRs, which contains mGlu4, 6, 7 & 8. These receptors are mostly presynatic and are present in most structures implicated in pain neuraxis including the spinal cord (except mGlu6). Previous studies have shown that group-III mGluRs spinal activation has an antihyperalgesic effect in neuropathic and inflammatory pain models but no effect in acute pain models in rodents (Goudet et al. Pain 2008). However, little is known about the precise role of the different group-III mGluRs subtypes, partly due to the poor pharmacology for these receptors. The aim of this study is to discriminate the role and the cellular and molecular mechanisms of mGlu4 in pain regulation at the spinal cord level. This study includes electrophysiology and behavioural pharmacology experiments.
Selective activation of mGlu4 by a specific agonist (LSP4-2022) has an antihyperalgesic effect on chronic pain models (Carrageenan and unilateral peripheral mononeuropathy) comparable to the hyperalgesia observed following whole group-III mGluRs activation (ACPT1). The antihyperalgesic effect of ACPT1 in rats traited by mGlu4 antisenses is strongly reduced. Furthermore we observe no effect of this compound in mGlu4 KO mice. Regarding spinal cord transmission in lamina II neurons, LSP4-2022 application reduces strongly the evoked excitatory postsynaptic currents amplitude (eEPSCs) (in the same range that a group-III mGluRs agonist (L-AP4)).
These results suggest a crucial role of mGlu4 within group-III mGluRs in the modulation of pain. This antihyperalgesic effect of mGlu4 activation could be attributed to an inhibition of glutamatergic synaptic transmission in lamina II neurons of spinal cord.

P3.180

Differences in serotoninergic metabolism contribute to differences in respiratory phenotype in FVB/N and C57BL/6J mice

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Numerous studies described the respiratory phenotype of mutant mice with targeted deletions of genes involved in respiratory rhythmogenesis or regulation. C57BL/6J and FVB/N inbred strains are often used as background strains for producing mutants and the background strain phenotype affects the mutant phenotype. However, little is known concerning the respiratory phenotype of C57BL/6J and FVB/N mice, especially in a developmental perspective. Here, we compared their breathing pattern from birth to one year of age. At birth, *in vivo* and *in vitro* approaches revealed robust respiratory rhythm in FVB/N but not C57BL/6J neonates. With aging, rhythm robustness difference persisted and inter-strain differences in tidal volume, minute ventilation, breathing regulations and blood gas parameters were observed. As serotonin affected maturation and function of the medullary respiratory network, we examined the serotoninergic metabolism in the medulla of C57BL/6J and FVB/N neonates and aged mice. Inter-strain differences in serotoninergic metabolism were observed at both ages, possibly contributing to inter-strain differences in breathing. We recommend taking into account the distinct serotoninergic and breathing phenotypes of C57BL/6J or FVB/N mice when examining mutant models from either strain.

P3.181

Beta-band oscillatory activity during the planning and execution of visually-guided reach-to-grasp movements

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The involvement of the beta-band (15-35 Hz) oscillatory activity in voluntary movement is well established. However, the functional role of these oscillations is still unclear. In the present study we investigated how they contribute to the planning and execution of visually-guided reach-to-grasp movements.
Subjects performed a precuing reaction time task in which they had to reach, grasp and pull an object as fast as possible after a visual GO signal using 1 of 4 possible responses. The response types combined 2 movement parameters: the hand shape (precision grip or side grip) and the overall force (high or low) to grasp and pull the object. 3 s before GO onset a cue provided advance information about force, hand shape, both parameters or no information at all. Beta synchronisation (ERS) and desynchronisation (ERD) were computed from EEG signals (64 electrodes). Their modulations between the 4 precuing conditions and the 4 response types were analyzed for the preparation period (PP), reaction time (RT), movement time (MT) and object holding time (HT). We also compared the modulations of ERS/ERD with those of the contingent negative variation (CNV), a slow negative-going shift in EEG potential recorded during PP.
Beta oscillations were characterized by an ERS in the early part of the PP followed by an ERD starting approximately 1 s before the GO. The ERS was sustained for the non-informative condition while it decreased rapidly for the 3 informative conditions. Similarly to the CNV, the ERD increased gradually during the late part of PP and peaked around GO onset. In contrast to CNV, beta ERD was not modulated by the precue. ERD was maintained during RT and MT and a short-lasting beta ERS (beta rebound) was observed during the HT. The precue and the response types had no effect on the relative amplitude of these signals. These results demonstrate that CNV and ERD/ERS reflect different processes. The sustained ERS observed in the non-informative condition argue for the ERS as an “idling rhythm” in the motor system. The absence of precue and response type effect on ERD during movement planning and execution suggests that beta oscillations are a global and unspecific indicator of the current state of the motor system.

P3.182

Acute intermittent hypoxia alters the respiratory network sensitivity to norepinephrine via an increase in synaptic inhibition

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Repeated periods of central hypoxia occur in sleep apneas and Rett syndrome. In these disorders, an imbalance of bioamines has been described. We hypothesize that the effect of a neuromodulator (norepinephrine (NE)) on the respiratory network can be altered by acute intermittent hypoxia (AIH). We exposed brainstem slices (P6-10 mice) to AIH with or without NE and we recorded the fictive respiratory activity generated in the preBötzinger complex (preBötC). Under control conditions, NE regularizes the respiratory rhythm. Following AIH respiratory activity is slightly unstable but becomes highly irregular if slices are exposed to NE at the same time. Using whole cell recordings, we measured excitatory and inhibitory postsynaptic currents (EPSCs, IPSCs) of inspiratory neurons in the preBötC. After AIH, the IPSCs amplitude and frequency increased, whereas no significant changes in EPSCs were observed. In the presence of NE, AIH induces an even larger increase of IPSC

amplitudes, whereas the frequency of EPSCs tended to decrease. Co-application of strychnine (STR, glycine-R antagonist) during AIH + NE prevented the instability. However, application of STR after AIH + NE did not restore a normal rhythm. We suggest that the AIH induced change in the responsiveness to NE has potentially important clinical implications and may convey state-dependency to various breathing disorders.

P3.183

Cortical processing underlying the tactile funneling illusion

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Cutaneous stimulation evokes topographically organized activation in the S1 cortex. The traditional view that somatotopic maps form a substrate of sensory perception is challenged by the funneling illusion: the simultaneous presentation of brief stimuli at multiple points on the skin produces a single focal sensation at the center of the stimulus pattern although no physical stimulus occurs at that skin site, rather than separate sensations at each of individual sites. Inputs at lateral sites are “funneled” centrally so that the perceived intensity at the central site is greater than that perceived in response to stimulation at the middle site alone. This tactile illusion raises the question of how sensation is encoded in the brain and whether cortical activation corresponds to the actual or to the perceived site of peripheral stimulation. Although the funnelling illusion has been studied for decades, its neural substrates remain unclear.
This study was designed to investigate the spatiotemporal dynamics of cortical integration underlying the funneling percept, using voltage sensitive dye (VSD) imaging in the S1 forepaw area of anesthetized rats. VSD signals reflect mainly cortical synaptic activity i.e., subthreshold membrane potential changes and thus can reveal the contribution of latent connections between functionally related cortical areas. Our preliminary results show that:
1) individual digital pad stimulation produces an initial depolarization spot that spreads across a large sector of the forepaw area, so that simultaneous stimulation of non adjacent digits results in overlapping activation sectors,
2) individual digit stimulation induces strong temporal correlation of VSD fluorescence profiles that tends to segregate neural responses evoked from each digital pad during simultaneous stimulation,
3) after simultaneous stimulation of non adjacent digits, fluorescence profiles were found to be similar to those induced by real stimulation of the intermediate digit.
These findings suggest that dynamic synchronization of synaptic inputs within overlapping zones of cortical activation is an important feature of tactile stimulus localization and that this synchronization may form a potential substrate of the funneling illusion.

P3.184

Activation of nesfatinergic neurons of nucleus of solitary tract after gastric dilatation

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Digestive functions of the stomach are under control of brainstem parasympathetic system including the sensory afferent vagal fibers projecting to neurons of nucleus of solitary tract (NTS). In turn,

neurons of NTS modulate the activity of preganglionic neurons of the dorsal motor nucleus of the vagus (DMNX). Then vagal efferent projections from DMNX control gastric functions. It has been established that this vago-vagal reflex involved a large number of neurotransmitters including glutamate and gamma-aminobutyric acid. However, the neuropeptidergic systems involved in this process remain unclear.
Nesfatin-1 was recently identified as a neuropeptide cleaved from the N-terminal part of NEFA/nucleobindin 2 precursor. Different studies suggest that Nesfatin-1 is involved in the energy homeostasis. Particularly central administration of this neuropeptide inhibits food consumption and gastroduodenal motility in mice. Very interestingly, NTS and DMNX contain a dense population of Nesfatin-1 cell bodies. These observations led us to investigate the possible involvement of Nesfatin-1 neurons in the brainstem neuronal pathways modulating gastric functions.
To address this question, we performed double immunohistochemistry to detect the expression of c-Fos, a marker of neuronal activation, and Nesfatin-1 in NTS of rat after gastric dilatation. We observed that the number of neurons expressing simultaneously c-Fos and Nesfatin-1 is higher in the NTS of rat after dilatation than in the NTS of control animals. In addition, using glutamic acid decarboxylase (GAD) 67-GFP mice, we found that numerous nesfatinergic neurons of NTS also expressed GAD67. When fluorogold was injected at stomach level, many retrogradely labeled neurons were found in DMNX and are positive for Nesfatin-1. Finally, we also found that the cholinergic-neurons of DMNX expressed Nesfatin-1.
Altogether, these observations suggest for the first time that Nesfatin-1 is involved in the organization and function of vago-vagal gastric control neurocircuitry.

P3.185

Acute immobilization stress alters metal trace elements distribution in rat brain

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Alterations in $GABA_A$ receptor expression and levels are well documented in rat brain following stressful conditions. Furthermore, we have reported that in response to a severe stressor such as immobilisation, applied acutely, the capacity of several $GABA_A$ receptor neuromodulators is declined. As some heavy metals have been reported to possess a receptor site on the $GABA_A$receptor complex and to inhibit the neuronal $GABA_A$ response, we decide to assess their endogenous content in stressed brain rats. To this aim, Wistar male rats (220 g) were stressed by attaching their appendages with adhesive tape on a piece of wood during 1 hour. After the end of the stress session, the animals were sacrificed by decapitation and the brains were removed, dissected, snap frozen in dry ice, and kept at -30°C until processing. After an appropriate chemical treatment, the solutions obtained were assayed for zinc (Zn^{2+}) and iron (Fe) by atomic absorption spectrophotometer. It is interesting to note that the endogenous Zn^{2+} concentrations show a significant decrease in the whole cerebral cortex, the prefrontal cortex, the hippocampal region and the medulla in the brainstem. They are 1.8 to 2 ($p< 0.01$) times higher in control rats than in stressed rats. Similar tendency is observed for endogenous iron. Interestingly, the topography of these alterations correlates well with the stress sensitive brain areas we reported previously. Taken together, these results support the alteration of the modulatory function occurring at the $GABA_A$ receptor level induced by stress. The concentration of the heavy metals investigated is not sufficient to modulate with efficacy the $GABA_A$ receptor.

P3.186

Effects of age and caloric restriction on brainstem satiety center signals in rats

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Age-related increase in body weight and adiposity, indicating dysregulation of food intake and/or energy expenditure, can be prevented in rodents by long-term moderate caloric restriction (LTMCR). The dorsal vagal complex (DVC) is the brainstem center mediating the satiety reflex and has recently emerged as a determinant effector of long-term feeding adaptations. The major food intake inhibitor on the long-term is the adipocyte-secreted hormone leptin. The anorexigenic actions of leptin are mediated via brain-derived neurotrophic factor (BDNF) expression in the DVC, as well as in the other feeding-regulatory center of the brain, hypothalamus.
To study the effects of aging and LTMCR on satiety circuits, leptin and BDNF signaling were compared among 2- and 19-month-old *ad-libitum*-fed (AL) rats and 19-month-old calorie-restricted (CR) rats. The age-induced hyperleptinemia in AL rats was correlated with elevated DVC BDNF immunoreactive concentrations and satiety threshold stability, suggesting functional desensitization of the DVC to these signals. To better understand the phenomenon, mRNA levels of receptor and post-receptor signaling effectors were measured by using real-time RT-PCR. Aging selectively increased BDNF receptors and suppressor of cytokine signaling-3 (SOCS-3) mRNA levels. LTMCR prevented age-induced increases in serum leptin, DVC BDNF and SOCS-3 mRNA levels, but not those of BDNF receptors. This study unravels a key role of DVC leptin post-receptor targets and BDNF signaling in the establishment of age-related dysfunctions.
This study was supported by research grants from Canadian Institutes of Health Research (CIHR to P.G.), CNRS, INRA and University Aix-Marseille-3; by PhD fellowships from the french Minister of Education (to SS and SM) and by a sabbatical year from Aix-Marseille-3 (CRCT to EM).

P3.187
Anti-inflammatory effect of vagus nerve stimulation in a rat model: mechanistic study

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High frequency vagus nerve stimulation (VNS) has already been approved as a treatment of epilepsy or depression in humans. VNS has also been successfully experimented on animals to cure different inflammation models such as septic choc or pancreatitis. Traditionally, the peripheral anti-inflammatory effect observed after VNS is mediated by a direct action of acetylcholine. However, this mechanism does not explain all the observed effects, and recent data has shown a crucial role of the spleen in the peripheral anti-inflammatory effect obtained after VNS. This study's aim is to better understand the role of the splenic cells in a rat model after VNS. Animals without a colitis. 5 groups (n=10 male Sprague-Dawley rats/group) anaesthetised with Isoflurane and 5 groups with Pentobarbital: 20 min or 3h stimulation, 20 min or 3h no stimulation, and control. The stimulation electrode is fixed around the cervical portion of the left vagus nerve. The stimulation parameters are the following: 0.5mA, 5Hz, 30s ON, 5 min OFF. The spleen and distal colon are removed after euthanasia in order to analyse lymphocytes sub-populations with flow cytometry technique. We observe a decrease of the total splenic cellularity for the animals anaesthetised with Isoflurane and stimulated for 3 hours. This trend is observed for each splenic lymphocyte sub-type. This result is not found in rats anaesthetised with Pentobarbital. The use of Pentobarbital brings to light an effect of the surgery: a significant decrease of the number of T lymphocytes for the groups of operated animals, and an increase of lymphocytes

activation for the operated and non-stimulated group. This increase is not observed for the operated and stimulated group. This last result would tend to show an inhibiting effect of VNS on lymphocytes activation. These preliminary results show 1) the importance of the anaesthetic choice: the use of Pentobarbital seems more adapted to study anti- and pro- inflammatory phenomenon, 2) a possible inhibiting VNS effect on the lymphocytes activation. The data obtained with animals without an inflammatory syndrome allows us to have control values which will be very useful for the on-going experiments on the study of splenic lymphocyte sub-types in rats with colitis treated with VNS.

P3.188
RAE-1, a marker and an actor of neurospheres cell proliferation

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Major histocompatibility complex class I (MHC-I) family comprises numerous members activating or inhibiting the functions of immune cells. MHC nonimmune functions in the central nervous system (CNS) have started to receive recent interest. The mouse MHC class-I family is divided into classical MHC-I, non-classical MHC-I coded within the MHC genomic region, and related molecules coded apart from this area (HFE, MR1, CD1d and the NKG2D ligands : RAE-1, MULT-1, H60).
RAE-1 (Retinoic Acid Early Induced transcript-1) transcripts are present in early embryonic stages particularly in the nervous tissue and embryonic stem cells express high levels of the protein. However, up to now, the function of RAE-1 in the CNS development remains unknown. So far, RAE-1 is only known for its immune functions as a ligand of the activating receptor NKG2D expressed by NK cells, NKT, Tγδ and some T CD8 lymphocytes.
We recently described that RAE-1 transcripts, expressed in the brain during development, persist in one of the main area of adult neurogenesis, the subventricular zone (SVZ) and are induced in several experimental models. Moreover, neurospheres derived from the SVZ neural stem/ progenitor cells constitutively expressed RAE-1 and CD1d but not MHC-Ia, Qa1 or β2m molecules. The expression of RAE-1 correlated with the rate of cell proliferation, the expression of cell cycle markers and disappeared when differentiation of neurosphere cells was induced. Interestingly, inhibition of RAE-1 expression in neurosphere cells reduced cell proliferation without alteration of cell viability, which argues for a nonimmune role for RAE-1. These results reveal an unexpected role of RAE-1 in regulating adult SVZ neurogenesis by supporting stem/progenitor cells proliferation .

P3.189
Escaping the prolactin feedback: plasticity of hypothalamic dopamine network

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Tuberoinfundibular dopaminergic (TIDA) neurons are the central inhibitors of prolactin (PRL) secretion from the anterior pituitary. The precise pulsatile patterning of PRL secretion is necessary for successful reproduction, and it is highly modulated over the course of the reproductive cycle. It is well established that dopamine (DA) tonically inhibits the release of PRL and that this, in turn, feeds back to the brain to stimulate TIDA, acting on PRL receptors. This short-loop negative feedback enables

PRL to regulate the timing of its own release. To allow for the hyperprolactinemia, necessary for the production and secretion of milk during lactation, TIDA neurons “escape” from this PRL feedback. The mechanisms involved are, however, still unclear.
To elucidate the mechanisms underlying this *silencing* we studied the electrical properties of TIDA neurones in males, females and lactating animals, using DAT-iCre x ROSA26-eYFP animals, expressing YFP in dopaminergic neurons. In virgin females, we identify an inherent rhythm of the TIDA population electrical activity, which was coupled to DA secretion episodes (measured by amperometry). During nursing, the electrical activity properties of TIDA neurons both spontaneous and PRL-induced remain almost unchanged. This is in sharp contrast with the output of the system in terms of DA secretion. PRL application, in fact, induced a strong elevation of DA in virgin females but no secretion during lactation. This was due to profound biochemical changes in the TIDA neurons during lactation: the lack of DA was found to depend on the lack of tyrosine hydroxylase (TH) phosphorylation. Finally, we found that the PRL stimulus has still a functional effect during lactation, being able to promote secretion of endogenous opioids, rather than DA, from TIDA neurons. In summary, lactation does not generate a change in the electrical activity of TIDA neurons, but rather a plastic change of phenotype from dopaminergic to opioidergic. TIDA neurons are therefore able to integrate the same stimulus (PRL) in a different way depending on the physiological status of the animal, drastically influencing the output of the system and its physiological outcome.

P3.190

Regulations and action processes of the chemokine RANTES/CCL5 in inflammation associated with obesity

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Nutrition-associated diseases and complications are a major challenge for health care systems in France, Europe and many other countries including USA. The rise in the prevalence of obesity has been accompanied by a consequent increase in associated pathologies as type 2-diabetes, liver and coronary heart diseases.
Accumulating evidences point to the existence of a low grade inflammation in adipose tissue of obese patients. Adipocytes and fat cell environment can then release in the blood different factors such as leptin, adipokines, but also proinflammatory cytokines and/or chemokines. These factors, transported to the brain, may act on various systems modulating feeding behavior and stimulate food intake increasing obesity through this vicious circle.
In sera of obese patients, we found a 6-fold increase in the chemokine RANTES/CCL5 levels as compared to control (lean) subjects. Among regulators of feeding behavior and metabolism, melanin-concentrating hormone (MCH) and orexin (ORX) expressing neurons of the lateral hypothalamus act as major orexigenic systems. In this study, quantitative PCR and immunoassays analysis have revealed a robust up-regulation of both orexigenic MCH and orexin mRNA expression and protein levels in the mouse hypothalamus after intraperitoneal and central acute CCL5 treatment. Using a patch clamp technique, we have also revealed a depolarizing effect of CCL5 (0.01-10 nM) on MCH neuronal activity in non-obese mouse hypothalamic slices occurring through glutamate release onto MCH neurons. These data were confirmed by experiments performed in crude synaptosomes from mouse hypothalami (containing both glia and synaptic fractions) suggesting that glutamate can be released both from neurons and glial cells. This glutamate release on MCH neurons should induce an increase in MCH neuronal activity and thus an increase in food intake-related behavior. Overall, our results suggest that CCL5 could participate, by maintaining overfeeding, in the deregulated central control of food intake found in obesity and could represent a potential therapeutic target in the treatment of eating disorders, such as diet-induced obesity.
This work was supported by the CNRS, INSERM and ANR.

P3.191

Neuroprotective effect of aretemisia absinthium against lead neurotoxicity in adult Wistar rats: an immunohistochemical study

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Lead has been recognized as a neurotoxic heavy metal, since it induces morphological and functional abnormalities in the brain. Several studies reported, in the rat brain, a neuroprotective effect of aqueous extracts of *Aretemisia absinthium* against lead neurotoxicity. Here, we investigated the effect of chronic lead exposure on the dopaminergic innervation together with the glial system. Using immunohistochemistry of tyrosine hydrosylase (TH), our data show a significant reduction of TH-immunoreactivity in substantia nigra pars compacta (SNpc) and its subsequent cortical projections in the intoxicated group, while the GFAP immunohistochemistry shows an hypertrophic immunoreactive asrtocytes in the frontal cortex and other brains structures of the lead treated animals. Treatment with *A. absinthium* extract restores most of the changes in the glial and dopaminergic systems which occur in lead intoxicated rats. Our finding demonstrate that *A. absinthium* might be beneficial for the treatment of the glial and neuronal alterations observed during chronic lead intoxication.
Keywords: Lead, rat, immunohistochemistry, *Aretemisia absinthium*, astrocyte, tyrosine hydroxylase. Substantia nigra pars compacta.

P3.192

A window into the brain: in vivo permeability of the median eminence and functional implications

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The median eminence is located at the junction between the pituitary and the hypothalamus. Neuroendocrine neurons located in the arcuate nucleus of the hypothalamus secrete their regulatory peptides at the level of fenestrated vessels of the median eminence. These neurohormones are carried to the pituitary where they act on their target cells to generate hormone pulses involved in a range of physiological responses. In addition to neurons involved in endocrine function, neurons involved in regulation of food intake are found in the arcuate nucleus. Blood-borne factors such as ghrelin, a hormone produced by the stomach and involved in both neuroendocrine function and food intake could enter through the fenestrated capillaries of the median eminence and diffuse into the arcuate nucleus, to provide retro-control on neurohormone secretion and/or food intake. However, it is not clear how blood-borne factors can reach their target neurons in the brain, as diffusion of molecules to neuronal cells bodies is controlled by tanycytes, specialized ependymal cells that line the critical junctions, and may therefore prevent molecules from the circulation from crossing the median

eminence/arcuate nucleus junction and diffuse into the brain. To address this question we analyzed dynamics of entry of fluorescent molecules injected in the circulation into this deep structure on the ventral side of the brain using state-of-the-art 2-photon microscopy and surgical approaches. We determined the cut-off size for vessels of the median eminence in vivo and investigated how far bioactive fluorescently labeled ghrelin diffused into the arcuate nucleus. We found that this small 4 kDa molecule was able to diffuse into the median eminence and the arcuate nucleus beyond the tanycyte extensions. It specifically bound to neurons, some of those corresponding to food intake regulatory neurons, and activated cells by induction of cFos expression in the area of diffusion. This data provides an unprecedented insight on the role of the median eminence in molecule entry into the brain and provides a model for studying molecular communications in different physiological stages or in pathological conditions such as deficits in pituitary hormone secretion or deregulations of energy balance and obesity.

P3.193

A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted *Magel2* gene

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The onset of feeding at birth is a vital step for the adaptation of the neonate to extra- uterine life. Prader-Willi syndrome (PWS) is a complex neurogenetic disorder caused by the alteration of several imprinted contiguous genes including *MAGEL2.* PWS presents with various clinical manifestations, including poor suckling behaviour and feeding problems in neonates. Hypothalamic defects have been proposed but the pathophysiological mechanisms remain poorly understood. Here we report that a *Magel2* deficient mouse with 50% neonatal mortality had an altered onset of suckling activity and subsequent impaired feeding, suggesting a role of MAGEL2 in the suckling deficit seen in PW newborns. The hypothalamus of *Magel2* mutant neonates showed a significant reduction of oxytocin. Furthermore, injection of a specific oxytocin receptor antagonist in wild type neonates recapitulated the feeding deficiency seen in *Magel2* mutants, and a single injection of oxytocin, three to five hours after birth, rescued the phenotype of *Magel2* mutant pups, allowing all of them to survive. Our study illustrates the crucial role of feeding onset behaviour after birth. We propose that oxytocin supply might constitute a promising avenue for the treatment of feeding difficulties in PW neonates and potentially of other newborns with impaired feeding onset.

P3.194

The GHS-R (Growth Hormone Secretagogue Receptor) and its constitutive activity: a new way in the treatment of the somatotroph adenomas?

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Pituitary tumors are most usual intracranial tumors, displaying hormonal hypersecretion with in some cases a sustained proliferation. The main objective of our work is to find a pharmacological alternative able to treat the patients insensitive to the somatostatinergic therapy. Ghrelin stimulates pituitary GH

release in vivo. Interestingly, the GHS-R, not only transduces signal via ghrelin binding, but also owns a high ligand-independant constitutive activity.
Human somatotroph adenomas expressed high level of GHS-R (mRNA and protein). Treatment of human somatotroph adenoma primary cultures with the GHS-R inverse agonist, Substance P Analog (SPA), induced a dose-dependent decrease of GH secretion.
We constructed cell clones from GH4C1, somato-lactotroph tumoral rat cell lines, performing overexpression of wild type or mutated GHS-R (respectively GHS-R WT and A204E-GHS-R). We used the genetically encoded FRET sensor EKAR to record the dynamic of ERK activity, in response to SPA.
SPA causes a YFP/CFP ratio decrease in the GHS-R WT and not in the A204E-GHS-R cells, giving first insights of the ERK1/2 MAPKinase pathway implication in the constitutive activity of the GHS-R. The involvement of a constitutive activation of ERK1/2, known as a significant mitogenic pathway, could participate to the tumorigenesis of adenomas. The fine knowledge of the different signalling pathways implicated in the constitutive activity of the GHS-R could permit to us to reveal a new potential treatment in the somatotroph adenomas.
The authors thank C Roche for her technical assistance and JD Troadec for his critical reading.

P3.195

Localization of kisspeptin neurons in the hypothalamus of prepubertal female lambs. Possible connections with GnRH and Neuropeptide Y

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One of the main roles of hypothalamic peptide kisspeptin (kiss) is attributed to its function in the initiation of the pubertal events in mammals, by activation of GnRH neurons. The aim of presented work was to investigate the localization of the perikarya, axons and axon terminals exhibiting immunoreactive (ir) expression of kiss in the hypothalamus of female lambs during the peripubertal period, before the first estrous cycle. These localizations were compared with the site of release of GnRH in the median eminence and also with the site of synthesis of neuropeptide Y in the arcuate nucleus . The experiment was conducted on eight 32-weeks old merino lambs. The distributions of the peptides were estimated using immunohistochemistry with antibody anti-kiss 10, anti-(2-10) GnRH and anti-NPY. The hypothalamic areas containing numerous kiss-ir perikarya and a dense network of kiss-ir fibers were localized in the caudal part of the arcuate nucleus in the medial basal hypothalamus. Single axons but not perikarya were observed throughout the hypothalamus from the area preoptica to the mammillary bodies. Distinct bundles of kiss-ir nerve terminals were seen in the lateral zone of the median eminence (ME) and in smaller amount dispersed in its medial zone. The localization of kiss-ir nerve terminals in the ME matched very closely with the distribution of GnRH-ir nerve terminals while the kiss-ir and NPY-ir cell bodies are observed in the same part of the arcuate nucleus . It is concluded that the exceptionally abundant presence of kiss-ir neurons could be attributed to their critical stimulatory action on the GnRH neuronal system during the peripubertal period. This influence could be direct in the site of the release of both peptides, indirect through connections with NPY neurons or at both levels.

P3.196

Microglia/neuron interactions in a murine model of 6-Hydroxydopamine-induced dopaminergic neurodegeneration

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Striatal injection of 6 hydroxydopamine (6-OHDA) has been widely used to induce dopaminergic neurodegeneration in rat models of Parkinson's disease. In contrast, systemic intoxication by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is preferred in mice and was shown to induce a robust inflammatory reaction along with a substantial loss of dopaminergic neurons. In the present work, we set up a murine model of 6-OHDA-induced dopaminergic neurodegeneration to assess neuron/microglia interactions in the substantia nigra (SN) of C57Bl/6 wild-type (WT) or DAP-12 knock-in (KI) mice. Functional alterations of the nigro-striatal dopaminergic system were quantified in vivo using an apomorphin-induced rotation test. Immunohistochemical analysis of TH (tyrosine hydroxylase)-immunopositive dopaminergic neurons and F4/80-immunopositive microglia was then performed at different time points following intra-striatal injections. Our histofunctional kinetic analysis showed that:
i) neuronal cell loss in the SN paralleled the increased density of microglia at early time points following injections (day 4 to day 7),
ii) functional alterations do not persist on the long-term despite irreversible TH+ neuronal cell loss and iii) microglial activation, as assessed by F4/80+ immunostaining is transient and characterized by the presence of close interactions between microglia and TH+ neurons.
Microglial ramifications detected within the soma of TH+ neurons suggest that microglia likely exert deleterious effects on TH+ neurons. In contrast, microglial activation was weak in 6-OHDA Dap-12 KI mice and neuronal cell loss in the SN was reduced by a factor of 40 % as compared to 6-OHDA WT mice. Accordingly, the rotational behavioral test showed that 6-OHDA Dap-12 KI mice were, at least partly, functionally protected from the neurotoxic effects of 6-OHDA. Overall, this study demonstrates that 6-OHDA-induced dopaminergic neurodegeneration can be considered as a valuable model to study neuron-microglia interactions in wild-type or genetically modified strains of mice. We are currently investigating the immune molecular mechanisms underlying microglia/neurons interactions in the SN of injured wild-type and DAP 12 KI mice.

P3.197
Perception of the impacted sounds prosody in schizophrenia

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Sounds are an essential way to interact with the surrounding world. In social interactions, emotional prosody is an important way to understand one others and for making mutual adjustment. In interactions with object, impact sounds can be considered as the equivalent of tone of voice. Impact sounds afford, in a very concise way, relevant information about surrounding objects in everyday situation. An extended literature analyzes the recognition of emotional prosody in schizophrenia, and reveals impairments in this ability.
But no studies at our best knowledge analyze possible equivalent impairments in the perception of impacted sounds. Yet, the parametric manipulation of these sounds was previously explored in healthy subjects, by creating sound continua (impacted sounds morphed into two materials categories) with analysis-synthesis and interpolation methods (Aramaki et al., 2011). With a reduced sound corpus from this previous study, we used the same experimental protocol based on a constrained categorization task of the perceived material and measure of the response curve for these transitions, to analyze possible perceptual impairments in patients with schizophrenia.
Data analysis highlighted shallower curves for all transitions with significantly smaller slopes in schizophrenics than in controls. The slope indicates how abrupt the categorization between materials is. Thus, this suggests that schizophrenics perceive category transitions in a more smooth way than controls. The acoustic parameters of the sounds (Aramaki et al., 2011) lead us to discuss the results in term of impairments in the perception of specific timbre features and in the internal representation of material sounds category in schizophrenia.
This is the first study to our knowledge that used calibrated and parameterized sounds to assess environmental sound perception in schizophrenia. This study confirms the importance of investigating

timbre perception in schizophrenia and constitutes a new step to better understand distortions related to this disorder, which induces deep handicap in the everyday situation.

P3.198

Chronic psychosocial stress (PSS) enhances high-fat diet intake and reverses the diet-induced increase of pro-opiomelanocortin (POMC) mRNA expression in hypothalamic arcuate nucleus of C57/BL6 mice

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Stressful life events can trigger physiological-emotional reactions and severe disruption of homeostasis. Chronic exposure to stressors and glucocorticoids(GCs)-related release may alter the processes regulating metabolic function. Since stressful events produce an impact on food intake and feeding motivation, stress may be considered a pathogenetic factor for the vulnerability to obesity. According to the idea of energy-dense diet as “comfort food”, we set up a murine model of chronic psychosocial stress (PSS) coupled to diet manipulations. In particular, we used a chronic PSS model in which two mice were housed in the same cage but separated by a grid partition that permitted only sensory, not physical, contact. To allow agonistic interactions to occur between mice dyads (a C57/BL6 mouse plus an outbred NMRI), such partition was removed twice a day. Inbred mice were always defeated by NMRI mice. Two control groups were included in the study: individually-housed (I) C57/BL6 mice and paired (P) C57/BL6 housed mice. To assess whether PSS may alter feeding behavior and metabolic rate as well as the expression of the anorexigenic peptide pro-opiomelanocortin (POMC) in the brain arcuate nucleus, we exposed mice to the following different diets:
i) standard (S);
ii) high-fat (HF) and
iii) high-fat + cafeteria (HF+C).
We found that, socially-stressed animals ingested more calories regardless the different diets and this effect was particularly pronounced in HF+C fed mice. However, in spite of the diet used, body weight did not increase as for control group. In mice fed with HF diet we evidenced an increase of POMC mRNA expression, an effect that was reversed by PSS. In conclusion, these data support the idea that chronic social stress may alter the expression of the anorexigenic POMC peptide in the brain arcuate nucleus.

P3.199

Disentangling visual and motor contributions to the dynamic pre-saccadic shift of attention

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A shift of visual selective resources anticipates the shift of the gaze during saccadic eye movement. We have previously put in evidence a characteristic time-course for such shift (Castet, Jeanjean, Montagnini, Laugier, & Masson, Journal of Vision 2006; Montagnini & Castet, Journal of Vision 2007). However, little is known about the mechanism which triggers this shift of attention in a classical visually-guided saccadic paradigm: it could be the onset of the visual cue pointing to the saccadic

target (cue-locking) or the initiation of the saccadic movement (saccade-locking). With the present study we aimed at investigating these two possibilities at the behavioral level. We tested human subjects on three variants of a dual-task paradigm, involving a perceptual task (orientation discrimination) at the saccadic target position or away from it, and a saccadic task following three different instructions (simple visually-guided saccade, delayed saccade and Go-Nogo task). We analyzed the time-varying performance in the perceptual task as a function of two possibly crucial variables, the Cue-to-Target Onset Asynchrony (CTOA) and the Saccade-to-Target Onset Asynchrony (STOA). We found that both variables affect visual performance, although in a different way and quantity, and we highlight different roles for the the two of them.

P3.200

Semantic priming effects in Arabic-Moroccan: comparison for vowelled and not-vowelled word pairs

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The semantic priming paradigm has traditionally provided a powerful tool for the investigation of cognitive processes related to memory and language, perception or attention. In a typical version of this paradigm, participants are required to make a semantic judgment of response to a target stimulus, which is preceded by either an unrelated word or a semantically related word prime.
The aim of this experiment was to record the EEG from human subjects and to analyze the ERPs elicited by visually presented vowelled words, and unvowelled words in Arabic-Moroccan.
While semantic priming effects have been investigated in many languages, to our knowledge it has not yet been investigated in Arabic.
We hypothesized that:
Semantically related words should be associated with shorter Reaction Times, with lower percentage of errors and with smaller N400 components than semantically unrelated words (semantic priming effects).
The semantic priming effect may be larger for vowelled words that are visually more complex than for Unwovelled words.
Results showed that:
At the behavioral level: results are in line with our hypothesis: RTs are faster for semantically related than for semantically unrelated words. However, participants also made more errors for semantically related words (speed-accuracy trade-off…).
At the electrophysiological level! larger N400 components for semantically unrelated than for related words for vowelled vs unvowelled words with no differences between these two conditions.
These results are discussed within the framework of semantic processing and cross-linguistic comparisons.

P3.201

Locomtor effects of Moroccan propolis on rat in open field test

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Propolis is a sticky resinous material that honey bees (*Apis mellifera* L.) collect from various plants, and mix with wax and other secretion. Propolis has been used as a folk medicine in many countries since ancient times because of its peculiar biological properties in the treatment of cancer, as an antioxidant, anti-microbial, anti-inflammatory, and antibiotic material. Several investigations on propolis have been done in Eastern Europe and South America, but there is no report about the water extract of Moroccan propolis sample previously.
Aim of study: Evaluation of locomotors activities in open field in the rats using different concentration of Moroccan propolis .
Results: The extract increase the locomotor activity (central and peripheral locomotor activities) and decrease the time of immobility and the time of grooming in the rats.
Conclusion: The extract of propolis has an effect on locomotor activity in rat.

P3.202

Long-term consolidation of declarative memory in temporal lobe epilepsy

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Consolidation theory assumes that memories are initially labile before undergoing a series of processes that render representations more stable. Several experiments carried out with a subset of temporal lobe epilepsy (TLE) patients have demonstrated normal memory performance at standard delays of recall (i.e., minutes to hours) but impaired performance over longer delays (i.e., days or weeks), suggesting altered long-term consolidation mechanisms. These mechanisms were specifically investigated in a group of five adult-onset pharmaco-sensitive TLE patients, exhibiting severe episodic memory complaints despite normal performance at standardized memory assessment. In a first experimental section, the magnitude of autobiographical memory loss was evaluated using retrograde personal memory tasks, respectively based on verbal and visual cues. In both conditions, results showed an unusual *U*-shaped pattern of personal memory impairment, encompassing most patients' life, sparing however the childhood, early adulthood and the past several weeks periods. This profile was suggestive of a long-term consolidation impairment of personal episodes, adequately consolidated over “short-term” delays but gradually forgotten thereafter. Therefore, in a subsequent experimental section, patients were submitted to a protocol specifically devised to investigate short and long-term consolidation of contextually-bound experiences (episodic memory) and context-free information (semantic knowledge and single-items). In the short term (1 hour), performance at both contextually-free and contextually-bound memory tasks was intact. After a 6-week delay, however, contextually-bound memory performance was impaired while contextually-free memory performance remained preserved. This effect was independent of task difficulty and the modality of retrieval (recall and recognition). Neuroimaging studies revealed the presence of mild metabolic changes within medial temporal lobe structures. Taken together, these results show the existence of different consolidation systems within declarative memory. They suggest that mild MTL dysfunction can impede the building and stabilization of episodic memories but leaves intact long-term semantic and single-items mnemonic traces.

P3.203

Sexual response of male rats to a mixture of odorant molecules potentially indicating oestrus in mammals

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In many mammalian species, odours given off by oestrous females exert an attracting effect upon male conspecifics. Rampin et al. (2006) showed that such a behavioural effect is not restricted to conspecifics. Indeed, besides being sexually aroused by the odour of oestrous rat faeces, male rats are also aroused by that of faeces of oestrous vixens and mares. This suggests that a common set of odorous molecules may characterize the hormonal status of receptive females in these three species. In search for these molecules, gas chromatography-mass spectrometry analyses were performed on faeces samples from male, di-oestrous and oestrous females of the three species. Five molecules, all short chain carboxylic acids, were found in lower concentrations during oestrus in faeces from female rats, horses and foxes. We tested the behavioural response of 12 male rats during a 30-min exposure to a mixture of these five molecules in the same proportions as found in female oestrous faeces. Different dilutions of this odorant mixture were tested and the behavioural responses of the rats were compared to those displayed when exposed to water (negative control) and faeces from oestrous female rats (positive control).
Sexual responses were found to be significantly dependent on mixture dilution. A bell-shaped curve was observed: Sexual responses to higher and lower dilutions did not differ from those observed with water, whereas those to intermediate dilutions did not differ from those obtained during exposure to oestrous female faeces.
These results support our hypothesis that a small set of odorous molecules may be involved in signalling sexual receptiveness in mammalian females. However, it is not yet clear whether all or only some of these molecules contribute to the behavioural effects and to what extent the odorous environment plays a role. These questions will be addressed in order to use these molecules in studies on the neurobiological basis of the control of behaviours by olfactory signals.

P3.204

Reward size and motor effort modulate neuronal activity in the external segment of the globus pallidus in monkey

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Motivation to perform an action can be defined by the subjective value of this action, and quantified by the ratio between its cost and its benefit. It requires convergence and integration of limbic, motor and associative information to adapt behaviours accordingly. The basal ganglia (BG) are known for their implication in processes involving these different types of information. The external segment of the globus pallidus (GPe) is usually considered as a relay structure between the striatum, the main input structure of the BG, and the two output structures of this subcortical system, the substantia nigra *pars reticulata* and the internal segment of the globus pallidus. It is well positioned to play a key role in the integration of information from the different striatal territories before their transmission to the output structures and, consequently, to encode information about motivation to perform an action. We recorded the activity of 48 neurons in the GPe of two monkeys performing a visuomotor task which requires a motor effort (development of a force) to get a reward. Four distinct associations of visual stimuli determine four cost (force to develop: small or big)/benefit (reward size: small or large) ratios for the animal. Behavioural data (error rates and reaction times) and GPe neurons activity were compared among these different conditions. Our behavioural results showed a decrease of the reaction time in the large reward conditions and an increase of the error rate in the conditions which require the development of the big force. The neuronal activity was modulated, by an increase or a decrease in the firing rate, in response to the occurrence of the visual stimuli (69%), the initiation of the movement (77%) and/or the reward (90%). Most of the GPe neurons recorded responded to multiple events of the task (92%). Seventy percent of the neurons were modulated by the task conditions. That reflects an integration of the information about the force to develop, the size of the reward or both of them in the different phases of the task. Our results suggest that the GPe is not only a relay structure

in the BG but can also play a role in the integration of motor and limbic information to give rise to adapted behaviours.

P3.205
Theory of mind and frontal lobe lesions

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Experimental and clinical studies lack of consensus about the role of frontal lobe in theory of mind. The purpose of current work was to study the impact of focal frontal lesions on theory of mind. 20 patients with focal frontal lesions (12 with right lesions and 8 with left lesions) were compared to 12 patients with posterior lesions and 32 healthy participants matched by age, gender and level of education. Subjects were assessed with a set of neuropsychological tests and two tasks of theory of mind: Reading the Mind in Eyes task and Character intention task. Results showed that patients with frontal lesions differs significantly from healthy controls in the two theory of mind tasks [$F(1,50) = 54.76$; $p < .0001$] and differs from posterior patients lesions only in the character intention task [$F(1,30) = 54.76$; $p < .0001$]. Laterality of frontal lesions had no incidence on performance [$F(1,18) = .008$; $p = .9774$]. However, in frontal lesions group, those with ventromedial lesions (n = 9) showed impairment in the Reading the Mind in Eyes Task and the character intention task relative to those with dorsolateral lesions (n = 11) [$F(1,18) = 2.22$; $p < .05$]. These results are discussed with findings of clinical studies and in terms of general deficit of social cognition.

P3.206
Effects of duration of melatonin and L- tryptophan treatment on anxiety levels in rats

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Melatonin (MEL) is the principal hormone of the pineal gland where it is synthesized in a circadian rhythm with maximum secretion at night. The synthesis of melatonin occurs from L-tryptophan (TRP), the precursor of all indoles of the pineal. Either, deficiency or excess of TRP affects concentration quantities of serotonin (5HT) in the brain. This suggests a possible involvement of TRP in the regulation of affective disorders. The purpose of this study is to examine the effect of TRP and MEL on the level of anxiety assessed in the elevated plus maze (EPM).
Rats were submitted to a long photoperiod (16 L/8D) and received chronic injections of MEL (4 mg / kg), TRP (4 mg / kg) or NaCl (9 ‰) during 2, 4 or 8 weeks. After treatment, the anxiety level is evaluated using EPM test. The number of entries (EAO) or time spent in open arms (TOA) is inversely correlated with anxiety level. The total number of entries in the arms (TEA) reflects locomotor activity. The results were analyzed by ANOVA followed by the test "t". Preliminary results showed that:
1 - MEL and TRP increased the EAO. Therefore, the two molecules affected the anxiety level of rats. However, a treatment period of 4 weeks is necessary to obtain an effect.
2 - TOA observed in rats treated with TRP (4mg/kg) for 8 weeks were significantly higher than those observed after 2 weeks $p < 0.01$ or 4 weeks $p < 0.01$.
3 - TOA of rats treated for 8s with MEL (4mg/kg) were significantly higher than those observed after 2 weeks $p < 0.05$ or 4 weeks $p < 0.001$.
4 - TEA is not affected by different treatments; locomotor activity was then not altered.
Keywords: Melatonin, Tryptophan, EPM, anxiety.

P3.207

Odor fear conditioning in rats: How do amygdala and olfactory cortex interact?

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Fear conditioning paradigm has been widely used for studying the neurobiology of learning and memory. It consists of pairing an initially neutral stimulus (CS) with an aversive unconditioned stimulus (US). Subsequent re-exposure to the CS induces a learned fear response. We previously showed that olfactory fear conditioning induces synaptic changes in the basolateral amygdala (BLA) and posterior piriform cortex (pPC) suggesting an involvement of these structures in the learning. More recently, we used 1-min resolution microdialysis on freely-moving Long-Evans rats to investigate the variations of glutamate (Glu) concentrations in both BLA and pPC during odor fear acquisition. Briefly, the animals received six pairings of peppermint odor as CS (20 s) and 0.4-mA footshock as US (last 2 seconds of odor presentation), with an inter-trial interval of 4 min. We observed a differential temporal dynamic of activation in BLA and pPC during the course of the successive pairings of the acquisition session, with an early response of the BLA during the first CS-US association preceding neurotransmitter release in pPC after which pPC alone shows training-related modifications. These data suggest that the projection pathway between BLA and pPC could play a crucial role in sustaining the observed dynamics. Therefore, in the present experiment, we investigated the consequences of an artificial increase of Glu in BLA, on the variations of Glu in pPC. For that purpose, microdialysis experiments were performed on anesthetized rats using probes implanted in pPC and ejection capillary for drug delivery (PDC, a Glu uptake inhibitor) into the ipsilateral BLA. Dialysates were analyzed for Glu content using capillary electrophoresis and laser-induced fluorescence detection. Results showed that injection of PDC in BLA induced a transient and delayed increase of Glu concentration in pPC. Those data are in agreement with an activation of the pPC by the BLA, and suggest that the variations observed in pPC during odor-fear acquisition in our previous study have been triggered by the amygdala.

P3.208

Cortical reorganization following a right or a left occipital lesion

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Background: Following unilateral occipital damage of the primary visual cortex one of the more common visual field defects observed is Homonymous Hemianopia (HH), which refers to a meticulously symmetrical loss of vision in the two eyes (see Zihl, 2000 for review).
The present study wanted to assess the cerebral network responsible for natural scenes perception, in healthy adults and hemianopic patients suffering from an occipital cortex lesion. Furthermore, the cognitive demand of the visual task is studied by contrasting a visual detection task (low cognitive demand) and a natural scene categorization task (high cognitive demand) presented in the central visual field .
Methods: Two left brain-damaged patients (Right Homonymous Hemianopia, RHH) and four right brain-damaged patients (Left Homonymous Hemianopia, LHH) were compared to fourteen healthy participants, in detection task (i.e. “Is there an image on the screen?”) and categorization task (i.e. “Is

it an image of a highway or a city?") during a block-designed functional magnetic resonance imaging (fMRI) recording session.
Results: Regarding the occipital cortex, healthy individuals showed a bi-hemispheric activation in the detection task but only in the left occipital lobe in the categorization task.
Conversely, in patients, the same network of activity was observed in both tasks. We found a bilateral activation (in the occipital lobes) in right brain-damage (LHH) patients whereas in left brain-damaged (RHH) patients the activation was mainly seen in the right (intact) hemisphere (occipital lobe and posterior temporal areas).
Conclusion: Results suggest that different cortical reorganization take place depending of the occipital lesion side.
Keywords: Hemianopia; Detection; Categorization; Natural scenes; fMRI.

P3.209
The neural basis of visual illusions in the larva zebrafish

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One of the main goals in neurosciences is to understand how cognitive functions, such as sensory perception are encoded by the dynamics of large neuronal networks. The main stream of perception research has mainly focused on sensory stimulation and recordings of the induced neural responses. An alternative approach is the use of sensory illusions, in which sensory perception takes place in absence of physical external stimulus, and therefore help to better isolate the neuronal circuit activities underlying sensory perception.
One example of these sensory illusions is the motion after-effect (MAE), in which exposure to coherent motion for a certain period of time, will induce motion perception in the the opposite direction following the end of the stimulus.
Here, we propose to study the neuronal mechanisms behind visual perception using the zebrafish larva, which enables us to monitor the activity of large neuronal networks (representing a relevant portion of the whole brain), still with single cell resolution, in an intact, behaving vertebrate.
Upon the presentation of a coherent motion visual stimulus, covering a large portion of its field of view, the zebrafish larva will move its eyes in the direction of the moving stimulus in order to to stabilise the moving external world on the retina. This behaviour is known as optokinetic response.
We have found that following the presentation of a moving stimulus for a duration longer than 250 s, the zebrafish larva performs, in absence of any sensory stimulation, eye movements in the exact opposite direction of the preceding visual stimulation.
In correspondence to humans that perceive a MAE with a slower velocity and amplitude, the zebrafish larvae show spontaneous eye pursuits of smaller amplitudes and at a lower frequencies than those induced by the visual stimulation.
We are now performing two-photon Ca2+ imaging in intact behaving larvae to study what are the patterns of neuronal activity that precede the spontaneous-reverse-eye-movements towards the elucidation of the neuronal mechanisms behind sensory perception.

P3.211
Honeybees' height control and optic flow

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There have been extensive studies on the use of optic flow (OF) cues in insect flight control processes such as height and speed control.

Honeybees were trained to fly along a high-roofed tunnel, part of which was equipped with a moving floor. Honeybees followed the stationary part of the floor at a given height. On encountering the moving part of the floor, which moved in the same direction as their flight, honeybees flew at a lower height, thus restoring their ventral optic flow to a similar value that they had experienced when flying over the stationary part of the floor. This was therefore achieved not by increasing their speed, but by lowering their flight height. These observations show that the ventral optic flow is directly involved in height control process.
These results can be accounted for by a control system called an optic flow regulator that we proposed in previous studies. This model may replicate the behavior of the honeybees and their safe navigation along tunnels on the sole basis of optic flow measurements, without any need to measure either their speed or the clearance from the surrounding surfaces.

P3.212

Lateralized movement inhibition in human and non-human primates

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Prevailing theoretical models for saccade-countermanding task posit a race between two noisy processes, those associated with "go" and "stop" respectively (Logan & Cowan, 1984). These models and their extensions (Boucher et al, 2007) have been used to account for some key behavioral measures and neurophysiological data. However, these models do not account for any possible spatial selectivity of the inhibitory stop signal. In order to further investigate this question, we explore the spatial tuning of the inhibitory processes that permits human and non-human primates to selectively countermand prepared movements. In a series of experiments we spatially allocated the probability of stop trials to distinct spatial target locations when keeping unchanged the global proportion of stop trials (from 30 to 80%). The results show that human and non-human primates are capable to adapt their performance to inhibit a movement at selective target location. Response times in no-stop trials increased at the location of larger probability of stop trials when response times remain unchanged at others target locations. Relative and absolute differences of proportion of stop trials appear to be capable to modify response times and performance to inhibit prepared movement. Then, we tested a race model (Boucher et al., 2007) consisting of movement (GO) and fixation (STOP) units, based on neurophysiological observations in the frontal eye field of behaving monkeys (Hanes & Schall, 1995). The model has distinct GO and STOP units according to target location that are capable to dynamically interact. Our results show that a spatially interacting race model fit the behavioral performance quantitatively. Altogether, we provide evidence that, as excitatory signal, inhibitory signal of movement is spatially tuned and our network model let us to propose that distinct inhibitory networks within the fronto-median cortex might encode spatially tuned probability of stopping.

P3.213

Motor performance is improved by action verb production and mental calculation

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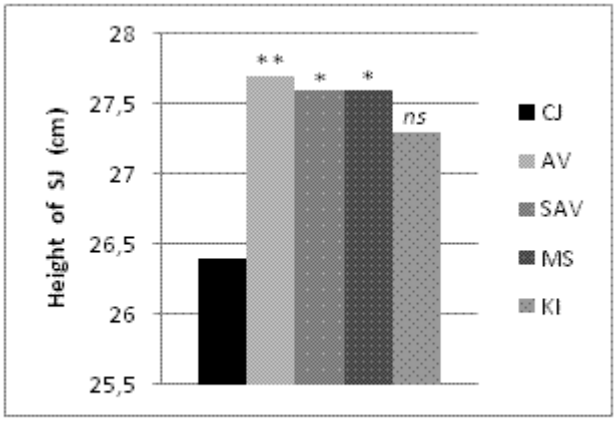
Several results show that motor imagery, language production, mental calculation and motor execution share the same or closely related brain motor cortical areas. The present study aimed at studying the possible influence of wording, language and calculus production upon motor performance. Novice subjects in mental imagery performed a squat jump after a cognitive task (Action Verb, Silent Action Verb, Mental Subtraction and Kinaesthetic Imagery). The results show (Figure 1) that the cognitive tasks improve the vertical jump, except Kinaesthetic Imagery that ameliorated the height but not significantly. The results indicate that the generation of an action verb (*saute* = jump) can improve vertical jump performance in the same way than mental calculation and more significantly than kinaesthetic imagery in mental imagery novice subjects. It is possible that the involvement of motor areas in language processing and in mental calculation may contribute to the pre-activation of these areas and thus facilitate the execution of the movement.



[Effect of various conditions on squat jump (SJ) pe]

Fig.1. Effect of various conditions on squat jump (SJ) performance. Subjects were asked to perform three trials of squat vertical jump without (control jump, CJ) and after the four cognitive conditions randomly ordered for each subject. The results are expressed as height of the jumps. * $P < 0.05$, ** $P < 0.01$, *ns*: not significant; n = 29 (7 females, 22 males).

P3.214

Chronic cannabinoid exposure during adolescence induces cogntive impairments in rats at adulthood: comparison between Lister Hooded and Wistar rats

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The adolescent period represents a critical phase of development where the endocannabinoid system plays an important role in influencing the functioning of different neurotransmitters. Human studies show that cannabis consumption during adolescence induces persistent brain and behavioural alterations which could lead to psychiatric diseases such as depression, anxiety and schizophrenia. In rats, chronic exposition to cannabinoid induces cognitive disorders similar to these observed in schizophrenia.
Lister-Hooded rats are known to develop a robust cannabinoid intake in self-administration paradigms in comparison to albino rats that have been commonly used to investigate long-term behavioral abnormalities induced by pubertal chronic cannabinoid treatment.
Thus, the aim of this study was to compare the long-term cognitive consequences of chronic cannabinoid exposure during adolescence in Lister-Hooded rats to those observed in Wistar rats. To do so, rats of both strains were injected throughout the adolescence period (postnatal days 29-50)

with vehicle or incremental doses of the synthetic cannabinoid CB1 receptor agonist CP55, 940 (CP) once daily for 21 consecutive days. Following a 28-day drug free period, visual short term and spatial working memories were assessed in object recognition and object location tasks respectively. For both tasks, 2 delay intervals between learning phase and testing phase were experienced: 30 min and 2 h. In the object recognition task, CP impaired visual short term memory after the 2 delays in both strains. In the object location task, after a delay of 30 min, CP also impaired spatial working memory in Wistar rats whereas in Lister-Hooded rats a similar effect was observed but only when the delay was increased to 2h.
Taken together, our results first confirm that adolescence is a critical period for deleterious effects of cannabinoids on memory processes. They also suggest that these deleterious effects on spatial working memory are more strain dependant than those observed on short term memory.

P3.215

Effect of dopamine depletion on the building of cognitive maps: a pilot radiotelemetric approach in the rodent

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Parkinson disease (PD) results from a degeneration of the dopaminergic neurons of the substantia nigra pars compacta. It is now recognised that, apart from the motor aspects that are relatively well controlled by pharmacological or surgical therapies, PD produces cognitive disorders amongst which is a form of spatial disorientation. This is increasingly problematic because of the increased autonomy enjoyed by Parkinsonian patients thanks to therapeutic improvements. In order to be able to deal with these cognitive dysfunctions, it is necessary to elucidate the pathophysiological mechanisms that underlie them. We hypothesize here that functional alteration of the hippocampus contributes to this disorientation, the brain structure implicated in spatial navigation with reference to an external frame. To this purpose, we study in awake and behaving rodent the influence of dopamine depletion upon the building of cognitive map in the hippocampus. A second important aspect of this study is the following: it is of importance for us for further experiments to dispose of a wireless device in order to be able to perform experiments in very large scale environments, we therefore used the opportunity of this pilot study to test the telespike, a prototype 16 channel radiotelemetric recording device developed by Alpha-Omega engineering (Nazareth-Illit, Israel). We recorded CA3 neurons in normal and dopamine depleted rats during a behavioural task in a Y maze. Our results show that our protocol allows us to evidence spatial learning impairments in DA-depleted animals. Sham rats are more efficient to learn the task than DA-depleted rats. Moreover, some effects of the dopamine lesion are lateralised. Finally, electrophysiological results suggest that the ability to learn the task could be related to a disruption of the encoding of the decision and that the encoding of the reward location [T1] is modified in DA-depleted rats.

P3.216

Neuroticism modifies psychophysiological responses to films

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Background: High neurotic people tend to be more psychologically reactive to stressors. This emotional reactivity might be assumed to have physiological correlates, and various works have

studied autonomic nervous system (ANS) responses of neurotic subjects using negative and positive stimuli without distinguishing between emotions.
Given the differences of ANS reactions according to emotional valence and arousal, our study aims at comparing the physiological activity (ANS and facial muscles activity) of neurotic subjects using specific and distinct categories of emotion.
Methods: Fourteen low neurotic subjects and eighteen neurotics were assessed on an emotional attending task using film excerpts inducing happiness, peacefulness, fear, disgust and sadness. We evaluated physiological measures such as the skin conductance response (SCR), heart rate (HR) and the activity of zygomatic (smiling) and corrugator (frowning) muscles as an index of emotional expression.
Results: Neuroticism increased corrugator activity and SCR during the fear-inducing film. Also, we found a decrease in HR during the happy and peaceful films in neurotics subjects.
Conclusion: Following decades of evidence that individual higher in neuroticism experience more intense emotional reactions to even minor stressors (Larsen & Ketelaar, 1991),our results indicate that these individuals have greater expressive and SCR reactivity to aversive stimuli. We also show for the first time that they have less HR reactivity to positive happy and peaceful stimuli. The observed effects of emotion on autonomic responses indicate that neuroticism has selective effects on psychophysiological parameters. These effects differ according to the presented emotion suggesting further research have to consider the emotion category in the study of individual differences.

P3.217

Unconscious cognitive control: correcting unaware (partial)errors

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In conflicting situations, such as Stroop task, incorrect responses are (more or less) automatically activated. When such activations reach the response threshold and an error is committed, subjects consciously and reliably detect such errors (>90%, Rabbit, 1996). Electromyographic recording in such tasks reveal that most of the incorrect activations remain subtreshold, however. In such trials, since the correct response was nonetheless given, the subliminal activations were interrupted and corrected, revealing the involvement of cognitive control mechanisms. The question as to whether those subliminal incorrect activations are consciously perceived remains an open issue. It has indeed been argued that cognitive control processes require conscious experience (Dehaene & Naccache, 2001). However, such "partial errors" might not have been consciously detected, since an event-related potential, called error positivity (Pe) reported after aware errors, is absent on such partial errors (Vidal et al. 2000).
Awareness of incorrect response activation was assessed by asking the participants, after every trial, to report how much sure they were to have activated the incorrect response (6-levels scale, 1: absolutely sure to have not activated the incorrect response, 6: absolutely sure to have activated the incorrect response).
To better caraterize subject detection performance, we calculated two parameters from Signal Detection Theory (Green and Swets 1966): d´ measuring the subject detection sensibility and beta the subject response bias.
d´ were overall very low, indicating that subjects were not aware of their partial errors. Their detection, although very low, was however higher for incompatible trials than for compatible ones. A significant effect on beta was also obtained: subjects tend to say more often that they have produced a partial error for incompatible trials.
The partial error latencies distribution revealed that correction is slower when subject level of awareness was high (5 or 6), suggesting that conscious detection delays correction.
Thank to a Bayesian approach, we also evaluated to what extend detection depends on the EMG burst parameters.

P3.218

A high-resolution EEG study of the role of SMA/preSMA in resolving conflict and action switching

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The supplementary motor area (SMA) is often assumed to be involved in movement planning. However, more recent studies suggested that the SMA and more precisely the pre-SMA is involved in the resolution of the conflict between motor actions plans (Ullsperger 2001, Nachev et al. 2007). Interestingly, LFP study in monkeys showed that the pre-SMA is also engaged in action switching (Isoda and Hikosaka 2007).
Thanks to appropriate processing techniques applied to EEG data, Vidal et al. (2003) have reported the existence of a fronto-central negativity occurring around 40 ms before EMG activity in choice reaction time task (called N-40), and which likely arises from SMA/preSMA. This opens the possibility to study the role of SMA/preSMA non invasively in Humans, at a high temporal resolution.
If the SMA/preSMA is responsible for the resolution of the competition between responses, a larger N-40 should be observed in conflicting situations leading to response co-activation. It should also be larger on trials in which a response switch is necessary. Particularly, the N-40 should be bigger in trials where the required response is different from the one at the previous trial (alternated responses) compared to the situation where the response is repeated.
We tested these predictions with two EEG studies (64 channels, Current Source Density estimation) by manipulating the response repetition and the stimulus-response compatibility. In both experiments, an activity was recorded over SMA/preSMA, starting about 70ms, and peaking about 30 ms, before voluntary EMG onset. It was largely reduced in compatible trials where the response was repeated. Interestingly, the highest activity of the N-40 was observed in incompatible trials where the response was alternated. We conclude that the SMA involvement is related to the difficulty of response selection, modulated by response conflict and response alternation.

P3.219

Contributions of dorsal striatal subregions to spatial alternation behavior

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Considerable evidence has shown a clear dissociation between the dorsomedial (DMS) and the dorsolateral (DLS) striatum in instrumental conditioning. In particular, DMS activity is necessary to form action-outcome associations, whereas DLS is required for developing habitual behavior. However, very few studies have investigated whether a similar dissociation exists in more complex goal-directed learning processes. The present study examined the role of the two structures in such complex learning, by analyzing the effects of excitotoxic DMS and DLS lesions during the acquisition and extinction of a spatial alternation behavior, in a continuous alternation T-maze task. We showed that DMS and DLS lesions have opposite effects, the first impairing and the second improving animals performance during learning and extinction. The DMS lesions may impair the acquisition of a spatial alternation behavior by disrupting the signal necessary to link a goal with a specific spatial sequence. The DLS lesions may accelerate goal-driven strategies by minimizing the influence of the external stimuli on the response, thus increasing the impact of action-reward contingencies. Taken together, these results suggest that DMS- and DLS-mediated learning strategies develop in parallel and compete for the control of the behavioral response early in learning.

P3.220

The antidyskinetic effect of exercise on L-DOPA-treated hemiparkinsonian mice

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The motor symptoms of parkinsonism respond to dopamine replacement with L-3,4-dihydroxyphenylalanine (L-DOPA), but most patients develop dyskinesias that are well experimentally modeled. Evidences indicates that a 'dopamine and cAMP-regulated phosphoprotein of 32 kDa' (DARPP-32) signaling misbalancing in the striatal medium spiny neurons modulates these dyskinetic responses to L-DOPA. We found that exercise influences DARPP-32 signaling and attenuates L-DOPA-induced dyskinesia in hemiparkinsonian mice. Four weeks after unilateral striatal lesion with 6-hydroxydopamine, C57BL6 mice presented a dramatic assimetry in forelimb use and apomorphine-induced rotations, and reduced striatal tyrosine hydroxylase immunoreactivity in the lesioned side. After it, all animais received daily treatment with L-DOPA (25 mg/kg, i.p.) and the peripheral DOPA decarboxylase inhibitor benserazide (12.5 mg/kg, i.p.) for two additional weeks, while half of the animals had simultaneously free access to voluntary running wheels. The L-DOPA administration produced a partial recovery in the forelimb use, with similar effects in sedentary and exercised animals. Sedentary mice presented severe dyskinesias until two hours after the L-DOPA administration. However, these dyskinesias were very discreet in the exercised animals. This significantly decrease was attributable to a marked reduction of axial, limb, orolingual and locomotor abnormal voluntary movements in the exercised animals. As expected, dyskinetic mice presented increased cAMP levels and upregulation of phosphorylated form of DARPP-32 at Thr34 and Thr75 in the striatum, but the exercise group displayed significantly lower values. Exercise also increased striatal cell cycle and the expression of the cell division cyclin-dependent kinase 5 (cdk5) that modulates the L-dopa-induced dyskinesias. We inhibited this exercise-induced increased cell cycle with radiation, where the striatal cdk5 levels returned to sedentary levels, and the antidyskinetc effects of exercise disappeared. Cdk5 inhibition with roscovitine showed similar effects in the exercised animals. These findings suggest that cdk5 upregulation may compensate the increased DARPP-32 phosphorylation in the striatum of dyskinetic mice.

P3.221

Dissociation between perception and eye movement for rich visual stimuli

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In order to analyze the characteristics of a rich dynamic visual environment, the visual system must integrate information collected at different scales through different spatiotemporal frequency channels. Still, it remains unclear how reliable representations of motion direction or speed are elaborated when presented with large bandwidth motion stimuli or natural statistics. Last year, we have shown that broadening the spatiotemporal frequency content of a textured pattern moving at constant speed leads to different results on a reflexive tracking task and a speed discrimination task. Larger bandwidth stimuli increase response amplitude and sensitivity of ocular following, consistently with a maximum-

likelihood (ML) model of motion decoding. In contrast, larger bandwidth stimuli impair speed discrimination
performance, suggesting that the perceptual system cannot take advantage of such additional, redundant information. Instead of ML, a gain control decoding mechanism can explain the drop in performance, suggesting that action and perception rely on different decoding mechanisms. To further investigate such task-dependant pooling of motion information, we measured pattern discrimination performance using these textured stimuli. Two noise patterns were presented sequentially for 250ms on a CRT
monitor (1280x1024@100Hz) and covered 47° of visual angle with identical properties (mean SF, bandwidth SF, speed) except for a randomized phase spectrum. A test pattern was then presented and subjects were asked to match it with one or the other reference stimulus (ABX task). At small bandwidth and optimal mean spatial frequency (0.3cpd), subjects were able to discriminate the two patterns with high accuracy. Performance dropped to chance level as spatial frequency bandwidth increased. Increasing the mean spatial frequency decreased the overall performance. Again, these results suggest that perceptual performance is deteriorated in presence of larger information.

P3.222

Implication of epigenetic factors in epileptogenesis and cognitive deficits associated with temporal lobe epilepsy

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Temporal Lobe Epilepsy (TLE) is the most common form of epilepsy in adults. Experimental models of TLE can be obtained after kainic acid-induced status epilepticus (SE), which triggers epileptogenesis, the process leading to the construction of an epileptic brain. The initial SE induces considerable network modifications, associated with a loss of theta rhythm (4-12 Hz) in vivo, and spatial memory deficits, well before the occurrence of the first spontaneous seizure. Identifying the mechanisms responsible for these alterations may provide ways to delay/stop epileptogenesis and/or restore cognitive function. The Neuron Restrictive Silencer Factor (NRSF), which can potentially control the expression1800 genes, has been identified as one key mechanism. Its expression is increased after SE, affecting gene expression. Preventing its action with decoy oligodeoxynucleotides restores theta rhythm and dampens epileptogenesis. Since NRSF recruits histone deacetylases (HDAC) for its action on gene expression, we hypothesized that SAHA (suberoylanilide hydroxamic acid), an FDA-approved HDAC inhibitor, was disease-modifying.
Using the kainic acid model of TLE, we tested the effect of SAHA on epileptogenesis, theta rhythm and cognitive performance,. We performed a longitudinal study to assess the spatial cognitive performance of the animals during epileptogenesis (7 days before SE, and 5, 10, 20, and 30 days post SE). In parallel, we monitored seizure activity, as well as theta oscillations in the hippocampal CA1 region of the rats treated with SAHA versus non-treated ones.
Our results provide evidence that SAHA treatment delays epileptogenesis, reduces seizure number and prevents the degradation of theta rhythm and cognitive performance. Although HDACs are ubiquitous, and their blockade by SAHA must have multiple consequences that remain to be assessed, its disease-modifying action opens the way to early treatment of at-risk patients.

P3.223

A neurocognitive model of meditation based on ALE meta-analysis

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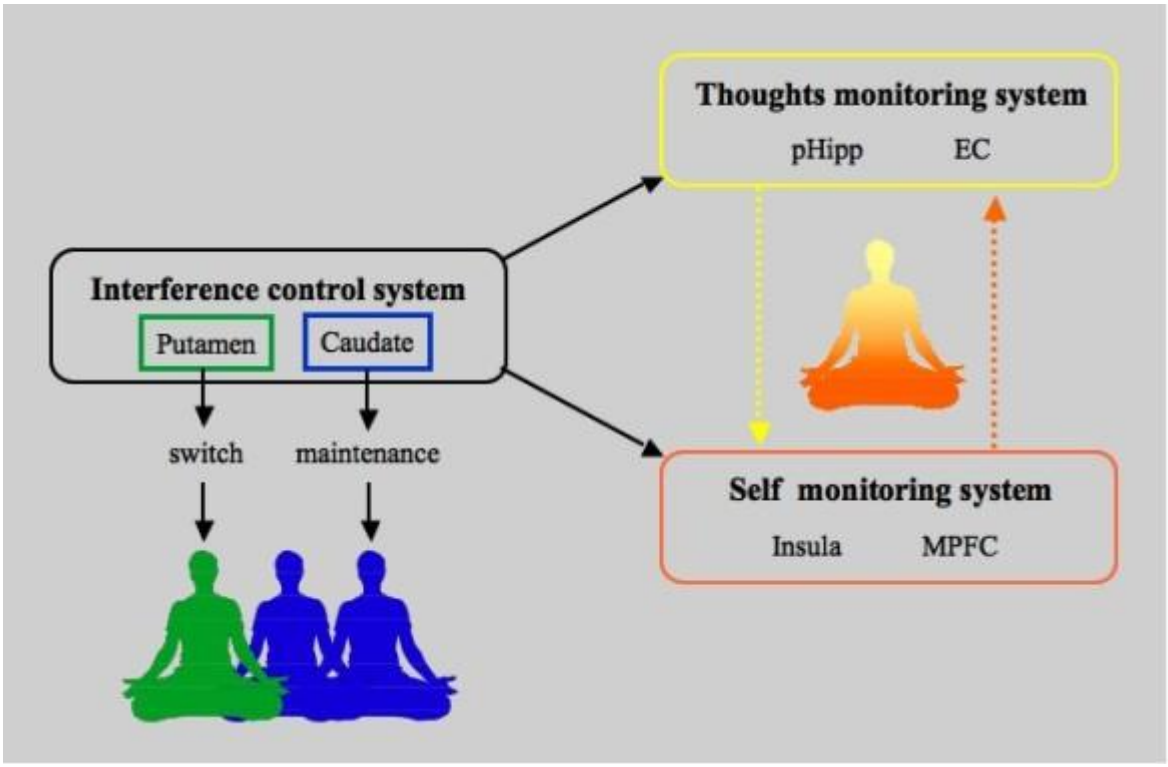
Meditation comprises a series of practices aiming at controlling emotions and enhancing attentional processes. Beyond anecdotical reports, meditation has been shown to improve cognitive functions in long-term practitioners and has been efficiently used in the treatment of several psychiatric disorders [1]. The neural correlates of meditation have been studied in the last fifty years with electrophysiological and neuroimaging techniques showing modulation of alpha and theta activity and activation of the fronto-parietal network and medial structures, involved in attentional control, and subcortical structures known to play a role in emotional processes [2]. Nevertheless results on neural correlates of meditation are controversial. Here we conducted a quantitative meta-analysis based on activation likelihood estimation (ALE) [3] of 10 neuroimaging studies (91 subjects) to evidence the core cortical network subserving meditation. We showed activations of basal ganglia, limbic system, insula and medial prefrontal cortex (MPFC), while activations in the cortical attentional and control systems were not found. We propose a neurocognitive model (Fig. 1) supporting meditation comprising: an *interference control system* (putamen and caudate body) subserving the inhibitory control required during meditation to block irrelevant stimulations; a *self monitoring system* (insula and MPFC) would reflect the engagement of an inward oriented attention; and a *monitoring thoughts system* (parahippocampal and enthorinal cortex) would play a role in the generation and control of the stream of thought necessary during meditation to pass by irrelevant thinking.



[Fig. 1]

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[3] Turkeltaub P.E et al. (2002). NeuroImage 16: 765-780.

P3.224

Similar muscular responses following TMS applied over SMAp or M1 during the preparation of a motor perturbation

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In everyday life, our actions can be perturbed by rapid variations of environmental external forces, and we have to react “well and fast” to these movement perturbations in order to achieve our goals. The Long Latency Stretch Reflex (LLSR), generated by a transcortical loop, is the fastest reaction that can be modulated according to subjects´ intention : its amplitude increases when subjects resist the perturbation compared to when they ´let-go´. This reflex adaptation is known to involve cortical anticipatory processes, especially in the primary motor cortex (M1). During the preparation to a motor perturbation, a role of the Supplementary Motor Area proper (SMAp) has also been suggested. Here, we used neuro-navigated TMS to stimulate SMAp (in order to temporarly disrupt its activity) at different delays (-50, -100, -150ms) preceding a mechanical perturbation of the wrist position, and analyzed the effect of SMAp stimulation on the intention-related modulation of the LLSR. Two major results were found when contrasting SMAp results with those obtained after M1 stimulation. First, surprisingly, TMS application on SMAp elicited Motor Evoked Potentials (MEPs) very similar in both latency and amplitude to those elicited by M1 stimulation. Morever, as for M1 stimulation, SMAp MEPs were followed by a Silent Period. It is important to remember that the SMAp hand area and the M1 hand area are separated by several centimeters, thus it is very unlikely that SMAp stimulation directly activated corticospinal neurons of the M1 hand area. Therefore, MEPs observed after SMAp stimulation most probably reflect direct corticospinal connections descending from SMAp. Second, with respect to the effect of SMAp (and M1) TMS-induced inactivation on the intention-related reflex modulation, the latter was affected by both SMAp inactivation and M1 inactivation when TMS was applied 50 ms before the perturbation, but not for longer delays between the TMS and the mechanical perturbation (100 and 150 ms). This result indicates that SMAp, as M1, is involved in the anticipatory processes leading to the intention-related reflex modulation.

P3.225

Cognitive modulation and long term stability of sensorimotor related oscillations in macaque monkeys

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Electroencephalography studies of human movement reveal characteristic patterns in the beta and gamma bands of cortical oscillations. Specifically, the preparation and execution of a movement leads to an event-related desynchronization (ERD) in the beta band over sensorimotor cortex, whilst the movement is followed by a beta event-related synchronization (ERS). Moreover, studies have shown gamma-ERS over sensorimotor regions, in parallel to the beta-ERD. Here, we use a chronic preparation in the monkey to test whether this pattern is responsive to the different elements of a cognitive task, and also to study whether oscillatory interactions between sensorimotor and prefrontal cortex subserve motor planning.
Two rhesus macaque monkeys learned the Problem Solving Task, a test of cognitive control in which they had to search amongst several targets for the rewarded one (the search phase), and then repeat the correct response a number of times (the repetition phase). A change signal then instructed monkeys to begin a new problem by searching again. Monkeys were chronically implanted with 22 electrodes, inserted through the skull to rest on the dura mater, covering prefrontal and sensorimotor cortex. Electroencephalographic recordings were referenced to a post-frontal electrode, and analyzed in the time-frequency domain to reveal the ERD/ERS pattern as changes in power.
We found the characteristic sensorimotor ERD/ERS pattern related to touch responses in the task, as expected. We demonstrated that the ERD/ERS pattern is modulated by the demands of a cognitive task, revealed by significant differences in power and latency of the ERD and ERS between the two phases of the task. Additionally, we found a coherent oscillatory network between prefrontal and sensorimotor cortex when monkeys wait for target appearance. Finally, we demonstrated the stability of this pattern over recordings spanning a number of months.

These findings contribute to an increasingly detailed characterization of the sensorimotor ERD/ERS pattern, and suggest that it could prove suitable for the longitudinal characterization of motor impairments. In addition, its potential role in cognitive control suggests suitability for studying some cognitive functions, impairments, and networks.

P3.226

Does response inhibition require attention? Yes, but motor specific!

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Executive functions refer to processes that are necessary to the selection and coordination of elementary information processing operations and that are thought to be implemented by a supervisory system. Inhibition of inappropriate response elicited by irrelevant information is one of these processes. The aim of the present study was to investigate whether the supervisory system needs attention to implement inhibition.
In the Simon reaction time (RT) task, particularly well-adapted to study inhibition, subjects have to choose between a left- and a right-hand keypress according to the color of a visual stimulus presented either in the right or the left visual field. It is then classically observed that RTs are shorter for ipsilateral (congruent) stimulus-response associations than for contralateral (incongruent) stimulus-response associations. Nowadays, one admits that in the congruent stimulus-response association, the non-required response activated by the spatial attribute of the stimulus must be inhibited and the attention refocus on the relevant response to be implemented. Interestingly, the efficiency of selective response suppression can be evaluated through the analysis of RT distribution (particularly the slope of the delta plots).
In two different experiments, we compared RT distributions obtained when the Simon task was performed either as a single task or concurrently to a secondary task (dual-task condition, DT) aimed at catching attentional resources. In Experiment 1, the secondary task was a temporal production task known to be very attention demanding. Delta plots analysis revealed that the DT did not alter the inhibition process suggesting either that attention was not necessary for inhibition process, or that time processing and inhibition tap into different attentional resources. In Experiment 2, the secondary task was a visual tracking task involving more motor control. Results show that the DT impaired the efficiency of inhibition, suggesting that attentional resources caught by the tracking task are necessary for inhibition. Therefore inhibitory processes do require attentional resources but only drawn from motor resources rather than perceptual ones.

P3.227

Effects of vitamin A status on stress reactivity and emotional behaviour

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Vitamin A and its derivatives, *all-trans* retinoic acid (RA), are involved in the control of hippocampal synaptic plasticity and in cognitive functions. Indeed, RA treatment can counteract the effects of vitamin A deficiency (VAD) on spatial memory deficits and on adult hippocampal neurogenesis disruption (*Bonnet et al. PloS ONE, 2008).* Furthermore, recent data have revealed interaction between retinoid and glucocorticoid signalling pathways. Particularly, an inhibitory effect of RA has been observed on the 11 bHSD1 expression, enzyme which regenerates active glucocorticoids. This

modulation might contribute to the beneficial effects of retinoids on memory since it has been recently shown that hippocampal 11bHSD1 expression increases by ~30% with aging and correlates with spatial memory deficits.
The present study aimed to elucidate the effects of vitamin A status on hippoccampal glucocorticoid activity and the consequences on cerebral plasticity and emotional behaviour. Thus, in order to characterize HPA axis function we firstly examined the effects of VAD (10 weeks) on plasma corticosterone levels in basal conditions and in stimulated conditions after open field test. To address the role of vitamin A status on stress reactivity, half of the vitamin A deficient rats and controls were supplemented with a vitamin A enriched diet during the 4 last weeks. Then, we examined the effects of long term VAD (14 weeks) and the preventive effects of 4 weeks of nutritional vitamin A supplementation on exploratory activity and anxiety-like behaviour. Finally, we studied the different steps of hippoccampal neurogenesis by immunohistochemistry and the expression of retinoid and glucocorticoid target genes in the hippoccampus by quantitative RT-PCR.
Our results showed that VAD induced a significant increase in plasma corticosterone levels compared to controls after open field test, indicating HPA axis dysfunctions. Moreover, VAD rats exhibited anxiety-like behaviour and vitamin A supplementation induced an increase in the exploratory activity and the number of entries in an 'aversive-like' context. Finally, these behavioural changes were associated to modulation of hippocampal neurogenesis and expression of genes involved in glucocorticoid and retinoid signalling pathways.

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Optimisation of opsins expression in specific subclasses of cortical interneurons using recombinant Adeno-Associated Virus and *Cre*-driver mice

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Optogenetics is a high-precision tool for controlling the activity of nerve cells on a millisecond-by-millisecond timescale via viral mediated expression of activator (ChR2) or inhibitors (NpHR/Arch). This toolbox offers a new powerfull approach for understanding how brain works but the use of viruses required an optimization of the administration techniques to maximize gene distribution and gene expression for *in vivo* experiments and to ensure a physiological response after light activation of neurons.
Our study was conducted to evaluate recombinant adeno-associated virus serotype 2-mediated expression and safety of channelrhodopsin-2 in a defined cortical interneurons population of somatosensory cortex. *Cre* recombinase-dependent Adeno-Associated Virus (FLEX rAAV) vectors carrying a fast kinetic variant of ChR2 and Yellow fluorescent protein (cHETA-YFP) under the control of a hybrid cytomegalovirus early enhancer and chicken beta-actin (CAG) promoter were injected in *Cre*-driver mouse in three different ways (IV, ICV, Local) with or without osmotic agents such as mannitol to increase the area of distribution throughout the CNS.
The expression of ChR2 was stable for up to 6 months with a high level of specificity in interneurons expressing *Cre* recombinase. At the highest virus concentration (2×10^{12} GC/ml), we improved by several orders of magnitude the infection rate and size in interneurons (ie up to 80% of FS-PV in the somatosensory cortex located 250um around injection site) compared to results obtained with standard experimental approach.
Our results show that AAV-mediated gene delivery may produce, long-term gene expression following prenatal or adult brain administration. Additional advantages of FLEX-rAAV as gene transfer vectors include a very high specificity of opsin expression, the availability of procedures for high titer, a lack of toxicity and reduced immunogenicity.

P3.229

Influence of exogenous and endogenous factors on proactive inhibitory control

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Recent studies show that we exert a proactive control upon our movements in order to prevent undesired responses triggered by external events. This inhibitory proactive control (IPC) depends upon the context. For example, if we need to respond to a target stimulus while knowing that no other stimulus can appear, this IPC will not be implemented. On the other hand, if another stimulus (a cue) can in some instances appear prior to the target, an IPC will be required to allow for a proper analysis of whether the stimulus that has just appeared is indeed the target or not. The IPC can then be lifted and a fast response to the target can be produced. These observations raise the question of the interaction between proactive inhibition and 1) exogenous factors (e.g. the visual perception of the cue versus the target stimulus), and 2) endogenous factors (e.g. the context of the task). Here, we provide evidence for such an interaction.
Human subjects were asked to press a button as fast as possible in response to the presentation of a visual target. In some conditions, the target was preceded by non-spatial cues indicating the close onset of the target. We varied either 1) the saliency of the cues and/or of the target; 2) the proportion of trials in which no target was actually presented; 3) the proportion of trials in which the cues did precede the target. The level of IPC was assessed by comparing the reaction times to target presentation (RT) on blocks of trials in which only the target was presented (pure target blocks) with RT in target-only trials embedded in blocks of trials in which the cue could also precede the target (mixed blocks). The release of IPC induced by the cues was assessed by comparing these latter RT with those obtained when the target was preceded by a cue.
Not unexpectedly, we show that saliency influences reaction times. However, we also show that it also affects the overall level of IPC, this level being higher as the cues get more salient than the target, indicating an interaction between perceptual decision and motor control variables. Moreover, we show that the level of IPC is also modulated by endogenous factors: the higher the probability of occurrence of the cues before target presentation, the higher the level of exerted IPC.

P3.230

High-throughput screening for psycho-active molecules in zebrafish embryos

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Small psychoactive molecules are indispensable tools for treating mental illnesses as well as dissecting nervous system function. However, very few novel psycho-active drugs only have been discovered in last decades and most of them were discovered serendipitously. Given the unmet need for new psycho-active molecules, we have developed a simple approach that combined an automated motility recording system with the biological complexity of living animals to identify *in vivo* novel psychoactive molecules using zebrafish embryos. Indeed, high-intensity light stimulus triggers a stereotypic series of motor behaviours in zebrafish embryos the photomotor response (PMR), which can be divided into four phases: a pre-stimulus phase, a latency phase, an excitation phase and a refractory phase. Interestingly, diverse classes of psychoactive molecules induce distinct, quantifiable and highly reproducible modifications of the PMR. In turn, these modifications make it possible to classify psychoactive drugs and to infer their activity. We have established a screening procedure using the PMR and an automated behaviour recording platform that allows to record the motility of hundreds embryos simultaneously in 48-well plates. So far, all drugs tested with psychotropic effects in humans also caused reproducible behavioural changes on zebrafish embryo PMR.

This approach allows us to rapidly identify novel psychotropic molecules and to predict their molecular targets, toxicity and psycho-activity.
Keywords: Zebrafish; Small molecules; Psycho-active molecule; High-throughput in vivo screening; vertebrate model
This service (MT0478) is proposed on the site MIGRATECH database: https://migratech.inserm-transfert.fr

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Spatio-temporal integration of written words during phonological and semantic tasks. High Resolution EEG sources reconstruction assessment

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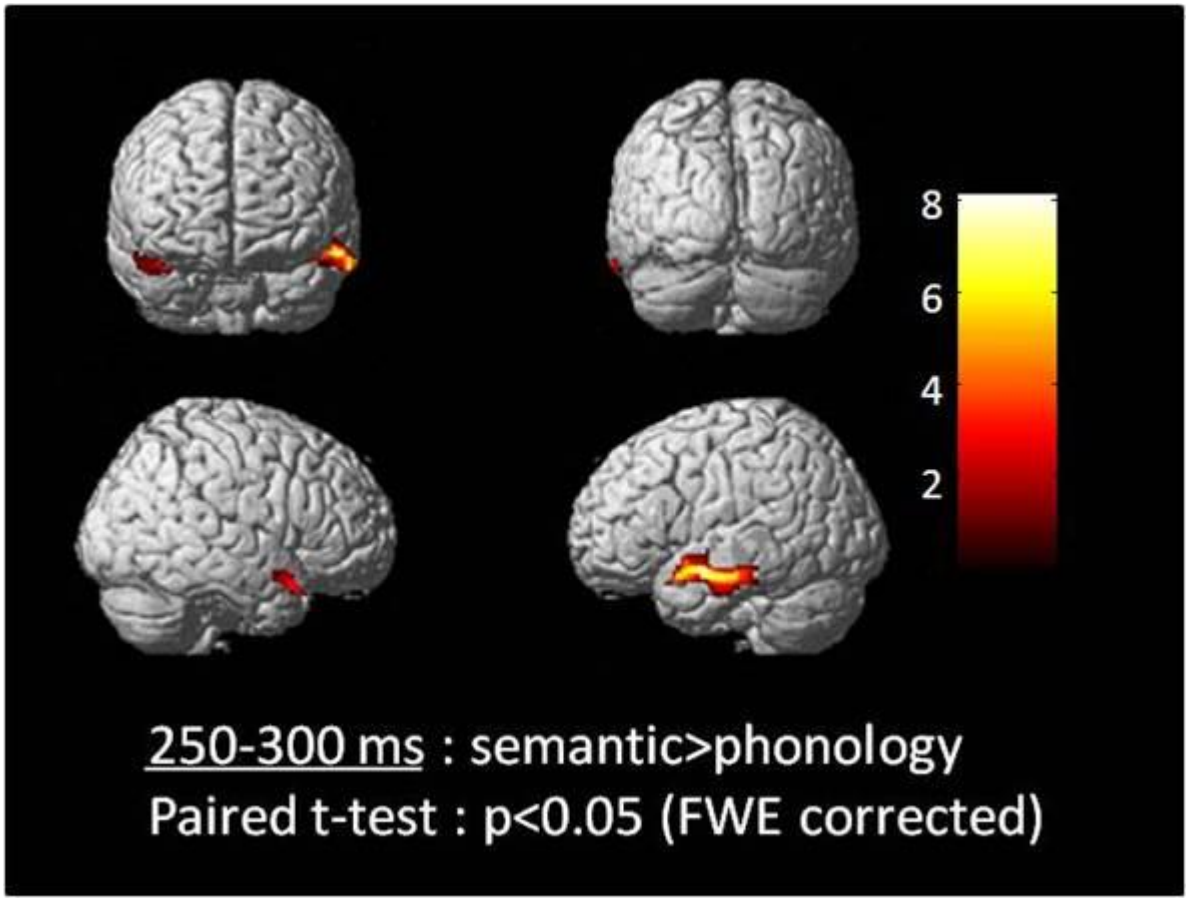
The goal of this study was to assess the spatio-temporal integration of written word using EEG source localization. The temporal lobe is the main candidate for the integration of written words, from shape analysis to lexicality discrimination. EEG and MEG studies have shown that the workflow of information processing occurs between 150 and 400 ms after stimulus onset. Here, fifteen healthy subjects performed phoneme detection and semantic categorization tasks. EEG activity was recorded with 96 active electrodes. Reconstruction of sources was subsequently performed on the event-related potentials (ERP) using SPM8. To limit inter-individual variability, we used a group-based reconstruction scheme that constrains the sources to be identical for all subjects. ERP showed significant task-related differences on left temporal electrodes between 250 ms and 300 ms peri-stimulus time, ERP amplitude for semantic task being stronger than for phonological task. After source localization, it was possible to highlight the travel of information from the infero-posterior temporal gyrus until the inferior frontal gyrus passing by the anterior temporal gyrus from 250 to 300 ms where a statistical difference was observed between both tasks.(figure 1) Our results confirm that spatio-temporal integration of word processing involves the left temporal lobe. They also suggest that activation of word processing moves from posterior (lexicality access) to anterior (semantic retrieval) regions of the left temporal lobe.



[figure 1]

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Comparative study of two prenatal stresses by forced swimming on behavioral development in mice pups

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Animal experiments have convincingly demonstrated that prenatal maternal stress affects pregnancy outcome and results in early programming of brain functions with permanent changes in neuroendocrine regulation and behavior in offspring.
The aim of this study is to evaluate the effect of prenatal maternal stress by swimming on neurobehavioral change during early postnatal life in mice. After confirming their pregnancy, the pregnant mice were divided into three groups: control group, forced swimming (FS) group wish is forced to swim for 10 min once a day from the 10th day of gestation until delivery, and forced swimming against the current (FSAC) group for 5min once a day, or fewer if the pregnant mouse can't resist to this duration, from the 10th day of gestation until delivery. The mice pups were examined from the 6th postnatal day (PND6), for their motor and vestibular functions using the righting test, and cliff avoidance reflexes.
The cliff avoidance test of pre-stressed offspring's, show a fewer latency (- 57.06% in FS group, and -77.88% in FSAC group) for taking away their paws and head from the board compared with control.

Furthermore, the righting test shows an improvement of straighten in pre-stressed offspring's. In addition, pups of the FSAC stressed group respond significantly faster than offspring's from FS dams. Our present study provides the evidence that maternal swimming during the gestational period is not harmful to the offspring development and can represent an exercise that may enhance the brain functions of the mothers' offspring.