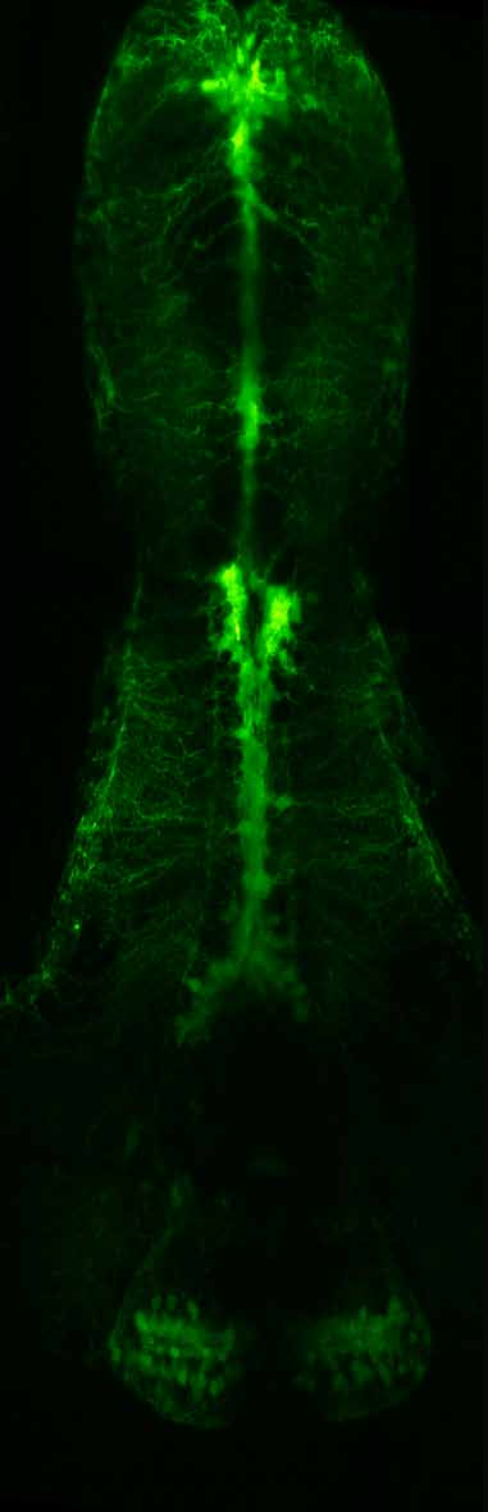

15th Annual Meeting of the LARC-Neuroscience Network

Le Diapason, Rennes, France

October 28, 2011

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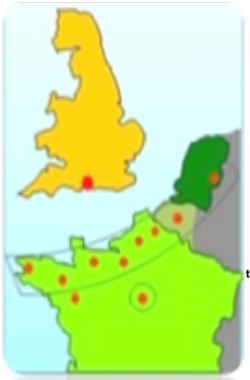

Legend to cover picture: 3D reconstitution of the brain of an 8 day-old zebrafish larva showing the radial glial cells expressing GFP under the control of the cyp19a1b (aromatase B) promoter. This picture was obtained by combining 1000 confocal images (Photo Yann Le Page)

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Contents

Programme

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10 :30-10:45: **Yannick Tanguy** (Rouen) Selenoprotein T function in brain : a neuron-to-glial switch in neuroprotection.

10:45-11:00: **Anne-Lise Pitel** (Caen) Specificity of macrostructural abnormalities in Korsakoff's syndrome compared with uncomplicated alcoholism.

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17:15-17:30 : Awards ceremony - End of the day

Oral communications

Local delivery of PACAP improves functional recovery after brain stroke in mice

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Despite intense research efforts, cerebral stroke remains the third leading cause of death and the first cause of long term disabilities, in industrialized countries. Several studies highlighted the neuroprotective effect of the neuropeptide Pituitary adenylate cyclase-activating polypeptide (PACAP) in brain ischemia models, based on its neurotrophic, anti-apoptotic and anti-inflammatory properties. However, the clinical use of PACAP is compromised by a very short half-life and the difficulty to reach the ischemic area where the vascularisation is disrupted. In this study, we propose a combined strategy using embryonic stem cell (ES) to locally deliver the neuropeptide PACAP in the infarct area. Thus, we have established a PACAP-over expressing ES cell line (ES-P cells) and assessed the therapeutic potential of the targeted delivery of PACAP by these cells, in a mouse model of focal permanent cerebral ischemia.
Our results show that the local delivery of PACAP improves functional recovery, one and two weeks after brain stroke. More precisely, mice transplanted with ES-P cells present reduced Neurological Severity Score (NSS) and reduced motor coordination deficit in the hole board test, compared to mice transplanted with wild-type ES cells or injected with Saline. Interestingly, the functional benefits are correlated with the modulation of local inflammatory response. Indeed, we report a strong decrease of the neurotoxic, pro-inflammatory TNF-□cytokine production. In parallel, we find a significant increase of the IL-10 anti-inflammatory cytokine and the neuroprotective protein Ym1 levels.
Taken together, these data suggest that the neuropeptide PACAP could exert a local neuroprotective effect by skewing the local inflammatory response toward a neuroprotective phenotype.

This work is supported by INSERM, TC2N Interreg Project, LARC-Neurosciences Network and the Region of Haute-Normandie.

Key-words: Cerebral ischemia, PACAP, Embryonic stem cells, Inflammation.

Selenoprotein T function in brain : a neuron-to-glial switch in neuroprotection

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Excess of reactive oxygen species (ROS) is a facilitating factor of neuronal death expansion during neurodegenerative diseases. Although most cells harbor a powerful antioxidant defense network, neurons rely on their coupling to astrocytes to combat oxidative stress. This system includes major antioxidant enzymes such as superoxide dismutases and several selenoproteins, a selenium-containing protein family. Indeed, the active site of these proteins encompasses a selenium atom, whose nucleophilic activity is essential for their enzymatic activity, like ROS reduction for instance. A pangenomic screening of the targets of the neuroprotective factor PACAP, permitted to identify a novel selenoprotein, the selenoprotein T (SelT), which is localized in the endoplasmic reticulum where it participates in the regulation of Ca2+ homeostasis. SelT is widely and strongly expressed during embryogenesis and neurogenesis, but vanishes in most adult tissues. We demonstrated that global knockout of SelT gene is lethal during embryogenesis at midgestation, and that its deficiency in nervous cells leads to cell death and increased intracellular levels of ROS. Thanks to a complementary approach of directed mutagenesis, we showed that the prosurvival effects of SelT on neuroblasts relies on the presence of a selenocystein residue within its active site, called “thioredoxin-like domain”. Moreover, in the adult, SelT levels were strongly induced in reactive astrocytes following cerebral ischemia. In fact, SelT also promotes astrocyte cell viability through inhibition of caspase-3 activity. These results indicate that SelT is a prominent antioxidant selenoprotein which is required for embryogenesis and which ensures a pivotal role in the neuron-to-glial switch for neuroprotection.

Supported by grants from Région Haute-Normandie, INSERM and University of Rouen.

Specificity of macrostructural abnormalities in Korsakoff's syndrome compared with uncomplicated alcoholism

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While neuropathological studies initially reported that shrinkage of the thalamic nuclei and mammillary bodies characterized Korsakoff's syndrome (KS), neuroimaging investigations revealed widespread cerebral damage including notably brain abnormalities in the frontal cortex, hippocampus and cerebellum. Reduced volume in these brain regions has also been shown in alcoholics without ostensible neurological complications (AL). The goals of the present study were therefore to identify 1) brain volume decrease related to chronic alcohol consumption and common to both AL and KS, and 2) regions specifically damaged in KS compared with AL. To this end, we conducted a whole brain analysis of gray and white matter volume in KS, AL and control subjects from T1-weighted MRI scan obtained in each participant. A conjunction analysis in AL and SK compared with control subjects indicated decreased gray matter volume bilaterally in the prefrontal cortex extending to the parietal lobe, and in the insula, hippocampal formation, caudate, thalami, hypothalami and cerebellum. Gray matter volumes were reduced in KS compared with both controls and AL bilaterally in the thalami, hypothalami (mammillary bodies) and left insula. There were graded effects of volume deficits in the thalami, mammillary bodies and insula from mild or moderate in AL to severe in KS. Regarding white matter, the conjunction analysis showed lower volume in both AL and SK compared to controls bilaterally in the fornix, stria terminalis, cingulate bundle and corona radiata, and in the corpus callosum and mesencephalic fibers. Compared with AL, greater decrease of white matter volume was found in KS bilaterally in the genu of the corpus callosum with graded effects of volume deficit in AL and KS. Our results indicate therefore that AL and KS present a common pattern of widespread alcoholism-related volume deficits affecting especially the limbic and frontocerebellar networks. In agreement with the continuity theory, the specificity of gray and white matter abnormalities in KS seems to lie in the exacerbation of alcoholism-related alterations in the thalami, mammillary bodies, insula and genu of the corpus callosum.

Supported by PHRC Korsakol, ANR Retour Post-doctorant 2010

Glutamate stimulation evokes t-PA secretion from brain vascular endothelial cells: dual effect of t-PA on neuron death depending on immaturity and cortical layers.

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Despite recent progress made in obstetrics and newborn intensive care, neurological disabilities of perinatal origin do not decrease. Excitotoxicity has been pointed out as a common pathway of most deleterious processes in neonate brain injuries. The detection of NMDA-receptor on brain microvascular endothelial cells (BMEC) support the hypothesis that BMECs participate in excitotoxic processes. We have recently demonstrated phenotypic and functional differences between newborn (n) and adult (a)-derived BMECs that could be relevant for enhanced glutamate (Glu) sensitivity of nBMEC. In particular, nBMEC released more tissue plasminogen activator (t-PA) and gelatinases than aBMEC under Glu stimulation.

In the present study using Evans Blue (EB) in vivo loaded microvessels, abluminal Glu stimulation induced EB-albumin extravasation more efficiently in microvessels isolated from newborn than from adult cortices. Conditioned media (CM) from aBMEC exposed to Glu increased the Glu-induced death of mature neurons in vitro. This effect was more pronounced with CM from nBMEC. The effects of CM were blocked by MK801 (a NMDA receptor blocker). Potentiation of Glu toxicity by CM was reversed by recombinant plasminogen activator inhibitor-1 indicating t-PA as the released-factor responsible for this effect. In contrast, t-PA decreased neuronal death of immature neurons. Ex vivo, in brain slices from 2, 5 or 10 day-old mice, t-PA inhibited caspase-3 activity and cleaved caspase-3 immunolabeling in superficial layers (II-III) containing immature neurons. Glu did not modify this effect. Infraliminal Glu and t-PA when used in association induced necrosis (LDH activity and 7AAD labeling) in the deep cortical layers (IV-VI) containing mature neurons.

These data suggest that upon parenchymal Glu challenge, BMEC-secreted t-PA in high amount by neonatal vessels may participate to BBB permeabilization, and exacerbate excitotoxicity. Reciprocally t-PA anti-apoptotic effects independent of Glu may coexist in immature neuron populations. These data point out stage-dependant microvessel sensitivity to Glu and potential deleterious reaction in the immature brain (see also abstract by Lecointre et al.), justifying the development of vessel oriented neuroprotection strategies in the neonate.

Supported by Rouen University, FEDER, Région Haute-Normandie, Les Gueules Cassées and ELA Foundations, ANR.

Tracking the fate of memories using fMRI: role of the hippocampus in the retrieval of recent and remote 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 suggest that the hippocampus is always required for the retrieval of episodic, but not semantic, memories. In this prospective study, 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 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 over time of the posterior part of this latter structure (confirmed by Region of Interest analyses), classically involved in retrieval processes. 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.

Supported by Inserm and a PhD grant from the French « Ministère de la Recherche (contingent ACI) »

Glun2D-containing NMDA receptors mediates tissue type Plasminogen Activator-influenced spatial memory.

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Tissue-type plasminogen activator is a pleiotropic serine protease of the central nervous system, critically involved in many brain functions and dysfunctions such as synaptic plasticity and neuronal death, respectively. Among the various mechanisms proposed to explain the multiple effects of tPA, its interaction with the GluN1 subunit of the N-methyl-D-aspartate receptor (NMDAR) leading to a potentiation of NMDAR-mediated calcium influx has been evidenced. Recently, we reported that the pro-neurotoxic effect of tPA is especially mediated by its interaction with GluN2D-containing NMDAR. Thus, the aim of the present study was to determine whether GluN2D-containing NMDAR might also drive tPA-mediated cognitive functions. To address this issue, combination of an immunization strategy to prevent interaction of tPA with NMDAR in vivo as well as GluN2D-deficient mice were used in a set of behavioral tasks. Our results show that while immunization totally blocks spatial memory in wild-type mice, it does not affect GluN2D-deficient mice memory performance. Taken together, our data provide the evidence in vivo that tPA affects spatial memory through its preferential interaction with GluN2D-containing NMDAR.

Nitric Oxide Neurons in the Preoptic Area of the Hypothalamus are a Direct Target of Leptin: Implications for the Reproductive Axis

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Reproduction in mammals requires adequate energy availability to enter into puberty and for ongoing fertility during adulthood. Leptin, an adipocyte-derived hormone, provides information about peripheral energy stores to the central reproductive axis, yet its site(s) of action remains unclear. Mapping of the leptin receptor (LepRb) in the mouse brain shows expression within the preoptic area (POA) of the hypothalamus, the region where gonadotropin releasing hormone (GnRH) neurons reside. While leptin does not act directly on GnRH neurons themselves, it is possible that neurons within the POA act to relay information provided by leptin to GnRH neurons. A recent study has shown that nitric oxide (NO) neurons throughout the hypothalamus respond to leptin treatment with phosphorylation of STAT3. Interestingly, administration of exogenous leptin during fasting conditions has the ability to phosphorylate neuronal NO synthase (nNOS), the enzyme required to form NO. In the present study, we pursued the influence of leptin on NO neurons within the POA. We first repeated the demonstration that leptin induces pSTAT3 in nNOS neurons. We next sought to examine whether leptin can induce the phosphorylation of nNOS (pnNOS) and the potential kinetics of leptin-induced activation of nNOS within the POA. Indeed, leptin increases levels of pnNOS at 15minutes post injection, which is also correlated with an increase of luteinizing hormone levels. Immunofluorescent analyses show an increase in pnNOS at the level of the OVLT/MEPO. Intriguingly, leptin induces pSTAT3 activation only 45 minutes post injection, while pnNOS is activated as early as 15 minutes post injection, suggesting that STAT3 is not the primary pathway through which leptin stimulates pnNOS. This is, however, in contrast to the activation of pAKT, which is seen as early as 15 minutes following leptin injection, providing a tentative link between the AKT pathway and leptin-induced pnNOS within the POA. Together these data show that leptin has the ability to stimulate pnNOS neurons during diestrous within the POA and the activation of nNOS potentially involves the AKT pathway.

Grant : University Lille2-Région Nord Pas de Calais Doctoral fellowship

The organization of two kisspeptin systems in zebrafish and sea bass brain shows evident species specific differences.

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Kisspeptins are fascinating actors in the neuroendocrine regulation of reproduction. In vertebrates, the number of kiss genes varies from none to three. This study aims to characterize kisspeptin systems in two fish species commonly used for reproductive studies: a freshwater Cypriniform, the zebrafish (Danio rerio) and marine Perciform fish, the European sea bass (Dicentrarchus labrax). Both fish have two kiss genes, kiss1 and kiss2, and two kiss receptors (GPR54 or Kissr), kiss1r and kiss2r. To elucidate the organization of kiss systems in zebrafish, antibodies were raised against the zebrafish preproKiss1 and preproKiss2 sequences. Immunohistochemical findings were fully confirmed by in situ hybridization data. Kiss1-expressing neurons are exclusively located in the habenula in zebrafish and sea bass, exactly where kiss1r mRNA-containing cells are also detected. During the breeding season sea bass shows an additional kiss1 population into the mediobasal hypothalamus, where kiss2-containing cells are shown in zebrafish. Nevertheless, the main kiss2 mRNA-positive population is observed in both species in the dorsal hypothalamus and in the preoptic area. Immunohistochemistry reveals that kiss2-expressing cells in zebrafish project widely into the forebrain and midbrain. These regions also strongly expressed the kiss2r mRNA in zebrafish, as well as in sea bass. Moreover, in both species kiss2 fibers or kiss2r-expressing cells of the preoptic region make close appositions with the respective hypophysiotrophic GnRH neurons of each species (GnRH3 in zebrafish and GnRH1 in sea bass). Kiss2 populations of the ventral and caudal hypothalamus are estrogen sensitive in juvenile zebrafish, whereas in sea bass it is the kiss1 population of the mediobasal hypothalamus that expresses ERα and slightly ERβ2. Furthermore, in this latter species during the breeding season, a strong kiss1 expression is observed in the pituitary FSHβ immunopositive cells. Altogether our results suggest that kiss1 in sea bass and kiss2 in zebrafish could participate in the regulation of reproduction through the hypothalamic kiss population sensitive to estrogens, proving that kisspeptin systems show evident species specific differences.

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Early life adversity and serotonin transporter gene variation interact to shape the adult hypothalamo-pituitary-adrenal axis

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Based on the recently postulated "three-hit concept", it is hypothesized that a genetic factor, the polymorphic region in the promotor of the serotonin transporter (SERT) gene, will interact with early life adversity (hit 1 x hit 2) to alter the brain's neurocircuitry that is involved in the stress response; the resulting altered stress sensitivity can predispose an individual to depression when exposed to hit 3: a psychological stressor in later life. It has been established that a polymorphism in the human SERT gene modulates the influence of early life adversity on the occurrence of depression. To elucidate the neurobiological basis underlying this gene x environment interaction, we used the SERT heterozygous knockout (SERT+/-) rat to model the polymorphism in the SERT gene, while the frequently used maternal separation (MS) paradigm was our model of choice for early life adversity.
Our results show independent effects of MS on maternal care behaviour, and of SERT genotype and MS on postweaning body weight. For the expression of key components in the adult hypothalamo-pituitary-adrenal (HPA-) axis, which is crucially involved in stress adaptation, we found strong gene x environment interactions at the level of the adrenal gland, where opposite effects of SERT genotype were found in the MS versus control groups, with respect to the mRNA levels of the adrenocorticotropic hormone receptor and 3β-hydroxysteroid dehydrogenase.
We expect that the interaction between MS and SERT gene variation will also affect the dynamics of members of the corticotropin-releasing factor (CRF) family of neuropeptides (e.g. CRF and urocortins) in brain areas that modulate the activity of the HPA-axis. This hypothesis is currently tested by immunohisto-chemistry and in situ hybridization

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Immunization with the CD8-specific epitope of myelin oligodendrocyte glycoprotein induces a mild form of experimental autoimmune encephalomyelitis.

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Multiple sclerosis (MS) is an autoimmune, demyelinating and degenerative disease of the central nervous system (CNS). Anti-myelin CD4 T cells are strongly associated with disease development in several animal models of MS such as experimental autoimmune encephalomyelitis (EAE). However, CD8 T cells often outnumber CD4 T cells in the CNS parenchyma of MS patients and recent studies suggest that anti-myelin CD8 T cells may be also implicated. In order to better understand the contribution of pathogenic CD8 T cells, C57Bl/6 female mice were immunized with a recently described epitope of myelin oligodendrocyte protein (MOG37-46) specifically presented by MHC-I to CD8 T cells. Only, one third of the mice immunized with MOG37-46 developed EAE with mild clinical signs. In contrast, all mice immunized with MOG35-55 developed hindlimb paralysis, as expected for this classical EAE model. Proliferation and FACS analysis of T cell reactivity using splenocytes isolated from MOG37-46-immunized mice confirmed that immunization led to the emergence of specific MOG-reactive CD8 T cells in vivo. Yet, the presence of this T cell autoreactivity was not necessary correlated with disease development. Moreover, immunohistochemical analysis of the spinal cord, cerebellum and optic nerve in mice that developed the first clinical sign (clasping reflex or tail weakness) indicates that CD8 T cells infiltrated the white matter of the central nervous system (CNS). Strikingly, the CD8 T cell infiltration was weak compared with CD4 T cells in the same lesions. Further characterization of the CNS lesions in this EAE model is underway. Taken together, these data indicate that a CD8 epitope in the MOG sequence can initiate mild EAE in a subset of mice unlike MOG35-55 that induces CD4 T cell autoreactivity and severe EAE in all mice. The fact that the development of anti-MOG CD8 T cells is not systematically associated with EAE symptoms suggest that anti-myelin CD8 T cells are insufficient to trigger sustained disease in mice. Further work is needed to better delineate the fate of autoreactive CD8 vs. CD4 T cells in the CNS during autoimmune neuroinflammation.

Supported by Region Pays-de-la-Loire (to ABN).

Gastric electrical stimulation modulates gastric distension-induced visceral pain and -activated brain centres.

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Gastric electrical stimulation (GES) is an effective therapy to treat patient with nausea and vomiting refractory to medical management. However, its mechanism of action remains poorly understood. The aim of this study was to assess whether GES could modify gastric sensitivity to gastric distension.
We monitored the variation of arterial blood pressure (BP) as a marker of visceral sensitivity in anesthetized rats in response to graded gastric distension from 20 to 80 mmHg during either GES or sham GES. In addition, c-fos protein expression was quantified by immunohistochemistry within the T9 dorsal horn of the spinal cord, tractus solitary nucleus (NTS) and hypothalamic paraventricular nucleus (PVN) in response to 60 mmHg gastric distension during either GES or sham GES. Moreover, in a same way, c-fos protein was quantified in rats distended and transected at thoracic level T5/T6, with or without GES.
Gastric distension induced a rise in BP (20 mmHg: 4.84 ± 0.54 mmHg; 80 mmHg: 11.2 ± 1.35 mmHg), and an increase in c-fos protein expression within the T9 dorsal horn of the spinal cord (11 ± 1 at baseline vs 23 ± 2; at 60 mmHg; p=0.0012), the NTS (55.36 ± 7 at baseline vs 96 ± 4; p=0.0002) and the PVN (179 ± 31 at baseline vs 373 ± 15 at 60 mmHg; p=0.0025). GES prevented gastric distension increase in BP from 20 (3.74 ± 0.51 mmHg; p=0.0039) to 80 mmHg of distension (8.14 ± 0.85; p=0.0002), while not affecting gastric compliance. Stimulation of the caecum using the same parameters did not affect BP response to gastric distension. In addition, GES prevented the rise of c-fos protein expression induced by gastric distension within the T9 dorsal horn of the spinal cord (15 ± 1; p=0.0047), the NTS (69 ± 4; p=0.001 and the PVN (212 ± 52; p=0.046). Moreover most of 60% of c-fos IR cells are CRF+. In addition, T5/T6 transection does not affect GES effect on the spinal cord, but abolished the rise induced in c-fos by the distension in the NTS and the PVN.
In conclusion, we demonstrated that GES decreases gastric sensitivity to gastric distension by recruiting brain centres involved in visceral sensation, and seems to act directly on splanchnic afferent fibers. Further investigations are necessary to determine mediators involved both at the medullar and gastric levels.

Keys words: gastric electrical stimulation, gastric distension, c-fos protein, dorsal horn of spinal cord

Activation of SRE and AP1 by olfactory receptors via the MAPK and Rho dependent pathways

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2School of medicine and public health, Unit pulmonary medicine, Madison, Wisconsin, U.S.A.

Whereas the activation of MAPKs (mitogen activated kinases) and Rho dependant pathways by GPCR (G protein coupled receptors) has been the subject of many studies, the implication of olfactory receptors (OR), which constitute the largest GPCR has been far less analyzed. Using an in vitro heterologous system, we showed that odorant activated ORs stimulate SRE containing promoters via the ERK pathway. We demonstrated that RhoA and Rock kinases but not Rac were involved in ORs-induced SRE/SRF activation and that AP1 was activated, via JNK and p38 MAPKinase. By using real time PCR, we found that mOR23, RnI7 and CfOR12A07 induced elevated levels of transcription factors ELK-4, srf, c-fos and c-jun mRNAs whereas mOREG induced an elevated transcription levels of c-fos and c-jun mRNA only. The primary cultures of rat olfactory sensory neurons (OSNs) lead us to confirm that activated ORs stimulate the downstream pathways namely MAPKs, Rho and AKT ones. Similar data were observed with olfactory epithelium extracts freshly obtained from rats exposed to a cocktail of odorants. Given the importance of the MAPK and Rho pathways in the survival and differentiation of neurons in general, we hypothesize that the binding of an odorant onto its OR is the very first key event that regulate OSNs homeostasis.

Summary of all presentations

Behavioral Neurosciences

1 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.

2 That's all right: Lateralization of food imprinting in the cuttlefish Sepia officinalis

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Lateralization of function is a well-known phenomenon in humans. The two hemispheres of the human brain are functionally specialized such that certain cognitive skills (e.g. language or musical ability, conspecific recognition, and even emotional responses) are mediated by one hemisphere more than the other. Lateralization occurs in other vertebrate species as well as insects. Although much attention has been brought to perceptual and motor lateralization, few studies addressed lateralization of learning, particularly in invertebrates. In the current study, we investigated lateralization of food imprinting in the cuttlefish Sepia officinalis. In this species, early juveniles innately prefer shrimps to crabs, except when they have been visually familiarized with crabs shortly after hatching. Cuttlefish were exposed to crabs for 2-h shortly after hatching and were able to see them only with their right eye, left eye or both eyes. Their prey preference was then tested 7 days later. We showed that their innate food preference was altered in cuttlefish exposed to crabs with their right eye, but not with their left eye. Our results reveal that cuttlefish display a lateralization while learning the visual characteristics of potential prey. As in many species of vertebrates and in honeybees, the right visual field may play a predominant role in foraging and feeding behaviour. This result is a strong argument in favour of the conservation of lateralization throughout different taxa.

3 Comparison of the impact of the targeted therapy everolimus (Afinitor®) and the chemotherapy 5-FU on cognitive functions and cerebral plasticity in an animal model

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Background
Cancer and treatments can induce cognitive impairments such as deficits of visual and spatial memories, and of psychomotor processing speed in patients, symptoms referred to as “chemofog”. The targeted therapy Everolimus (Afinitor®), which blocks the mTOR pathway, alters cell proliferation, metabolism and neoangiogenesis. Thus, we used a validated behavioral animal model to evaluate the potential cognitive impairments induced by Everolimus and to compare its effect with the 5-fluorouracil (5-FU) chemotherapy.
Methods
Everolimus (5 mg/kg) was daily administered for two weeks and 5-FU (37 mg/kg) was injected once a week during 3 weeks in adult C57BL/6J Rj mice. Learning and memory processes were then evaluated by means of the object recognition and the Morris water maze tests. Ex situ, hippocampal neurogenesis and vascularization processes were investigated by immunohistochemistry in each group of mice. In vitro, neural stem cells (NSC) and/or endothelial cells (EC) in culture were treated with Everolimus.
Results
Everolimus slowed body weight gain from the last day of the treatment period until the end of behavioral sessions. Although 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, behavioral flexibility and object recognition memory were not impaired by Everolimus. These data correlated with absence of altered neurogenesis in Everolimus-treated mice. In vitro, increasing concentrations of Everolimus induced a significant EC death without affecting NSC survival.
Conclusion
At short term after the end of the treatment, Everolimus did not modify mice cognitive functions evaluated by means of the hippocampal-dependent behavioral tasks. These observations differ from our studies demonstrating that chemotherapy (5-FU) led to selective long-term cognitive deficits, i.e. behavioral flexibility and recognition memory. These results reinforce the interest of developing targeted therapies for cancer treatment.

4 The neurotopography of length and frequency effects in written production. Role of the left premotor dorsal cortex in graphemic buffer substrates

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Written language production (spelling) plays an important role in daily routines such as taking notes, filling out applications, writing checks, etc. Compared to reading and spoken language, relatively little research has examined the neural substrates of spelling. Deficit/lesion correlation studies have emphasized the role of the superior parietal cortex, superior premotor region and the angular gyrus. More recent work has added the left inferior frontal gyrus/junction and the left mid fusiform gyrus. At least some of these areas may subserve orthographic processes recruited by both reading and spelling (Rapp & Lipka, 2011; Roux et al., 2009). While these various regions are implicated in the spelling process, their specific roles are not well understood. The neuropsychological literature indicates that acquired dysgraphia may selectively disrupt different components of the written language system. The hallmark of deficits affecting the orthographic lexicon (orthographic long term memory) are lexical frequency effects, while deficits affecting graphemic buffering (orthographic short term memory) are associated with letter length effects. On this basis, we used fMRI with neurologically intact participants to examine the sensitivity of the writing substrates to the factors of lexical frequency and word length. We demonstrated that there exists in the superior frontal sulcus a region sensitive to length effects. We also discovered that this premotor region was not activated by frequency effects; a non-intervention that has been proposed for the graphemic buffer during reading tasks. Finally, we repeated anterior results showing that this region is part of the central processes of writing revealing its activation in the contrasts of writing and circle drawing tasks.
The set of these clues makes this cerebral region a good candidate for the role of substrate of the graphemic buffer or that of one of its numerous parts.
By demonstrating its sensitivity to the length effects, we add an important cue in favor of this hypothesis.

Keywords: Spelling, graphemic buffer, lexical frequency, letter length, fMRI
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5 Brain lateralization of auditory processing in a songbird

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In 1970, Marler set the idea of a parallel between human speech and birdsong, emphasizing the many common properties in vocal development of both infants and songbirds. One of the best-known features of human speech is the hemispheric specialization of the brain for its perception and production. The lateralization for the production of song that has been observed in a number of passerine birds is reminiscent of this hemispheric asymmetry. However, although evidence exists for a lateralization of song production, very few studies have focused on the perceptual aspect of lateralization in songbirds. In the present study, we investigated the central processing of communicative and artificial signals at different levels of the song system in male starlings. Neuronal responses to a variety of species-specific and artificial nonspecific stimuli were recorded in both hemispheres of awake and anesthetized birds. Recordings were made in the primary auditory area of the songbird brain, namely the Field L, which is the main auditory input of the song system, and in the vocal control nucleus HVC, which is a highly integrative part of this system. In both cases, the right hemisphere exhibited significantly more responsive units than the left hemisphere when the birds were awake and this difference was reduced or even suppressed under anesthesia. Moreover, our results showed a complex and state-dependent hemispheric specialization towards behaviorally-relevant classes of stimuli. These results are in agreement with the suggestion that the two hemispheres of the brain exhibit different general functions in terms of attention or the nature of the cognitive task performed and they add to the many parallels between the avian and human systems that have become paradigmatic of vocal communication. Hemispheric specialization may therefore be a ubiquitous property of the brain of species exhibiting vocal learning abilities.

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6 Tracking the fate of memories using fMRI: role of the hippocampus in the retrieval of recent and remote 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 suggest that the hippocampus is always required for the retrieval of episodic, but not semantic, memories. In this prospective study, 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 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 over time of the posterior part of this latter structure (confirmed by Region of Interest analyses), classically involved in retrieval processes. 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.

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7 Motor Cortical Maps in Adult Rats Submitted to Hindlimb Unloading

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Body immobilisation in humans results in muscle atrophy and weakness as well as impairment in motor tasks performance, which is correlated to a change in corticospinal excitability. In rats, hindlimb unloading (HU) is commonly used to mimic the effects of confinement to bed in patients. Several studies have reported changes in the representation on the somatosensory cortex in rats submitted sensorimotor restriction: remapping and enlargement of receptive fields, changes in the response of layer 4 neurons to peripheral stimulation. However, we have no data about motor cortical maps. Thus, the objectives of the present study were (1) to determine, in control rats and in rats submitted to a 14-day period of HU, the size and organization of hindlimb representation in the M1 cortex; (2) to evaluate the overall excitability of M1 cortex by determining the stimulation thresholds.

Rats were anesthetized with ketamine and acepromazine. The M1 cortex was stimulated by monophasic cathodal pulses (0.2 ms duration, in 100 ms trains at interval of 1 s). The stimulation was progressively increased until a movement was evoked. Identification of joint movements was performed by visual inspection and/or palpation. The minimal threshold required to elicit a movement of any joint of the hindlimb ("Absolute threshold") was recorded. Then, current intensity was gradually increased until a movement of the different joints was detected ("Relative threshold"). Penetration sites that failed to elicit a movement of the hindlimb at current intensity up to 80 µA were defined as unresponsive.

We have shown that HU led to a dramatic decrease in the hindlimb representation on the M1 cortex (-61%, $p<0.01$). Comparison with previous data obtained on the somatosensory cortex reveals that the motor cortical maps are more reactive to disuse than somatosensory ones (-15%, Dupont et al. 2011). In addition, absolute and relative current thresholds for eliciting a movement were increased. However, these effects were not identical according to the joint considered: the hip was more strongly affected by HU than the toes. Our main conclusion is that HU dramatically affects the organization and functioning of cortical motor maps and decreases corticospinal excitability.

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8 Neurogenesis and cognitive functions in adult mouse: Potential involvement of a glio-vascular neuropeptide?

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In two neurogenic adult brain area, i.e. the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus, neuronal stem cells (NSC) are closely associated with both endovascular components and undifferentiated glial cells. Signals inducing proliferation of NSC, their migration and their differentiation in neurons, astrocytes or oligodendrocytes are poorly understood. The localization of NSC in close contact with endothelial system within the niche suggests that vasoactive factors, released by the vascular compartment, control neurogenesis and induce modifications of the emotional and cognitive status in physiological and pathophysiological conditions. The present study aims to determine in adult mice, the role of the vasoactive neuropeptide urotensin II (UII) on NSC fate in vivo and in vitro (neurospheres), and to assess its impact on cognitive functions involving hippocampal-dependent tasks.
Intracerebroventricular administration (icv) of UII increased the number of proliferating cells (BrdU+) in the SVZ and in the SGZ of adult C57/Bl mice, suggesting a stimulatory effect of UII on adult neurogenesis. The effect of UII was also studied in vitro on growth and differentiation of NSC in cultured neurospheres. These structures are composed of a minority of cells located at the periphery which are involved in the neuro-epithelial process (nestin expressing cells), and a majority of precursor cells expressing IQGAP-1 and the receptor for UII, a GPCR named UT. Indeed, more than 70% of the cells forming the neurospheres were GFAP+ and expressed UT. Our data also indicate that UII did not affect neurosphere growth cultured in the absence or presence of growth factors. In contrast, UII may initiate a process to promote adhesion and cell orientation towards a phenotype of proliferating GFAP+ precursors. Behavioral experiments show that icv injection of UII did not affect long-term emotional behaviors such as anxiety or depression. However, UII altered the kinetic of responsiveness to novelty, 15 days after injection. These preliminary data need to be pursued by i) increasing the cohort of animals, ii) focusing research on the behavioral tasks associated to exploration and attention, and iii) increasing the delay between peptide injection and behavioural evaluation in order to wait for integration of newborn neurons in hippocampal circuits.

9 Differential effects of the duration of environmental enrichment exposure on long-term memory, anxiety- and depressive-like behaviour in adult mice.

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Environmental enrichment (EE) stimulates brain functions and constitutes an interesting model to elicit brain plasticity1. It improves learning and memory performances and decreases anxiety- and depressive-like behaviour. However, minimal duration of EE exposure to induce such beneficial effects is not well determined.
The aim of this study was to explore whether different durations of EE may differentially influence long-term memory (LTM) performances, anxiety- and depressive-like behaviour in male mice. To this end, adult mice were continuously housed in standard or enriched environment for 24 hours, 1 week, 3 weeks or 5 weeks before behavioural experiments (n=12 per group). LTM performances were tested in the passive avoidance task. Anxiety-like behaviour was assessed in the dark–light box and elevated plus maze tasks while depressive-like behaviour was assessed in the tail suspension and forced swimming tests.
An improvement of LTM performances was only found after a 3-week exposure to EE, whereas no modification of anxiety-like behaviour was observed in the dark–light box test whatever EE duration. Additionally, an anxiolytic-like effect, as shown in the elevated plus maze task, appeared after 3 weeks of EE; as for memory, this beneficial effects of a 3-week-long EE disappeared after longer exposure. Interestingly, EE led to an anti-depressive like effect in the tail suspension test, but not in the forced swimming test, whatever its duration (i.e. from 1 day to 5 weeks).
The present findings reveal a major influence of EE exposure duration on memory and anxiety-like behaviour, with a particular efficiency for a 3-week duration. In contrast, the anti-depressive-like effects of EE seem to be independent of the duration. These findings suggest that the mechanisms underlying the behavioural effects of EE are different. It also suggests that the heterogeneity of the EE effects reported in the literature depends on the different EE exposure durations. Mechanisms of these differential effects of EE will be further assessed with a particular interest on neurogenesis.

1 Van Praag H. et al. (2000) Nature reviews. Neuroscience, vol. 1, 191-198.

10 Glun2D-containing NMDA receptors mediates tissue type Plasminogen Activator-influenced spatial memory.

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Tissue-type plasminogen activator is a pleiotropic serine protease of the central nervous system, critically involved in many brain functions and dysfunctions such as synaptic plasticity and neuronal death, respectively. Among the various mechanisms proposed to explain the multiple effects of tPA, its interaction with the GluN1 subunit of the N-methyl-D-aspartate receptor (NMDAR) leading to a potentiation of NMDAR-mediated calcium influx has been evidenced. Recently, we reported that the pro-neurotoxic effect of tPA is especially mediated by its interaction with GluN2D-containing NMDAR. Thus, the aim of the present study was to determine whether GluN2D-containing NMDAR might also drive tPA-mediated cognitive functions. To address this issue, combination of an immunization strategy to prevent interaction of tPA with NMDAR in vivo as well as GluN2D-deficient mice were used in a set of behavioral tasks. Our results show that while immunization totally blocks spatial memory in wild-type mice, it does not affect GluN2D-deficient mice memory performance. Taken together, our data provide the evidence in vivo that tPA affects spatial memory through its preferential interaction with GluN2D-containing NMDAR.

11 Specificity of macrostructural abnormalities in Korsakoff's syndrome compared with uncomplicated alcoholism

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While neuropathological studies initially reported that shrinkage of the thalamic nuclei and mammillary bodies characterized Korsakoff's syndrome (KS), neuroimaging investigations revealed widespread cerebral damage including notably brain abnormalities in the frontal cortex, hippocampus and cerebellum. Reduced volume in these brain regions has also been shown in alcoholics without ostensible neurological complications (AL). The goals of the present study were therefore to identify 1) brain volume decrease related to chronic alcohol consumption and common to both AL and KS, and 2) regions specifically damaged in KS compared with AL. To this end, we conducted a whole brain analysis of gray and white matter volume in KS, AL and control subjects from T1-weighted MRI scan obtained in each participant. A conjunction analysis in AL and SK compared with control subjects indicated decreased gray matter volume bilaterally in the prefrontal cortex extending to the parietal lobe, and in the insula, hippocampal formation, caudate, thalami, hypothalami and cerebellum. Gray matter volumes were reduced in KS compared with both controls and AL bilaterally in the thalami, hypothalami (mammillary bodies) and left insula. There were graded effects of volume deficits in the thalami, mammillary bodies and insula from mild or moderate in AL to severe in KS. Regarding white matter, the conjunction analysis showed lower volume in both AL and SK compared to controls bilaterally in the fornix, stria terminalis, cingulate bundle and corona radiata, and in the corpus callosum and mesencephalic fibers. Compared with AL, greater decrease of white matter volume was found in KS bilaterally in the genu of the corpus callosum with graded effects of volume deficit in AL and KS. Our results indicate therefore that AL and KS present a common pattern of widespread alcoholism-related volume deficits affecting especially the limbic and frontocerebellar networks. In agreement with the continuity theory, the specificity of gray and white matter abnormalities in KS seems to lie in the exacerbation of alcoholism-related alterations in the thalami, mammillary bodies, insula and genu of the corpus callosum.

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Functionnal and autonomic Neurosciences

12 Hydrogen sulfide affects cardiorespiratory reflexes and behaviour in trout.

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For several years hydrogen sulfide (H2S) has only been known as a highly toxic environmental pollutant but, along with nitric oxide and carbon monoxide, H2S has recently emerged as a third member of endogenous signaling molecules termed gasotransmitters. In mammals and in most vertebrate species, H2S is present not only in peripheral tissues but also in the brain where its relatively high concentration suggests that this gasotransmitter is involved in central modulatory functions. However, little attention has been paid to the possible eco-physiological action of H2S on cardiorespiratory functions. In this preliminary study, we investigated the effect of environmental H2S, and the actions of intra-arterial (IA) and intra-cerebroventricular (ICV) injections of H2S, on cardiovascular and ventilatory functions in the unanesthetized rainbow trout. Intrabuccal administration of 5 µmol sodium hydrosulfide (NaHS), a H2S donor, evoked a hyperventilatory action, a bradycardia and a consecutive fall in dorsal aortic blood pressure (Pda). IA injection of NaHS (0.5-2 µmol) provoked a significant and dose-dependent tachycardia without significant change in either Pda or ventilation. This tachycardia is mediated by the sympathetic nervous system since only a sympatholytic drug (sotalol), but not a para-sympatholytic agent (atropine), blocked H2S-induced tachycardia. ICV injection of 10-50 pmol NaHS profoundly affected the behaviour of the trout. Struggling occurred accompanied by a hyperventilatory response but the changes in the cardiovascular parameters could not be accurately quantified.
In conclusion, environmental H2S affects cardiorespiratory reflexes and mimics cardiorespiratory responses to environmental hypoxia in fish. In addition, the actions of exogenous H2S administered peripherally and centrally suggest that the endogenous molecule may play an important role in cardiorespiratory regulation and behaviour. Further studies are needed to clarify the role of H2S in cardiorespiratory regulation in fish.

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13 Central effects of trout tachykinins on baroreflex sensitivity, heart rate and blood pressure variabilities in the unanesthetized trout.

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Orthologs of the mammalian tachykinins have been isolated and structurally characterized in teleost fishes but little is known regarding the physiological effects of these peptides in their species of origin. Our recent studies have shown that in trout, intracerebroventricular (ICV) injection of picomolar doses of trout neuropeptide gamma (NPg) and to a lesser extent substance P (SP), but not neurokinin A (NKA), produced a significant increase in heart rate variability (HRV) with no significant change in mean heart rate and in mean dorsal aortic blood pressure. The increase in HRV with no change in the other cardiovascular parameters suggests that the baroreflex sensitivity (BRS) may be increased following ICV NPg and SP. Consequently, the aim of this study was to examine in our experimental model, the unanesthetized rainbow trout Oncorhynchus mykiss, the central actions of a standard dose (50 pmol) of trout NPg, SP and NKA on BRS, HRV and blood pressure variability. Cross spectral analysis techniques using a fast Fourier transform algorithm were employed to calculate the coherence, phase and transfer functions between spontaneous fluctuations of systolic arterial blood pressure (SAP) and R-R intervals (R-Ri) of the electrocardiogram. The transfer function provides a measure of the degree to which the input signal (SAP), at a given frequency, appears in the output (R-Ri) energy. The BRS was estimated as the mean of the gain of the transfer function between SAP and R-Ri when the coherence was high. The power spectral density of R-Ri and SAP reflecting respectively HRV and the SAP variability were also determined. Compared with the vehicle-injected group of trout (n= 16), ICV administration of NPg (n=8) did not significantly change the baroreflex sensitivity. However, the HRV and the spontaneous variability of the SAP were significantly increased and the coherence between these two signals was high. In contrast, central injection of the same dose of SP and NKA were without any significant effect on any parameter. In trout, the parasympathetic nervous system is the main, or even the exclusive, contributor to HRV while the SAP variability might correspond to rhythmic sympathetic vasomotor activity.In conclusion, the increase in HRV associated with the increase in the SAP variability following ICV injection of NPg suggests that this peptide might be directly or indirectly implicated in central neuroregulatory control of vasomotor sympathetic activity in trout, a physiological mechanism which in turn affects HRV.

This study was supported by the Interreg IV ‘2 seas’ project Trans Channel Neuroscience Network (TC2N).

14 Gastric electrical stimulation modulates gastric distension-induced visceral pain and -activated brain centres.

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Gastric electrical stimulation (GES) is an effective therapy to treat patient with nausea and vomiting refractory to medical management. However, its mechanism of action remains poorly understood. The aim of this study was to assess whether GES could modify gastric sensitivity to gastric distension.

We monitored the variation of arterial blood pressure (BP) as a marker of visceral sensitivity in anesthetized rats in response to graded gastric distension from 20 to 80 mmHg during either GES or sham GES. In addition, c-fos protein expression was quantified by immunohistochemistry within the T9 dorsal horn of the spinal cord, tractus solitary nucleus (NTS) and hypothalamic paraventricular nucleus (PVN) in response to 60 mmHg gastric distension during either GES or sham GES. Moreover, in a same way, c-fos protein was quantified in rats distended and transected at thoracic level T5/T6, with or without GES.

Gastric distension induced a rise in BP (20 mmHg: 4.84 ± 0.54 mmHg; 80 mmHg: 11.2 ± 1.35 mmHg), and an increase in c-fos protein expression within the T9 dorsal horn of the spinal cord (11 ± 1 at baseline vs 23 ± 2; at 60 mmHg; p=0.0012), the NTS (55.36 ± 7 at baseline vs 96 ± 4; p=0.0002) and the PVN (179 ± 31 at baseline vs 373 ± 15 at 60 mmHg; p=0.0025). GES prevented gastric distension increase in BP from 20 (3.74 ± 0.51 mmHg; p=0.0039) to 80 mmHg of distension (8.14 ± 0.85; p=0.0002), while not affecting gastric compliance. Stimulation of the caecum using the same parameters did not affect BP response to gastric distension. In addition, GES prevented the rise of c-fos protein expression induced by gastric distension within the T9 dorsal horn of the spinal cord (15 ± 1; p=0.0047), the NTS (69 ± 4; p=0.001 and the PVN (212 ± 52; p=0.046). Moreover most of 60% of c-fos IR cells are CRF+. In addition, T5/T6 transection does not affect GES effect on the spinal cord, but abolished the rise induced in c-fos by the distension in the NTS and the PVN.

In conclusion, we demonstrated that GES decreases gastric sensitivity to gastric distension by recruiting brain centres involved in visceral sensation, and seems to act directly on splanchnic afferent fibers. Further investigations are necessary to determine mediators involved both at the medullar and gastric levels.

Keys words: gastric electrical stimulation, gastric distension, c-fos protein, dorsal horn of spinal cord

Molecular and cellular Neurosciences

15 Activation of SRE and AP1 by olfactory receptors via the MAPKand Rho dependent pathways

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Whereas the activation of MAPKs (mitogen activated kinases) and Rho dependant pathways by GPCR (G protein coupled receptors) has been the subject of many studies, the implication of olfactory receptors (OR), which constitute the largest GPCR has been far less analyzed. Using an in vitro heterologous system, we showed that odorant activated ORs stimulate SRE containing promoters via the ERK pathway. We demonstrated that RhoA and Rock kinases but not Rac were involved in ORs-induced SRE/SRF activation and that AP1 was activated, via JNK and p38 MAPKinase. By using real time PCR, we found that mOR23, RnI7 and CfOR12A07 induced elevated levels of transcription factors ELK-4, srf, c-fos and c-jun mRNAs whereas mOREG induced an elevated transcription levels of c-fos and c-jun mRNA only. The primary cultures of rat olfactory sensory neurons (OSNs) lead us to confirm that activated ORs stimulate the downstream pathways namely MAPKs, Rho and AKT ones. Similar data were observed with olfactory epithelium extracts freshly obtained from rats exposed to a cocktail of odorants. Given the importance of the MAPK and Rho pathways in the survival and differentiation of neurons in general, we hypothesize that the binding of an odorant onto its OR is the very first key event that regulate OSNs homeostasis.

16 Selenoprotein T is involved in the protection of catecholaminergic neurons in vitro and in vivo

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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 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 the neurotoxin 1-methyle 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), to induce degeneration of dopaminergic cells through the liberation of ROS, as a model of Parkinson's disease, we sought to evaluate in this study the expression of SelT in this neurodegenerative disease model. After MPTP treatment, brain sections were processed for immunohistochemical studies. Using anti-tyrosine hydroxylase (TH) and confocal microscopy analysis, we observed a marked decrease in dopaminergic neurons at 2, 4 and 8 days post-intoxication by MPTP. Concurrently, a strong astrogliosis was found in the striatum based on glial fibrillary acidic protein (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 and dopaminergic neuron degeneration. To further understand the role of SelT in these conditions, we used an in vitro model of catecholaminergic cell (SH-SY5Y) degeneration after treatment by MPP+. We observed that transfection of SelT significantly increased survival of SY5Y cells after the MPP+ treatment compared to the controls. Thus, these in vivo and in vitro results strongly suggest that SelT has a protective role during neurodegeneration caused by oxidative stress.

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17 Phosphopeptides Detection and Identification Using a Chip Cube Interface With Electron Transfer Dissociation Ion Trap Mass Spectrometry

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A fundamental aspect of proteomics is the analysis of post-translational modifications, of which phosphorylation is an important class. Phosphorylation controls a wide range of biological processes, therefore a precise determination of the phosphorylation sites is crucial for the understanding of the cellular response. Due to phosphate group lability, mass spectrometers using collision induced dissociation (CID) are usually not appropriate to determine a specific phosphorylation location site. Using the electron transfer dissociation (ETD) technique, the phosphorylation remains on the amino acid, allowing its location. Thus, to detect phosphorylation sites, ETD method is favored, which has introduced into mass spectrometry new approaches based on a combination of CID and ETD analysis. In this study we compared several methods to identify phosphopeptides after enrichment on specific chromatography columns in order to optimize phosphopeptides detection and characterization. We observed that ETD after a neutral low mass (NLM) detection provides higher detection and identification scores than the use of both CID and ETD alternatively. The enrichment procedure coupled with the NLM ETD technique will allow the analysis of phosphopeptides in order to compare the phosphorylation status of proteins under various experimental conditions.

Keywords: Phosphorylation, Mass Spectrometer, Collision Induced Dissociation, Electron Transfer Dissociation, Neutral Low Mass

This work is supported by the TC2N Interreg Project and the Region of Haute-Normandie.

18 Regulated recycling of tissue Plasminogen Activator (tPA) by astrocytes.

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Astrocytes are key players in the brain, controlling many physiological and pathological processes notably by uptaking neurotransmitters such as glutamate or GABA. Tissue plasminogen activator (tPA) is a serine protease expressed by neurons and glial cells within the central nervous system. It plays critical roles in controlling brain homeostasis through its properties of neuromodulator. Indeed, when released into the synaptic cleft by depolarized neurons, it modulates the activity of glutamate receptors (NMDA receptors) involved in the cellular mechanisms of learning and memory (long-term potentiation, LTP) and in excitotoxic cell death. In our study, we try to understand the role of astrocytes in regulating the effects of tPA on glutamatergic neurotransmission. We were able to demonstrate a specific and active internalization of tPA by astrocytes. Following this internalization, tPA can then be released from astrocytes, so there is a phenomenon of recycling of tPA. This recycling is regulated by extracellular glutamate and influences excitotoxic neuronal death. Within the synapse, modulation of this phenomenon could be of great importance. Indeed, increasing the uptake of tPA would avoid the harmful effects of tPA on cell death in diseases such as ischemic stroke. In contrast, in Alzheimer's disease, increasing the release of tPA by astrocytes may be beneficial by stimulating the mechanisms of synaptic plasticity.

19 Proliferation, migration and differentiation in juvenile and adult Xenopus laevis brains

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In contrast to mammals, the brain of adult non-mammalian vertebrates exhibits a higher proliferative and/or neurogenic activity. To provide new models on this issue, we have examined origin, distribution and fate of proliferating cells in the entire brain of juvenile and adult Xenopus laevis. Using immunohistochemistry for the Proliferation Cell Nuclear Antigen (PCNA), and/or the thymidine analog, 5-Bromo-2' deoxyUridine (BrdU), the labeled cells are located in ventricular zones of the olfactory bulbs, cerebral hemispheres, preoptic region, ventral hypothalamus and cerebellum. Qualitatively, the highest level of proliferative cells was found in the telencephalic ventricles. By using in situ hybridization/immunocytochemistry double-labeling techniques, we demonstrate for the first time in post-metamorphic frog brain that the proliferative cells are localized in very close vivinity to the radial glial cells, progenitor cells that we have also identified in the ventricular layer using classical molecular markers (BLBP, Vimentin). In addition, after long post-BrdU administration survival times ranging between 14 and 28 days, BrdU labeling combined with immunohistochemistry for markers of cell migration (DoubleCortin) or radial glial cells (BLBP), reveals that the proliferative cells are able to migrate from the ventricular zone into the brain parenchyma, most likely by migrating along the radial processes. Finally, at survival time of 28 days and by using a combination of BrdU labeling and in situ hybridization for markers of differentiation states (Neuro-□-tubulin, Proteolipid Protein), we demonstrate that newborn cells can differentiate in large portion into either neurons or oligodendrocytes.

Key words : Proliferation, Neurogenesis, gliogenesis, radial glia, Xenopus, brain.

20 PACAP and acetyl-[Ala15, Ala20]PACAP38-propylamide protect rat brain from ischemia: insight into the mechanisms of action

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Pituitary adenylate cyclase-activating polypeptide (PACAP) exerts protective activities in numerous models of neurological disorders involving neurodegeneration. However, the use of PACAP as a clinically efficient drug might be limited due to its poor metabolic stability and adverse physiological effects. Thus, by combining identification of enzymatic cleavage sites with targeted chemical modifications, a metabolically stable and potent PACAP38 analogue, acetyl-[Ala15, Ala20]PACAP38-propylamide, was developed. The in vivo biological activity of this new compound was evaluated and compared to the native peptide using a rat model of middle cerebral artery occlusion (MCAO). The results show that as low as picomolar doses of PACAP38 and its analogue, administered intravenously, act on the same mechanisms to strongly reduce infarct volume and improve neurological impairment induced by stroke. In particular, these peptides inhibit the expression of Bad, caspase 3, MIP-1□Nos2, TNF-□ and NF-□B mRNAs, and increase ERK2, Bcl-2 and IL-6 mRNA levels. These results indicate that the neuroprotective effect of PACAP after MCAO is not only due to its ability to inhibit apoptosis but also to modulate the inflammatory response. Altogether, the present study highlights the potential therapeutic efficacy of very low concentrations of PACAP or its metabolically stable derivative for the treatment of stroke.

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21 Nuclear progesterone receptors are up–regulated by estrogens in neurons and radial glial progenitors in the brain of zebrafish

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In rodents, there is increasing evidence that nuclear progesterone receptors are transiently expressed in many regions of the developing brain, notably outside the hypothalamus. This suggests that progesterone and/or its metabolites could be involved in functions not related to reproduction, particularly in neurodevelopment. In this context, the brain of adult fish is of particular interest as it exhibits constant growth and a high neurogenic activity that is supported by radial glia progenitors. However, although synthesis of neuroprogestagens has been documented recently in the brain of zebrafish, information on the presence of progesterone receptor is very limited. In zebrafish, a single nuclear progesterone receptor (pgr) has been cloned and characterized. Here, we demonstrate that this pgr is widely distributed in all regions of the brain of zebrafish. Interestingly, we show that Pgr are strongly expressed in radial glial cells and more weakly in neurons. Finally, we present evidences, based on quantitative PCR and immunohistochemistry, that nuclear progesterone receptors mRNA and proteinare upregulated by estrogens in the brain of adult zebrafish. These data document for the first time the fact that radial glial cells are preferential targets for peripheral progestagens and/or neuroprogestagens. Given the crucial roles of radial glial cells in adult neurogenesis, the potential effects of progestagens on their activity and the fate of daughter cells require thorough investigation.

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22 Pleiotrophin plays a key role during the ontogenesis of cerebellar cortex in mice

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In rodent cerebellum, pleiotrophin (PTN) is present in high concentration in the pre-migratory zone of the external granule cell layer (EGL) and in the molecular layer (ML) during the first postnatal week, suggesting that this cytokine could participate to the development of the cerebellar cortex. However no work was performed up to date to precise the role of PTN during cerebellum ontogenesis. So we undertook a time dependant study between P0 (first postnatal day) and P21 to establish the profile of PTN expression during mouse childhood and to determine the effect of subarachnoid injections of PTN on the proliferation, migration and differentiation of granular neurons. Our immunohistochemical results confirmed the presence of PTN in the extracellular matrix of EGL and ML in young mice. At P12, a moderate cytoplasmic label appears in migrating granular cells and in Purkinje cells but becomes quite undetectable at P21. The two PTN receptors RPTPξ and Syn3 were also both localized in Purkinje cells and immature granule cells during the same period. In parallel, Western blot studies performed from P0 to P21 indicate that the levels of PTN, Syn3 and RPTPξ stay high until P12 before decreasing until adulthood. These data suggest that PTN could exert various effects during cerebellum ontogenesis depending on the receptor used and the temporal window considered. This diversity of action sites was demonstrated by in vivo experiments showing that injections of PTN at the surface of the cerebellar cortex induce an atrophy of Purkinje cells and exacerbate apoptosis of differentiated granule cells. Moreover, administration of PTN significantly reduces the rate of migration of immature granule cells on ex vivo organotypic slices since the cytokine stimulates the mobility of granular neurons in culture. Finally, we demonstrated that PTN is able to increase its own expression in vitro.

Altogether, our results show that PTN participates to the control of the major steps of cerebellum ontogenesis namely the migration, the differentiation and the apoptosis. However, administration of PTN into the cerebellum seems to induce a stimulation loop in the immature granule cells leading to an excess of PTN that is more deleterious than beneficial for cerebellum development. Nevertheless this work could open perspectives to detect and/or treat pathologies in which PTN expression would be impaired such as in ataxic Weaver mice. This work is supported by Interreg 4A, TC2N and LARC-Neurosciences.

23 Glutamate stimulation evokes t-PA secretion from brain vascular endothelial cells: dual effect of t-PA on neuron death depending on immaturity and cortical layers.

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Despite recent progress made in obstetrics and newborn intensive care, neurological disabilities of perinatal origin do not decrease. Excitotoxicity has been pointed out as a common pathway of most deleterious processes in neonate brain injuries. The detection of NMDA-receptor on brain microvascular endothelial cells (BMEC) support the hypothesis that BMECs participate in excitotoxic processes. We have recently demonstrated phenotypic and functional differences between newborn (n) and adult (a)-derived BMECs that could be relevant for enhanced glutamate (Glu) sensitivity of nBMEC. In particular, nBMEC released more tissue plasminogen activator (t-PA) and gelatinases than aBMEC under Glu stimulation.

In the present study using Evans Blue (EB) in vivo loaded microvessels, abluminal Glu stimulation induced EB-albumin extravasation more efficiently in microvessels isolated from newborn than from adult cortices. Conditioned media (CM) from aBMEC exposed to Glu increased the Glu-induced death of mature neurons in vitro. This effect was more pronounced with CM from nBMEC. The effects of CM were blocked by MK801 (a NMDA receptor blocker). Potentiation of Glu toxicity by CM was reversed by recombinant plasminogen activator inhibitor-1 indicating t-PA as the released-factor responsible for this effect. In contrast, t-PA decreased neuronal death of immature neurons. Ex vivo, in brain slices from 2, 5 or 10 day-old mice, t-PA inhibited caspase-3 activity and cleaved caspase-3 immunolabeling in superficial layers (II-III) containing immature neurons. Glu did not modify this effect. Infraliminal Glu and t-PA when used in association induced necrosis (LDH activity and 7AAD labeling) in the deep cortical layers (IV-VI) containing mature neurons.

These data suggest that upon parenchymal Glu challenge, BMEC-secreted t-PA in high amount by neonatal vessels may participate to BBB permeabilization, and exacerbate excitotoxicity. Reciprocally t-PA anti-apoptotic effects independent of Glu may coexist in immature neuron populations. These data point out stage-dependant microvessel sensitivity to Glu and potential deleterious reaction in the immature brain (see also abstract by Lecointre et al.), justifying the development of vessel oriented neuroprotection strategies in the neonate.

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24 ISOLATION, CHARACTERIZATION AND GENETIC PROFILING OF SUB-POPULATIONS OF OLFACTORY ENSHEATHING CELLS FROM THE OLFACTORY BULB

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Olfactory ensheathing cells (OECs) play a crucial role during neurogenesis of primary olfactory neurons. Transplantation of OECs is considered as a hopeful therapy for central nervous system repair. Nevertheless, OECs are constituted of distinct subpopulations and the role of each of them during neurogenesis is not clearly understood. In particular, OECs from the olfactory bulb (OB) constitute a heterogeneous, not yet isolated and characterized, population of cells.

In our study, flow cytometry analyses of primary OB cultures, based on cell surface expression of low-affinity nerve growth factor receptor (p75), reveal presence of two distinct populations of OECs. Indeed, some of them express a high level of p75 (P75High) and the other a low level of p75 (P75Low). Effects of OB microenvironment were assessed and we could show that fibroblasts mediate the induction of these two distinct populations through the secretion of soluble factors. In order to characterize P75High and P75Low OECs, cells were sorted based on their differential expression of p75. Microarray analyses revealed that P75High OECs overexpress genes implicated in modulation of extracellular matrix and cell sorting, whereas P75Low OECs overexpress genes involved in regulation of the inflammatory response and axonal guidance. Altogether, these results permit for the first time to isolate the two distinct subpopulations of OECs from OB, and to characterize, in part, their specific role during neurogenesis.

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Key Words: olfactory ensheathing cells, olfactory bulb, microenvironment, p75, neurogenesis.

25 What role do the highly conserved non-coding sequences play in the regulation of the midbrain arc genes expression?

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Specific and highly regulated gene expression creates a precise arc pattern in the ventral midbrain during vertebrate embryogenesis. Alterations to this arc pattern through gain and loss of function experiments on the arc genes show that neuronal differentiation and axon guidance in the developing brain are compromised. The most highly conserved tracts in the developing brain are the Tract of the Postoptic Commissure and the Medial Longitudinal Fascicle, creating a tract which runs along the ventral side of the neural tube. This tract is distorted when the arc genes are tampered with. One of the genes shown to be expressed in the arc formation is Emx2 which encodes a transcription factor with characterised function in areas such as the intermediate mesoderm and forebrain (Beachy & J. L. Rubenstein 1998) in Emx2 knockout mice the red nucleus fails to form, indicating that Emx2 is also necessary for ventral midbrain and pretectum development (Gangemi et al. 2006). The Sax1 gene and it's paralogue Sax2 are expressed in the ventral midbrain at the same time as Emx2 and the other arc genes and it has been shown that Sax1 is a downstream gene of Shh. Research carried out by Ahsan et al. 2007 showed that the expression of Emx2 was inhibited by the over-expression of Sax1. However, it is unclear whether the interaction between Sax1 and Emx2 is direct, or in fact how the expression of the midbrain arc genes are generally regulated. The aim of this study is to characterise Cis-regulatory elements controlling the expression of Sax1, Sax2 and Emx2.

Highly conserved non-coding sequences (HCNCS) were cloned into expression vectors and electroporated into chick embryos at developmental stages H&H 11. The embryos were incubated for a further 2-3 days up to the stage when acr genes are normally expressed. Specific expresion of the vector in some or all of the endogenous expression areas of the respective gene would indicate the HCNCS acts as an enhancer elements for the cell-type specific gene expression pattern.

From here deletion analysis to find the minimal sequence of the enhancer and electrophoretic mobility shift assay (EMSA) will show whether the previously observed genetic interaction between Sax1 and Emx2 is direct or indirect. This interaction will further be tested in vivo by co electroporating the reporter construct with a Sax1 and Emx2 expression construct.

26 P2X7 receptors in hippocampus of normal and dystrophic (mdx) mice –comparative analysis.

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Duchenne muscular dystrophy (DMD) is a lethal neuromuscular disorder characterized by muscle wasting associated with mental retardation.
We have recently shown that the level of expression and activity of P2X7 ATP receptors is higher in dystrophic mdx mouse muscles comparing to the control ones. In order to study P2X7 receptor expression in dystrophic mouse brains we performed Western blot analyses in both brain and hippocampi extracts from 3 month old wild-type (C57BL10) and dystrophic mdx mice. Densitometric analyses showed significant increases in P2X7 protein levels in mdx brains samples compared with wild-type controls. As a negative control, two P2X7 knock-out mice strains have been used: in one KO line the P2rx7 gene was knocked out by insertion of a lacZ transgene into exon1, whereas in the second line a neomycin cassette was inserted into exon 13. P2X7 immunoreaction has been found absent in brains from these KO lines.
In the next step we have attempted to study P2X7R distribution in the brain using immuno-localisation. Previous studies have shown that several of the commonly used antibodies are not fully specific to P2X7 proteins, because of the reported pseudoimmunoreactivity in hippocampal neurons of P2X7-/- (knock-out) mice. Although the novel C-terminal antibody used by us gave the most reproducible results in western blotting, the specificity of this antibody in the immunohistochemistry is still uncertain. We attempted to increase the specificity by applying different antigen retrieval and blocking methods and the results will be presented.
Knowing the cellular distribution of P2X7 receptors in the brain would be important for our understanding of this receptor function and may explain its involvement in DMD-associated mental retardation.

Key words: Duchenne muscular dystrophy, P2X7 ATP receptor, mdx mouse, mental retardation

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27 7Development of a Q-PCR panel to investigate oxydative stress induced by ethanol consumption

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A major cause of alcohol toxicity is the production of ROS generated during its metabolism into acetaldehyde and acetate. However, cells have an antioxidant system composed of 3 enzymes, i.e. SOD, catalase and GPX, which eliminate ROS. Oxidative stress occurs when the amount of ROS exceeds the capacity of the antioxidant system to eliminate the excess. ROS are known to influence the expression of a number of genes and signal transduction pathways such as the MAP kinase and NF-kB pathways, which are redox-sensitive and mediate many of the redox effects in cells. The respiratory chain of mitochondria and the NADH oxidase contribute to basal ROS production. Under oxidative stress conditions, their capacity to eliminate ROS production is reduced. ROS also induce proteins and lipids oxidation, and DNA damages responsible for inflammation or apoptosis. To get an overview of the oxidative stress induced by ethanol consumption at different stages of brain development, we have developed a qPCR panel composed of 84 oxidative stress regulated genes related to NF-kB and MAP kinase pathway, antioxidant enzymes, mitochondrial and NADPH oxidases proteins, oxidative stress responsive genes, and oxidative stress damages. We have designed the reverse and forward primers, for each gene by using the FastR application that we have developed (http://www.fastr.fr). The primers have been validated by high throughput qPCR to verify their efficacity and specificity, and the panel is now being used to address the effect of alcohol on oxidative stress.

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Keywords: Oxidative stress, Ehanol, Eain, qPCR, Pathway

28 A unique peptide receptor both regulating migration and adhesion of glioma

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High grade glioma represent the most frequent primitive cerebral tumor in adult. They are characterized by invasion of healthy brain tissue by tumoral cells and proliferation of endothelial cells (neoangiogenesis), two phenomena responsible of the high level of disease recur. Vasoactive neuropeptide participate in recruitment of cells and/or proangiogenic factor release mediating neoangiogenesis in situ. Among them, 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 controls astrocyte activities, promotes neoangiogenesis from brain microvessels and acts as a chemoattractant, stimulating migration of endothelial progenitor cells. 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 a 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 by means of the yeast two-hybrid strategy. Until now, several proteins have been characterized, including Filamin A, a protein known to be involved in cell migration and/or adhesion.

29 Immature brain microvessels less contributed to glutamate uptake and metabolism than mature brain microvessels

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In western countries, the neurological disabilities of perinatal origin remain unchanged for the last 60 years. The uncontrolled release of glutamate (Glu) in the Parenchyma is considered as a common pathway in most noxious conditions that affect neonatal brain, as well as in adult pathology. In physiological conditions, the blood brain barrier prevents high plasmatic Glu to cross the vascular wall. Moreover, specific uptake and metabolism of Glu at the endothelial level contributes to maintain Glu below toxic concentrations. Nowadays, only few data exist on Glu uptake and metabolism during brain development and none concerns the brain vascular endothelium.

In the present study, we propose to follow the expression of GLT-1 (a major Glu transporter in adult), glutamine synthetase (an enzyme implicated in Glu metabolism) and doublecortin (an indicator of cortical neurons immaturity) during brain development in newborns, 2 days-old (P2), P5, P10, P20 and adult (P50) mice cortex. Western Blot (WB) analyses showed an increase of GLT-1 and glutamine synthetase expression (50 fold from neonates to adults) correlated with ages ($r^2=0.992$, $p<0.001$ and $r^2=0.914$, $p<0.01$, respectively) and inversely correlated with doublecortin expression ($r^2=0.825$, $p<0.05$ and $r^2=0.902$, $p<0.01$, respectively).

In isolated cortical microvessels, GLT-1 expression was not detected either by immunolabeling or WB at P10, whereas it was by both techniques at P50. Preliminary WB analysis suggested a lower glutamine synthetase expression in P10 isolated microvessels compared to P50 vessels.

In vitro, Glu (50 µM) was applied on brain microvessels endothelial cells (BMEC) cultures. Glu assays in supernatants exhibited unchanged concentration after 6 hours in the presence of BMEC from P10 mice whereas Glu level was significantly reduced (35.2µM; $p<0.05$) in BMEC media from P50 mice.

These data reinforce our hypothesis that neonate and adult brain microvascular endothelium have different properties regarding Glu scavenging mechanisms (see also abstract by Henry et al.). Taken together our results suggest that microvascular immaturity may contribute to the neuronal tissue vulnerability of neonates.

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30 Memantine and UBP145, two treatments to prevent tissue plasminogen activator-promoted neurotoxicity in stroke

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Glutamate induces N-methyl-D-aspartate receptors (NMDA-R)-mediated neurotoxicity which represents one of the major causes of neuronal death after stroke. Despite its pro-excitotoxic effects which would be mediated by GluN2D-containing NMDA-R, recombinant tissue plasminogen activator (rtPA) remains the only acute treatment approved by the authorities. Memantine (1-amino-3, 5-dimethy-ladamantane), is an uncompetitive GluN2C- or GluN2D-containing NMDA-R antagonist used for the symptomatic treatment of the moderate to severe forms of Alzheimer's disease. Here, we investigated whether memantine could be an adjunct therapy to rtPA-induced thrombolysis for stroke. In vitro, memantine prevented the pro-neurotoxic effects of rtPA. Moreover, calcium video-imaging performed as an index of NMDA-R activity revealed that memantine was capable to prevent the rtPA-induced increase of NMDA-R-mediated calcium influx. In vivo, memantine reduced the noxious actions of rtPA in a thrombotic stroke model. In parallel, we tested the effects of UBP145 ((2R*,3S*)-1-(9-bromophenan-threne-3-carbonyl)piperazine-2,3-dicarboxylic acid), a proposed selective antagonist of GluN2D-containing NMDA-R. UBP 145 produced the same effects as memantine both in vitro and in vivo. In conclusion, we evidence that GluN2D is involved in the pro-neurotoxic effects of rtPA and our data suggest that memantine or UBP145 could be effective adjunct therapies to thrombolysis for stroke.

31 Tissue-type plasminogen activator immunoreactivity in cells constituting the neurovascular units: Effects of a colchicine treatment on mouse forebrain.

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Despite its primary vascular function linked to its endothelial expression, tissue-type Plasminogen Activator (tPA) is involved in numerous neurophysiological processes including neurogenesis, axonal growth, neuronal migration, apoptosis (neuronal or glial), necrosis, excitotoxicity, memory, endocrinology and so on ! Surprisingly, in vivo, brain cells expressing tPA are not unambiguously characterized in mammal.

In mice forebrains, we performed an immunohistological study with optimized fixative conditions and used various cellular markers associated, or not, to one of two selected specific antibodies recognizing mouse, rat and human tPA. In mice, these last immunological tools revealed tPA expressing cells but gave a surprising different subcellular location for tPA immunoreactivity in various type of embryonic and adult cells. Independently of this observation, which is probably linked to tPA maturation in cells, we provide a unambiguous description of tPA immunoreactivity among the cells types (ectodermal and mesodermal derivatives) encountered in neurovascular units. In mice forebrains, tPA-ir is detected in endothelial cells, some oligodendrocytes, ependymocytes but is undetectable in astrocytes, microglial cells, pericytes, most of the choroïde cells and in muscular arteriolar cells. Our neuronal distribution of tPA-ir is in accordance with the literature but colchicine treated mice showed, for the first time, new cortical and hippocampal neuronal populations expressing tPA immunoreactivity.

32 Vascular protective effect of Angiopoietin-2 during cerebral ischemia

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Cerebral ischemia is characterized by damage of the entire neurovascular unit with a neuronal death associated with an increase of the blood brain barrier (BBB) permeability. Accordingly, in response to permanent cerebral ischemia, we detected an increase in the endogenous expression of Vascular Endothelial Growth Factor (VEGF), a factor associated with vascular leakage. Interestingly, our results showed a concomitant overexpression of Angiopoietin-2 (Ang2) known as a collaborator to VEGF in vascular remodeling. Based on this observation, the aim of this study was to evaluate the role of the vascular growth factor Ang2 alone or in combination with VEGF, in the acute phase of cerebral ischemia. To this end, in a mouse model of focal cerebral ischemia, Ang2 and/or VEGF were intracerebroventricullary-administered at the time of permanent cerebral artery occlusion (MCAO) and the effect of these angiogenic factors were evaluated on the ischemic lesion volume using Magnetic Resonance Imaging (MRI). Consistent with its deleterious role in the acute phase of the ischemic insult, we observed that an early administration of VEGF exacerbates ischemic damage because of its effects on blood-brain barrier (BBB) permeability. In contrast, an administration of Ang2 leads to a significant reduction of the ischemic volume (Ang2 group = 64.8 ± 19 % of control group). In addition, this beneficial effect of Ang2 on the ischemic lesion is maintained in presence of VEGF and Ang2 blocks the BBB permeability effect of VEGF. We performed complementary studies to determine whether Ang-2 decreases the permeability of BBB by affecting the expression of tight junction proteins such as claudin-5 and adherens junction protein vascular endothelial (VE)-cadherin. As expected in regard to its permeability effect, VEGF leads to a decrease of both protein expressions, whereas Ang2 counteracts this effect.

This study reports for the first time a protective role of Ang2 during cerebral ischemia that is associated with a reduced BBB permeability. From these results, it could be proposed that Ang2 may represent an interesting molecular target for cerebral ischemia as a therapeutic strategy for this pathology should be to limit the acute damage by promoting not only protection of neural but also vascular compartments.

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33 Regulation of autophagy by urotensin II: potential involvement in glial tumorigenesis

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Growth factors, but also vasoactivepeptides, are endogenous compounds that have been shown to play an important role during tumorigenesis. We have previously demonstrated that the vasoactive peptide urotensin II (UII) and its receptor UT are expressed in several glioma cell lines. Moreover, we have found that UII stimulates tumor growth of heterotopical xenografts of U87 glioma cells in nude mice (*). Autophagy is a catabolic process that involves the entrapment of cytoplasmic components within characteristic vesicles for their delivery to and degradation within lysosomes. This process is induced by cellular stress, such as nutrient deprivation or hypoxia. When overactivated, however, autophagy can lead to cell death. At times, autophagic cell death is used as an alternative to apoptosis to eliminate unwanted, damaged, or transformed cells. Consistent with this, tumorigenesis is associated with a downregulation in autophagy, and many genes that mediate the execution of autophagy have been shown to be tumor suppressors. Together, these data prompted us to investigate if the oncogenic activity of UII could involve regulation of the autophagic process.
Using the HEK-293 cell line, we found that UII reduced the autophagic activity elicited by hypoxia. Moreover, inhibition of autophagy by UII correlated with reduced levels of mRNAs encoding the pro-autophagic proteins Bnip3 and Redd1, which were previously described as tumor suppressors in xenograft mouse models. This effect of UII might involve post-transcriptional mechanisms, since UII strongly induced the expression of the microRNA-221, an "oncomiR" highly expressed in several human cancers, which has been found to downregulate Redd1 mRNA by targeting its 3'untranslated region. Collectively, these data lead us to propose that UII, by activating a signaling pathway involving the oncomiR-221 and its downstream targets, may accelerate tumor growth by reducing the autophagic capacity of cancer cells exposed to their hypoxic microenvironment.

(*) V. Lejoncour et al., Poster: "Urotensinergic vasoactive peptides as new factors involved in tumor growth and neoangiogenesis of high grade glioma"

Keywords : glioma, urotensin, autophagy, tumorigenesis

34 Gene function studies using the Xenopus model – H2A.Z variant histones are required for correct neural and mesodermal development

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A systematic screen of the expression and function of variant histones in the early Xenopus embryo has revealed that, among others, H2A.Z1 and H2AZ.2 have critical roles in development. Loss of H2A.Z2 causes paralysis via a subtle neural development phenotype in which the number and behavior of neurons made in the embryo are altered. In situ hybridization of a variety of genes involved in neural development has identified some of the pathway that is likely to be disrupted by H2A.Z2 loss of function.

Loss of function of H2AZ.1 caused defects in the correct formation of several cell types, initially these appeared to be restricted to mesodermal lineages. The pan-mesodermal gene brachyury requires H2AZ.1 for its correct expression and chromatin IP in embryos confirms that this variant histone is found at the brachyury promoter. Further investigation has revealed that H2A.Z1 is required for the correct expression of a number of key developmental genes and that these are not restricted to the mesoderm. Identifying the direct H2AZ.1 target genes and then identifying the epigenetic or genetic marks that define these target genes are the next challenges and the system to take them on is now in place.

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35 Involvement of PACAP and tPA in cell survival during cerebellar development

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and tissue plasminogen activator (tPA) play important roles during cerebellar development. However, a direct link between the neurotrophic effects of PACAP and tPA has never been investigated. In the present work, we found that PACAP stimulates the expression of tPA mRNA (2.4 fold increase after 3 h of treatment) and the release of proteolytically active tPA in immature cerebellar granule neurons (CGN). Immunocytochemical labeling revealed the presence of tPA in the cytoplasm and processes of cultured CGN. Since PACAP has previously been shown to exert an anti-apoptotic effect on CGN, we have studied the effect of tPA and PACAP on CGN survival in depolarizing conditions (K25/N1 supplement-containing medium) or in K5 (5 mM KCl)/N1supplement-deficient medium. In depolarizing conditions, PACAP (10-7 M) displayed a neuroprotective effect (+43%) on CGN after 48 h of culture. tPA (10-7 M) induced a slight increase of cell viability while the tPA inhibitor PAI-1 (10-7 M) reduced partially the stimulatory effect of PACAP on cell survival. When CGN were transferred into a K5/N1 supplement-deprived medium (24 h of culture), tPA (10-7 M), in contrast to PACAP, displayed a neuroprotective effect (+26%) on CGN. In addition, application of PAI-1 significantly reduced cell viability, suggesting that the release of endogenous tPA participates to CGN survival. Altogether, these data demonstrate that, in depolarizing conditions, tPA mediates the neurotrophic effect of PACAP on CGN via a mechanism dependent of its proteolytic activity. Furthermore, in a K5/N1 supplement-deprived medium, spontaneous release of tPA exerts a neuroprotective effect on CGN through its proteolytic activity.

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36 PACAP and tPA exert key functions in the control of cell migration during cerebellum development

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In the developing brain, immature neurons have to migrate from their birthplace to reach their final destination. Excitatory and inhibitory neurons originate from distinct regions of the brain, and exhibit different modes of migration. In the developing cerebellum, the centripetal migration of granule cells (GC; excitatory interneurons) and the centrifugal migration of basket/stellate cells (BSC; inhibitory interneurons) have been extensively studied but little is known about the factors that regulate the velocity of these cells in the different layers of the cerebellum. In the present work, we have applied ex vivo imaging techniques to investigate the effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and tissue-plasminogen activator (tPA) on GC and BSC migration. Incubation of cerebellar slices from P10 mice with exogenous PACAP (10-6 M) decreased the speed of migration of GC in the molecular layer (ML) by 62%. Reciprocally, the PACAP antagonist PACAP(6-38) (10-6M) increased the rate of migration of GC by 68% in the Purkinje cell layer (PCL) and reduced the motility of BSC in the ML by 22%. Application of the tPA inhibitor, PAI-1 (10-7 M), reduced the migration velocity of GC and BSC in the ML by 70% and 27%, respectively. In contrast, neither PACAP nor PAI-1 had any effect on cell migration in the internal granular layer (IGL). These data indicate that both PACAP and tPA play a major role in the regulation of interneuron migration in the developing cerebellum.

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37 The effect of the P2X7 purinergic receptor knockout in the mdx mouse model of Duchenne muscular dystrophy.

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Duchenne muscular dystrophy is an X-linked recessive neuromuscular disorder affecting 1 in every 3000 males. It is characterised by a progressive weakening of nearly all muscle groups, a premature mortality, and a cognitive deficiency. The mdx mouse model of this disease presents many clinical phenotypes of the human disorder including a compromised muscle cell membrane, leading to increase in blood plasma levels of the cytosolic enzyme creatine kinase, a progressive and episodic disorganisation of tissue organisation and secondary cellular development, and a general biochemical and metabolic shift towards a dystrophic phenotype. Our laboratory has shown an increase in expression levels of several purinergic receptor sub-types in dystrophic mouse muscles compared to age-matched controls. The altered expression of the P2X7 purinergic receptor subtype has the potential to affect various cellular aspects of DMD, via cell growth or cell arrest pathways, either dependent on or independent of secondary cellular inflammatory response in diseased tissue.

To study this we have developed the double-negative mouse model mdx/P2X7-/-. Using biochemical, cellular, and molecular biological techniques we have identified differences in functional protein and histological markers of disease progression. At 4 weeks post-natal, coinciding with a peak in neuromuscular degeneration in the mdx mouse model, P2X7 and dystrophin double knockout mice present a reduced level of blood serum creatine kinase compared to their mdx counterparts. Immunoblot comparisons of muscle health markers suggest improvement of muscle functions in the mdx/P2X7 double knockout mice. These statistically significant and qualitative shifts in the progression of the neuromuscular pathology resulting from the genetic ablation of the major functional variants of the P2X7 receptor are indicative of a potential amelioration in the severity of disease.

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38 Characterisation of Tensin 2 expression in the mouse brain

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The Tensin family are large intracellular proteins which, through protein-protein interactions, serve as a bridge to link receptor tyrosine kinases and integrins to the actin cytoskeleton of the cell interior. This bridging function is thought to regulate signalling for cytoskeletal organisation, and may thereby control cell shape, vesicular transport, cell motility, adhesion and proliferation. Currently, the role of Tensin proteins in the brain and central nervous system is unknown. Therefore, the aim of this study was to determine the expression profile of Tensin2 in the mouse brain.

Immunofluorescence staining and confocal microscopy, and western blot analysis, were used to detect the expression of Tensin2 in different parts of the adult C57/BL mouse brain. In-house antibodies were used which were previously verified for specificity; moreover, we used different antibodies raised against Tensin2 in order to confirm each other's results as definitive.

Tensin2 showed a punctuate and intracellular expression, and it showed a prominent and discrete pattern in both the cerebellum and the hippocampus. In the hippocampus, strong expression of Tensin2 was present in the strata oriens and radiatum of the CA1 region. Furthermore, Tensin2 expression colocalised with other neuronal markers, including VGlut1, GAD67, cannabinoid receptor type 1 and parvalbumin, which indicate the expression of this protein in both inhibitory and excitatory axon terminals. Western blot analysis revealed high expression of Tensin2 in different regions of the neonatal and postnatal mouse brain, including cortex and cerebellum, with apparently two isoforms existing exclusively in the neonatal brain.

In conclusion, we have found Tensin2 expression to be highly and discretely expressed in the mouse brain, suggesting that it plays a significant role in neuronal functioning. We are currently continuing this research to elucidate the precise subcellular location of Tensin2, and looking for further potential interactors, including analysing brains from genetically Tensin2-deficient mice.

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39 Selenoprotein T function in brain : a neuron-to-glial switch in neuroprotection

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Excess of reactive oxygen species (ROS) is a facilitating factor of neuronal death expansion during neurodegenerative diseases. Although most cells harbor a powerful antioxidant defense network, neurons rely on their coupling to astrocytes to combat oxidative stress. This system includes major antioxidant enzymes such as superoxide dismutases and several selenoproteins, a selenium-containing protein family. Indeed, the active site of these proteins encompasses a selenium atom, whose nucleophilic activity is essential for their enzymatic activity, like ROS reduction for instance. A pangenomic screening of the targets of the neuroprotective factor PACAP, permitted to identify a novel selenoprotein, the selenoprotein T (SelT), which is localized in the endoplasmic reticulum where it participates in the regulation of Ca2+ homeostasis. SelT is widely and strongly expressed during embryogenesis and neurogenesis, but vanishes in most adult tissues. We demonstrated that global knockout of SelT gene is lethal during embryogenesis at midgestation, and that its deficiency in nervous cells leads to cell death and increased intracellular levels of ROS. Thanks to a complementary approach of directed mutagenesis, we showed that the prosurvival effects of SelT on neuroblasts relies on the presence of a selenocystein residue within its active site, called “thioredoxin-like domain”. Moreover, in the adult, SelT levels were strongly induced in reactive astrocytes following cerebral ischemia. In fact, SelT also promotes astrocyte cell viability through inhibition of caspase-3 activity. These results indicate that SelT is a prominent antioxidant selenoprotein which is required for embryogenesis and which ensures a pivotal role in the neuron-to-glial switch for neuroprotection.

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40 Molecular mechanisms controlling early brain development

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The early differentiating neurones in the embryonic vertebrate brain form an evolutionary well-conserved array of axon tracts, the early axon scaffold. The simple, regular organisation makes this an attractive model to study the molecular mechanisms that underlie initial patterning, neuronal differentiation and fate determination, and axon guidance. Furthermore, the precursors of the future brain vasculature start accumulating at the basal lamina of the neural tube at the time when the first neurones differentiate, making this system also a model for early neural-vascular interactions.

We have identified a number of homeodomain transcription factors that are expressed in precisely defined domains in the ventral midbrain and diencephalon during early stages of development. Gain-of-function experiments in our lab suggest that transcription factors like Sax1 or Emx2 are part of a genetic network that controls the dorsoventral organisation of the brain and influences the fate determination of the differentiating neurones. The outgrowing axons depend on extrinsic guidance cues for the correct navigation to their targets. The expression of the axon guidance molecules most likely is also governed by the early patterning events, as the axon trajectory of the longitudinal tracts is affected by misexpression of Sax1 and Emx2. An expression screening of axon guidance molecules in our lab has revealed a number of candidate molecules that could influence tracts of the early axon scaffold. By gain- and loss-of-function experiments we have shown that Sema3A acts as a repellent to stop MLF axons from projecting into the anterior diencephalon, while Netrin2 repulsion forms a channel that restricts the posterior commissure axons to the posterior pretectum. Current work in our lab focusses on the early genetic network, specifically how the precise expression of the early expressed transcription factors is regulated.

41 P2X7 receptor expression and function in the pathology of Duchenne Muscular Dystrophy

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Mutations in the DMD gene encoding the protein dystrophin are responsible for the phenotypes of the debilitating and as yet incurable diseases known as Duchenne and Becker Muscular dystrophies. Widely regarded as a scaffold protein, loss of functional dystrophin from the sarcolemma results in the dissolution of a large complex of dystrophin associated proteins, with vast functional consequences for the cell. One of the oldest observed hallmarks of Duchenne Muscular Dystrophy (DMD) is that of increased intracellular Ca2+ levels, the mechanisms of which are still to be fully elucidated. The P2X receptor family comprises seven membrane bound ligand gated ion channels involved in intracellular Ca2+ influx in response to binding Adenosine 5'-triphosphate (ATP). P2X7 in particular has been attracting avid attention in recent years due to its unique propereties: pore formation following prolonged stimulation and insensitivity to ATP, a feature which may be of great significance in the environment of an exercising/damaged muscles. Indeed, we have recently documented abnormalities in P2X7 receptor expression and function in muscles of the mdx mouse model of DMD (Young et al., 2011, in press).

We have demonstrated significant P2X7 receptor abnormalities in isolated primary muscle cells, immortalised myoblast lines and in dystrophic muscles in vivo. P2X7 mRNA and protein expression levels were significantly up-regulated in dystrophic muscles and this was associated with altered function of P2X7 receptors producing increased responsiveness of cytoplasmic Ca2+ and ERK phosphorylation to purinergic stimulation. Ca2+ influx and ERK signalling were stimulated and inhibited by specific agonist/antagonist combinations, confirming P2X7 receptor involvement. In dystrophic mice in vivo treatment with a P2X7 antagonist reduced the number of degeneration-regeneration cycles in mdx skeletal muscles. Altered P2X7 expression and function is thus an important feature in dystrophic mdx muscle and treatments aiming to inhibit P2X7 receptor might slow the progression of the disease.

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Neuroendocrinology

42 The Neuropeptide 26RFa is Expressed in Human Prostate Cancer and Stimulates the Neuroendocrine Differentiation and the Migration of Androgeno-Independent Prostate Cancer Cells

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Neuroendocrine differentiation in prostate cancer is reported to correlate with tumor malignancy, loss of androgen sensitivity, autocrine/paracrine activity and poor prognosis. Accumulating data suggest that neuropeptides produced by neuroendocrine cells play a crucial role in the progression and aggressiveness of the CaP in the absence of androgen stimulation. In the present work, we show that the neuropeptide 26RFa and its receptor, GPR103, are present in carcinomatous foci exhibiting a neuroendocrine differentiation and that the number of 26RFa-immunoreactive cancer cells increases with the grade of the CaP.
The in vitro studies reveal that 26RFa is expressed in the androgeno-dependent (AD) CaP cell line LNCaP as well as in the two androgeno-independent (AI) CaP cell lines DU145 and PC3 at similar levels. In contrast, GPR103 is only detected in the DU145 cells. Induction of a neuroendocrine differentiation in the three cell lines by db-cAMP/IBMX does not affect the expression of 26RFa nor GPR103 whatever the cell line considered. We further demonstrate that 26RFa stimulates the migration of native or transdifferentiated AI DU145 cells but has no effect on their proliferation. Finally, we show that 26RFa induces the neuroendocrine differentiation of the DU145 cells as assessed by the occurrence of neurite-like extensions and the increase of the expression of the neuroendocrine marker CgA. Taken together, the present data support the view that 26RFa, produced by neuroendocrine cells may participate to the development of the CaP at the AI state by promoting the neuroendocrine differentiation and migration of surrounding cancer cells via paracrine mechanisms.

Key words: neuropeptide, G protein-coupled receptor, neuroendocrine differentiation, advanced prostate cancer
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43 The accessory protein SET interacts with GnRH receptor and modifies its signaling

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The reproductive function is under the control of the hypothalamic neurohormone Gonadotropin-releasing hormone (GnRH) which activates a G protein coupled receptor (GPCR) in pituitary gonadotrope cells. In mammals, this receptor shows a unique structural feature among the GPCR family members, the lack of a carboxy terminal tail. Consequently, it is not internalized in response to agonist stimulation and is only poorly sensitive to the classical desensitization mechanisms of GPCR. The mechanisms that regulate the signal transfer between GnRH-R and heterotrimeric G proteins still remain unknown. Due to its atypical structure, proteins interactions at the level of receptor intracellular loops (ICL) are likely crucial for its regulation. Our goal was to identify ICL-interacting proteins which have never been characterized for GnRH-R and to elucidate their roles in regulation of GnRH-R signaling efficacy and/or specificity. In a first approach, we looked for protein-protein interaction domain within intracellular domains of GnRH-R. In silico analysis revealed the presence of a potential binding site for the protein SET. SET has been shown to regulate protein phosphorylation or gene transcription and to inhibit the signal transfer between M3 muscarinic receptor and Gq protein. We showed using GST pull down assay that SET interacts directly with the first and third intracellular loops of GnRH-R. Using site-directed mutagenesis, we delineated SET binding sites to a short sequence of basic amino acids within ICL1 (KRKK) and ICL3 (RK). Interestingly, SET binding sites are located next to the heterotrimeric Gs and Gq protein binding sites, suggesting that SET could impact GnRH-R coupling to cAMP and calcium signaling. To test this hypothesis, we first characterized the signaling pathways activated in response to GnRH. For the first time, we demonstrated that GnRH-R is not only able to couple to calcium signaling, but also to cAMP signaling in the gonadotrope □T3-1 cell line. We then assessed the impact of SET on GnRH-R signaling by decreasing its expression with small interfering RNA strategy. We showed that SET knockdown decreased receptor-mediated mobilization of intracellular cAMP by ~47% and increased receptor-mediated mobilization of intracellular calcium by ~44% in □T3-1 cells. Importantly, SET knockdown had no effect on the mobilization of intracellular cAMP and calcium by PACAP and oxytocin receptors, respectively, suggesting that SET selectively influences GnRH-R signaling via G-protein coupling. Overall, our results strongly suggest that docking of SET to intracellular domains of GnRH-R contributes to a switch of GnRH-R coupling from calcium to cAMP signaling.

44 Hypothalamic Tanycytes Are Direct Target for Leptin

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The arcuate nucleus of the hypothalamus (ARH) is a critical component of the neural circuits that regulate energy balance. However, little is known about how peripheral signals reach the ARH to mediate their central effects. The ARH is adjacent to the median eminence (ME), where highly specialized ependymal cells called tanycytes are found. The cell bodies of tanycytes are lining the floor of the third ventricle and their end feet are contacting the rich capillary plexus with fenestrated endothelium of the ME. Tanycytes have recently been proposed to play a role in blood-hypothalamus barrier regulation. Their privileged location at the interface between the blood and the brain suggests that these cells might be direct target for a variety of peripheral signals, including the adipocyte-derived hormone leptin. The understanding of leptin transport mechanisms is fundamental as it may provide new insights into cellular processes involved in leptin resistance linked to obesity.
In the present study, we hypothesized that tanycytes of the median eminence are direct target for leptin .
We first investigated whether tanycytes express leptin receptors in vitro. RT-PCR analyses indicated that isolated tanycytes express most isoforms of the leptin receptor. Consistent with these observations, western blot experiments showed that cultured tanycytes respond directly to leptin because leptin exposure of tanycyte with leptin in vitro results in increased phosphorylation of key leptin signaling pathways (STAT3, ERK1/2, and Akt). We are confirming these findings in vivo, and studying whether this putative tanycyte-leptin sensing is altered in animals that developed hypothalamic resistance to metabolic hormones.
All together this data may provide valuable information in the understanding of central leptin transport and may help to explain mechanism underlying obesity-associated leptin resistance.

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45 Nitric Oxide Neurons in the Preoptic Area of the Hypothalamus are a Direct Target of Leptin: Implications for the Reproductive Axis

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Reproduction in mammals requires adequate energy availability to enter into puberty and for ongoing fertility during adulthood. Leptin, an adipocyte-derived hormone, provides information about peripheral energy stores to the central reproductive axis, yet its site(s) of action remains unclear. Mapping of the leptin receptor (LepRb) in the mouse brain shows expression within the preoptic area (POA) of the hypothalamus, the region where gonadotropin releasing hormone (GnRH) neurons reside. While leptin does not act directly on GnRH neurons themselves, it is possible that neurons within the POA act to relay information provided by leptin to GnRH neurons. A recent study has shown that nitric oxide (NO) neurons throughout the hypothalamus respond to leptin treatment with phosphorylation of STAT3. Interestingly, administration of exogenous leptin during fasting conditions has the ability to phosphorylate neuronal NO synthase (nNOS), the enzyme required to form NO. In the present study, we pursued the influence of leptin on NO neurons within the POA. We first repeated the demonstration that leptin induces pSTAT3 in nNOS neurons. We next sought to examine whether leptin can induce the phosphorylation of nNOS (pnNOS) and the potential kinetics of leptin-induced activation of nNOS within the POA. Indeed, leptin increases levels of pnNOS at 15minutes post injection, which is also correlated with an increase of luteinizing hormone levels. Immunofluorescent analyses show an increase in pnNOS at the level of the OVLT/MEPO. Intriguingly, leptin induces pSTAT3 activation only 45 minutes post injection, while pnNOS is activated as early as 15 minutes post injection, suggesting that STAT3 is not the primary pathway through which leptin stimulates pnNOS. This is, however, in contrast to the activation of pAKT, which is seen as early as 15 minutes following leptin injection, providing a tentative link between the AKT pathway and leptin-induced pnNOS within the POA. Together these data show that leptin has the ability to stimulate pnNOS neurons during diestrous within the POA and the activation of nNOS potentially involves the AKT pathway.

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46 Functional characterization of a LFRFamide receptor and its ligand in the Pacific oyster Crassostrea gigas

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The pacific oyster Crassostrea gigas exhibits an annual cycle of reproduction. The regulation of this cycle requires the integration of multiple outdoor signals leading to the secretion of (neuro)hormones such as the neuropeptide Y (NPY). This neuropeptide is involved in the coordination of energy flows in relation with food intake and reproduction in various animal models.
Like most neuropeptide hormones, NPY binds receptors of the G protein-coupled receptor (GPCR) family. In silico screening of the “GigasDatabase”, a specific databank containing up to 80 000 independent sequences from C. gigas resulted in identification of 5 distinct GPCRs related to NPY receptors. Further phylogenetic and molecular studies identified one of them as the likely oyster NPY/F receptor. To determine whether C. gigas NPY/F-related neuropeptide actually represents the cognate ligand of this receptor, a reverse endocrinology approach was undertaken. As C. gigas NPY/F belongs to the RF-amide family of neuropeptides, a set of RF-amide neuropeptides expressed in the oyster were also tested. Although Cg-NPY/F did not activate the receptor at any concentration, two peptides expressed from the same precursor, GALFRFamide and GSLFRFamide, activated the receptor in a dose-dependent manner.
Both receptor and LFRFamide peptide precursor were essentially expressed in the central nervous system and to lower levels in other tissues. In contrast, LFRFamide peptides were widely distributed in various tissues.
The receptor was more expressed in female ganglia than in male ganglia, and in fasted animals compared to fed animals. In the gonad area, highest expression of the receptor was found at stage 1, when storage tissue activity is maximal. The possibility that LFRFamide pathway could play a role in energy storage or energy metabolism, maybe in promoting storage at the expense of reproduction in C.gigas is suggested.

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47 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. In order to further elucidate the role of urotensinergic system at the central level, we performed binding experiments on tissue slices to establish the first complete distribution of 125I-UII and 125I-URP binding sites in the adult rat brain. As compared to the UTR gene expression pattern (Jégou et al., 2006), it appeared that UII- and URP-binding sites have a more restricted distribution, identical for the two peptides. In addition to regions already described by others, we detected labeling in a limited number of other areas which are mainly the central amygdaloid nucleus and the subfornical organ of the forebrain, the midbrain sphenoid nucleus and discrete zones of the wall of the fourth ventricle (4V). UII/URP are known as vasoactive peptides acting at the interface between physiological fluids and tissue. In addition, other data have revealed the presence of both UII and URP in fish cerebrospinal fluid contacting neurons (CSF-like contacting neurons) lining the central canal of spinal cord and the 4V (Yulis and Lederis, 1988). Hence, we focused on binding sites detected in the wall of the 4V and in the sphenoid nucleus located below this ventricle aperture. With an immunohistochemical approach following intracerebro-ventricular injection of each peptide, we observed c-Fos activation in these two regions, thus showing that they contain functional receptors. The use of double-labeling, performed with c-Fos and NeuN antibodies, ruled out the possibility that in the rat brain UTR could be expressed in CSF-contacting neurons at the 4V border, as UII and URP in fish. Moreover, c-Fos positive cells were detected with antibodies directed against two cytoskeletal proteins, vimentin which is expressed by most cells lining the 4V and glial fibrillary acidic protein (GFAP) whose distribution is much more restricted in that region. In conclusion, we provide evidence for the existence of a few additional regions containing functional UII and URP receptors in rat brain. The presence of UTR in a subpopulation of vimentin+/GFAP+ ependymal cells suggests a distant action of these peptides in the brain, via the CSF.

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48 Composition of the migratory mass during development of the olfactory nerve and migration of gonadotropin hormone-releasing hormone neurons in early human embryos

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Gonadotropin hormone-releasing hormone (GnRH) neurons are essential for mammalian reproduction as they control the pituitary-gonadal axis. Embryological studies in various vertebrate species have shown that these cells migrate from the medial part of the olfactory placode up to the forebrain in association with the olfactory axon nerve. In mice, the coalition of both placode-derived migratory cells and olfactory sensory neurons is collectively termed “migratory mass’ (MM) and it has been suggested that they may play an important role in the establishment of the olfactory axonal scaffold and subsequently in the migration of GnRH neurons. This study examined the formation of the MM in early human embryos. We found that, at 48 days of gestation, a subpopulation of neurons emerge from the medial olfactory placode, preceding the emergence of olfactory axons. At this stage, only a few isolated olfactory sensory axons are detectable within the nasal mesenchyme, whereas the migratory mass is largely cellular. At 63 days of gestation, the MM is still present and robustly stained GnRH neurons are located mostly in the nasal region associated to NCAM-positive fibers. We next studied the distribution and the number of GnRH neurons at different embryological stages, during the entire migratory process. The data reported here begin to establish a spatiotemporal framework for the migration of molecularly heterogeneous placode-derived cells and provide new insights into the distribution and number of GnRH neurons throughout their migratory pathway in early human fetuses, demonstrating the genesis of a much greater number of GnRH neurons than previously thought.

49 Does Chromogranin A induce the recruitment of Myosin 1b to promote the biogenesis of secretory granules in neuroendocrine cells?

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Neuroendocrine cells produce and store neurohormones and neurotransmittors in dense-core secretory granules (SG). These organelles are generated by budding from the trans-Golgi network (TGN). At this level, soluble proteins named chromogranins aggregate with hormones, and these aggregates interact with the TGN membrane to induce the biogenesis of SG. Then, these organelles are routed along microtubules to reach actin cortex, where they are docked until their Ca2+-induced exocytosis. This process of hormone and neuropeptide release constitutes the regulated secretory pathway of neuroendocrine cells. In a previous study, we demonstrated that the expression of chromogranin A (CgA) in non-endocrine COS7 cells induces the biogenesis of vesicles with structural and dynamic characteristics of SG, and are able to release co-expressed hormones in a Ca2+-dependent manner (Montero-Hadjadje et al., 2009). To identify the mechanism of action of CgA, the proteome of purified granules from CgA-stably expressing COS7 cells (COS7-CgA) was analyzed, showing the occurrence of several cytoskeleton-associated proteins. Among these, we focus our attention on myosin 1b because this motor protein has been recently demonstrated to trigger the budding of post-Golgi carriers in HeLa cells (Almeida et al., 2011). Then, using Western blotting experiments, we demonstrated that myosin 1b is expressed in COS7 cells, as well as in COS7-CgA and corticotrope AtT20 cells. Immunofluorescence studies revealed that myosin 1b is mainly associated to the Golgi apparatus and to the plasma membrane of COS7 cells, while the motor protein co-localized with CgA-containing secretory granules in the cytosol of COS7-CgA and AtT20 cells. Interestingly, after cell stimulation, myosin 1b distribution follows that of CgA to the plasma membrane due to SG exocytosis. The inhibition of myosin 1b expression in COS7-CgA and AtT20 cells would inform us on the relevance of the role of this actin-binding protein in the biogenesis of CgA-induced secretory granules.

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50 Central and peripheral ghrelin expression in methotrexate-induced anorexia and anxiety- and depression-like behavior

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Background and aims. Peripheral acylated ghrelin has orexigenic effects, but its expression in the brain and roles of central ghrelin in feeding and anxiety are uncertain. Here we studied if peripheral and central ghrelin expressions are altered in chemotherapy-associated anorexia.
Methods. Preproghrelin and ghrelin O-acyltransferase (GOAT) mRNA were assayed in the stomach, hypothalamus and amygdala and plasma acyl- and des-acyl-ghrelin were measured in Sprague-Dawley rats treated with methotrexate (MTX, 2.5 mg/kg, SC for 3 days). Forced-swim (FST) and elevated plus maze (EPM) tests and tissues sampling were performed at day 5 after 1st MTX injection, corresponding to the maximal decrease in food and water intakes. Control rats received PBS; the EPM test was done in a separate series of rats.
Results. Preproghrelin mRNA expression levels were lower in the stomach but increased in the hypothalamus of MTX rats. GOAT mRNA was decreased in the stomach and amygdala but not in the hypothalamus of MTX rats. Plasma levels of acyl- and des-acyl ghrelin did not differ significantly from controls but acyl-/des-acyl ghrelin ratios was lower in MTX rats. No significant differences in FST were observed between MTX and control rats, but fewer entries in open and central zone of the EPM were found in MTX rats.
Conclusions. These data show that the hypothalamic preproghrelin gene expression is increased during MTX-induced anorexia. Considering activation of hypothalamic neurosecretory vasopressin neurons in MTX-treated rats (Hamze-Sinno M. et al Physiol. & Behav. 2010), and immunohistochemical detection of ghrelin in magnocellular neurons (own results), these data suggest that both acyl- and des-acyl-ghrelin can be secreted from the hypothalamus into the systemic circulation in order to compensate loss of gastric ghrelin and/or to counteract dehydration. However, the lower rates of acylation of systemic ghrelin in MTX rats may contribute to anorexia-cachexia and increased anxiety.

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51 Fasting induces acute and reversible reorganization of the tanycytic barrier in the metabolic brain via VEGF signaling pathway activation

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The arcuate nucleus of the hypothalamus (ARH) is a critical component of the neural circuits that regulate energy balance in response to peripheral hormones. This requires a dialogue with the periphery which seems to be facilitated by the median eminence (ME). Indeed, the ME is a circumventricular organ -located next to the ARH- characterized by numerous fenestrated vessels, making it a direct target for blood-borne molecules. However, the blood-brain-barrier properties would be shifted to specialized ME ependymal cells -called tanycytes- which express organized tight junction proteins. In the present study, we investigated whether energy imbalance induced by a fast of 24h alters blood/brain exchanges within the ME/ARH by studying both fenestrated vessels and tanycytes tight junction complexes. 24-h fasting in adult male mice induced a significant increase in the number of permeable capillary loops within the ME, when compared to controls. Small and long fenestrated capillary loops were visualized respectively in the ventral and lateral part of the ME in fasted animals. Remarkably, some of the lateral permeable microvessel loops reached the ARH directly. This effect was accompanied by a redistribution of tight junction proteins in ARH tanycytes. In fed conditions they exhibited disorganized apical expression pattern of occludin and ZO1, while these molecules organized as a continuous belt around the cell body of arcuate tanycytes in fasted animals. Importantly, these effects were reversible by 24-hours refeeding, by intraperitoneal injections of VEGFR inhibitor (Axitinib (25 mg/kg), or specific neutralizing antibodies against each VEGFR (40 mg/kg)) or by a rescue of glucose during the fast. Moreover, these effects could be induced in fed mice with VEGF injection (120 µg/kg) or 2-deoxyglucose (300mg/kg). Finally, permeability studies using intravenous injections of Evans blue dye and the study of food intake behavior showed that these reorganizations have an impact on the access of blood-borne molecules to the ARH. Our data show that energy deficit alters blood/brain exchanges in the “metabolic” brain: it raise the exciting possibility that fasting-induced structural changes in the tuberal region of the hypothalamus facilitate the access of blood born endocrine factors to ARH neurons and that VEGF signaling may be mediating this effect.

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52 Developmental expression and estrogenic regulation of Kiss2 using a transgenic zftg(kiss2-GFP) zebrafish line.

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The recent discoveries of the kisspeptin system and its involvement in reproduction have been major breakthroughs in neuroendocrinology. In zebrafish, two kisspeptins (Kiss1 and Kiss2) and two kisspeptins receptors (GPR54.1 = Kiss1r and GPR54.2 = Kiss2r) are expressed and each of them is encoded by a single gene. Recently, we have fully described the distribution of the kisspeptins and their receptors in the brain of adult zebrafish. Kiss2 expressing neurons are mostly localized in the dorsal and ventral hypothalamus. They project widely in the midbrain and anterior brain in regions expressing the Kiss2r and are likely in contact with GnRH3 fibers. In contrast, Kiss1 expressing neurons are exclusively localized in the habenula, which also expresses the Kiss1r, and project in the interpeduncular and raphe nuclei. These results suggest a gene speciation in zebrafish, with the Kiss2 system involved in reproductive processes and the Kiss1 system in interactions with others processes like food intake or environmental perception. Interestingly, exposure of juvenile zebrafish to estrogens up-regulates the kiss2 gene, suggesting that the Kiss2 system could mediate the neuroendocrine control of reproduction by estrogens. In order to study (i) the kiss2 expression in early development and (ii) the modulation of this expression in response to estrogens, we developed a transgenic zebrafish line, zftg(kiss2-GFP), expressing GFP under the control of the zebrafish kiss2 promoter. This zebrafish transgenic line, validated by immuno-histochemistry, expresses GFP as early as 4 dpf in the hypothalamus, demonstrating that the kiss2 system is functional widely before sexual maturation. Moreover, exposure of zebrafish embryos to estrogens enhances the number of Kiss2 immunoreactive-fibers, suggesting that estrogens modulate the development of kiss2 neurons. Taken together, these results reinforce the view that, in zebrafish, kiss2 is probably linked to the regulation of reproductive processes. Moreover, the zftg(kiss2-GFP) line appears to be a promising tool to study the role of Kiss2 in early development, notably its interaction with the GnRH system, but also its potential disruption by environmental estrogenic compounds.

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53 Mouse models of chronic food restriction: central and peripheral alterations

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Eating disorders, like anorexia nervosa (AN), are a group of complex disorders that currently lack effective pharmacological or other biology target-directed therapy. In AN, the severe chronic malnutrition leads to several endocrine, metabolic, bone and central alterations that can cause long term damages for the individual. To better understand how these alterations occur and modify durably the physiology of the organism, we developed two complementary mouse models of chronic food restriction that mimic most of the alterations observed in AN: separation-based anorexia (SBA) and activity-based anorexia (ABA) using C57Bl/6 female mice of 8 weeks-old (n=6-12 per group). In the SBA protocol a chronic stress is induced by the separation, leading to an increase in energy expenditure. In the ABA protocol, alteration of the activity is involved in the higher energy expenditure. After 15 days of protocol, ABA and SBA mice showed a stable 25% decrease of the body weight with a change in the body mass composition reflected by a significant decrease in the fat mass. The ABA mice displayed hyperactivity along the 24h and the SBA mice reduced their consumption of food. The plasma leptin and insulin levels were respectively undetectable and strongly reduced. Furthermore, alterations in the estrous cycle were also noticed associated with a reduction of the ovary sizes. Finally, in the hypothalamus, our models displayed an accumulation of AgRP in the arcuate nucleus cell bodies. These data reflect central alterations in the mechanisms that regulate food intake and/or energy homeostasis.
The present results show for the first time physiological changes in two food restricted mouse models both at peripheral and central levels.

Keywords: anorexia, mouse models, chronic food restriction, fat mass, central alterations

54 miRNA biogenesis is essential for GnRH system development and fertility.

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Micro-RNAs (miRNAs) constitute a new class of important regulators of gene expression at the post-transcriptional level. Although emerging evidence show that miRNAs are essential for fertility by controlling gonadal development and gametogenesis nothing is known about their role in the central control of reproduction, i.e. the hypothalamic-pituitary-gonadal (HPG) axis.
Here, we investigate the role of miRNAs in the development and function of the gonadotropin hormone–releasing hormone (GnRH) system using conditional knockout mice with a targeted deletion of Dicer, an essential protein for miRNAs biogenesis, in GnRH neurons. Neurons that synthesize the neuropeptide GnRH are the final output of the brain control of reproduction; disruption of GnRH neuronal ontogeny/migration and function causes several human neuroendocrine disorders characterized by hypogonadism and absent or reduced fertility.
The analysis of Dicer conditional knockout mice showed that a lack of miRNAs in GnRH neurons causes defective sexual development resulting in hypogonadotropic-hypogonadism and infertility in both males and females. Male Knockout mice had small testes and incomplete gametogenesis while females never reached puberty. Strikingly, immunofluorescent analyses revealed that this selective deletion of Dicer in GnRH neurons resulted in the loss of GnRH expression in the hypothalamus of adult mice. Even though further analyses are required to determine the causes of this GnRH deficiency, the present study is the first report indicating that miRNAs biogenesis in GnRH neurons is essential for the correct development/ physiology of the GnRH system.

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55 Appearance of the AGRP-system in three mouse models of anorexia/starvation

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The hypothalamic Arcuate nucleus (Arc) is central in the regulation of food intake and energy utilization. This nucleus harbors, among others, two populations of neurons critical for the energy homeostatic processes. One population coexpresses the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-gene related protein (AGRP). The other population coexpresses the anorexigenic cocaine- and amphetamine regulated transcript (CART) and the precursor protein proopiomelanocortin (POMC).
We have used immunohistochemistry (IHC) to compare the appearance of the AGRP system in three mouse models of anorexia/starvation; the anx/anx mouse, the separation- (SBA) and the activity-based anorexia (ABA) models.
The anx/anx mouse is a genetic model of anorexia that is due to a spontaneous, recessive mutation resulting in starvation and emaciation. These mice die around postnatal day (P) 21. Previous studies have shown that the anx/anx mice display an increased number of cell bodies immunoractive (ir) for AGRP in Arc at P21. AGRP-ir cell bodies are very rarely detectable in normal mice without the use of colchicine. In addition, at the same age anx/anx mice display a reduced density of fibers ir for AGRP in Arc and all the projection areas of these neurons, e.g. the paraventricular hypothalamic nucleus (PVN). We recently showed that this reduction is related to hypothalamic degeneration (Nilsson et al., 2011).
The SBA model is based on a protocol combining reduction of food access duration with stress induced by separating mice in cages with small plexiglas partitions, allowing them to see and smell each other, but not to have physical contact. The protocol results in reduced food intake and body weight. The other protocol used in this study, ABA, is combining food restriction with access to a running wheel. This results in extreme physical activity, reduced body weight and food intake. Both the SBA and ABA mice used here were exposed to a two weeks protocol. When performing immunohistochemical staining for AGRP in Arc and PVN, we saw the same increase in AGRP-ir cell bodies in Arc of the SBA and ABA mice as in the anx/anx mice. In contrast, in ABA and SBA-mice we did not detect any reduction in AGRP-ir fibers in Arc or PVN. These results are in agreement with previous studies in other anorectic/starving mice, e.g. during 24h food deprivation and Contactin-KO mice (Fetissov et al. 2005).

56 The organization of two kisspeptin systems in zebrafish and sea bass brain shows evident species specific differences.

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Kisspeptins are fascinating actors in the neuroendocrine regulation of reproduction. In vertebrates, the number of kiss genes varies from none to three. This study aims to characterize kisspeptin systems in two fish species commonly used for reproductive studies: a freshwater Cypriniform, the zebrafish (Danio rerio) and marine Perciform fish, the European sea bass (Dicentrarchus labrax). Both fish have two kiss genes, kiss1 and kiss2, and two kiss receptors (GPR54 or Kissr), kiss1r and kiss2r. To elucidate the organization of kiss systems in zebrafish, antibodies were raised against the zebrafish preproKiss1 and preproKiss2 sequences. Immunohistochemical findings were fully confirmed by in situ hybridization data. Kiss1-expressing neurons are exclusively located in the habenula in zebrafish and sea bass, exactly where kiss1r mRNA-containing cells are also detected. During the breeding season sea bass shows an additional kiss1 population into the mediobasal hypothalamus, where kiss2-containing cells are shown in zebrafish. Nevertheless, the main kiss2 mRNA-positive population is observed in both species in the dorsal hypothalamus and in the preoptic area. Immunohistochemistry reveals that kiss2-expressing cells in zebrafish project widely into the forebrain and midbrain. These regions also strongly expressed the kiss2r mRNA in zebrafish, as well as in sea bass. Moreover, in both species kiss2 fibers or kiss2r-expressing cells of the preoptic region make close appositions with the respective hypophysiotrophic GnRH neurons of each species (GnRH3 in zebrafish and GnRH1 in sea bass). Kiss2 populations of the ventral and caudal hypothalamus are estrogen sensitive in juvenile zebrafish, whereas in sea bass it is the kiss1 population of the mediobasal hypothalamus that expresses ERα and slightly ERβ2. Furthermore, in this latter species during the breeding season, a strong kiss1 expression is observed in the pituitary FSHβ immunopositive cells. Altogether our results suggest that kiss1 in sea bass and kiss2 in zebrafish could participate in the regulation of reproduction through the hypothalamic kiss population sensitive to estrogens, proving that kisspeptin systems show evident species specific differences.

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57 Early life adversity and serotonin transporter gene variation interact to shape the adult hypothalamo-pituitary-adrenal axis

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Based on the recently postulated "three-hit concept", it is hypothesized that a genetic factor, the polymorphic region in the promotor of the serotonin transporter (SERT) gene, will interact with early life adversity (hit 1 x hit 2) to alter the brain's neurocircuitry that is involved in the stress response; the resulting altered stress sensitivity can predispose an individual to depression when exposed to hit 3: a psychological stressor in later life. It has been established that a polymorphism in the human SERT gene modulates the influence of early life adversity on the occurrence of depression. To elucidate the neurobiological basis underlying this gene x environment interaction, we used the SERT heterozygous knockout (SERT+/-) rat to model the polymorphism in the SERT gene, while the frequently used maternal separation (MS) paradigm was our model of choice for early life adversity.
Our results show independent effects of MS on maternal care behaviour, and of SERT genotype and MS on postweaning body weight. For the expression of key components in the adult hypothalamo-pituitary-adrenal (HPA-) axis, which is crucially involved in stress adaptation, we found strong gene x environment interactions at the level of the adrenal gland, where opposite effects of SERT genotype were found in the MS versus control groups, with respect to the mRNA levels of the adrenocorticotropic hormone receptor and 3β-hydroxysteroid dehydrogenase.
We expect that the interaction between MS and SERT gene variation will also affect the dynamics of members of the corticotropin-releasing factor (CRF) family of neuropeptides (e.g. CRF and urocortins) in brain areas that modulate the activity of the HPA-axis. This hypothesis is currently tested by immunohisto-chemistry and in situ hybridization

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58 Effects of progesterone on neurogenesis in the brain of adult zebrafish

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Progesterone and progesterone derivatives have been shown to contribute to neurodevelopment and neuroprotection in mammals, suggesting a possible role for these compounds in the neurogenic activity of other vertebrates. The teleost brain provides an intriguing model for studying the role of progesterone in neurogenesis, as the capacity for widespread neuronal proliferation persists into adulthood in these species. This unique trait among vertebrates can be attributed to the presence of radial glial cells, which act as neural progenitors, throughout the teleost lifespan. It has been previously demonstrated that the estrogen-synthesizing enzyme aromatase B is only expressed in radial glial cells, where it exhibits high activity levels. Interestingly, nuclear progesterone receptors (nPRs) were recently shown to be more strongly expressed in radial glial cells than in neurons and upregulation of these receptors was found to be mediated by estrogens. Due to the identification of radial glial cells as preferential targets for progestagens and the role of these compounds in neurodevelopment, this study sought to identify the effects of progesterone on neurogenic activity in adult zebrafish. Immunohistological processing for proliferating cell nuclear antigen (PCNA) was performed on brain tissue of adult zebrafish exposed to progesterone (P4; 10-6 M) or an ethanol control (EtOH) for 48 or 100 hours. Our preliminary data indicate that 48 hours of P4 treatment significantly decreases cell proliferation in the olfactory bulbs (OB), but leads to an increase in the number of proliferating cells in the ventral periventricular pretectal nucleus (PPv). These results demonstrate regional dependency of proliferative activity in response to P4 treatment in adult zebrafish.

Key Words: progesterone, neurogenesis, zebrafish, radial glial cells, proliferation

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Neuropathologies

59 Distinct hyperexcitablity mechanisms promote a switch from Fast ripples to epileptic spikes

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In drug-resistant partial epilepsies, interictal events such as high frequency oscillations (Fast-ripple, FR) and epileptic spikes (IES) represent clinically relevant biomarkers, characteristic of the underlying epileptogenic networks, generally admitted to be produced by synchronous hyperexcitable cells. However, the precise neurophysiological mechanisms leading to either FR or IES, and the functional link between these two interictal events remain unclear. Moreover, the relationship between FR, IES and seizures is still a matter of a debate. Methods. Our computational model of CA1 hippocampal network (cellular level, including main pyramidal cells and interneurons) targeted by CA3 pcs was used to simulate realistic FRs and IESs as compared to our in vivo data. Results. Overall, results showed that quasi-synchronous discharge patterns of pyramidal cells are a common feature of these two events, but subtle balance changes of altered GABAergic and Glutamatergic synaptic transmission at network level could lead either to FRs or IES. In addition, differences could be revealed in terms of spatial features of hyperexcitability (uniformly vs. clustered increased excitability), the number of hyperexcitable cells involved, drift of GABA reversal potential, as well as the degree of synchronicity of pyramidal cells. The model could also predict an influence of the electrode size, which can impact the recording of FRs. Conclusion. Overall we suggest that the degree of alteration of the synaptic transmission, space and time feature of the hyperexcitability, is responsible for the progressive switch from FR to IES and finally to seizure.

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60 Significance of diaschisis in functional deficit and recovery following focal stroke in the non-human primate

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Introduction: The significance of diaschisis, defined as reductions in brain metabolism in remote regions after focal stroke, in functional deficits and their recovery is not well understood. Here, we addressed this issue using longitudinal positron emission tomography (PET) investigations and behavioural tests in a model of transient ischemia in the non-human primate.
Methods: Three marmosets were subjected to 3 hour transient middle cerebral artery occlusion (MCAO) (Bihel et al., 2010), and two were sham-operated. Each animal underwent 3 sessions of MRI at 60min, 7days and 42days following MCAO. Each MRI examination was followed by a PET scan with [18F]-FDG to measure glucose consumption (CMRglu). During 42days a battery of behavioral tests were performed weekly to analyse sensorimotor deficits.
Results: In sham-operated animals, CMRglu values were similar to those reported in human's brain (25-35 µmol/100g/min in the cortex). In animals subjected to ischemia, the MRI-defined lesion at the acute and subacute stages affected the striatum and in a lesser extent the cortex. At the chronic stage, no apparent lesion was visible on DWI and T2-MRI.
As early as 2h following the occlusion, a decrease in CMRglu was observed in the ipsilateral thalamus and substantia nigra (-13.9±4.3% and -17.4±3.8%, versus the contralateral structures, respectively) and the contralateral cerebellum (-9.1±15.1%, versus ipsilateral one). In the subacute stage (7 days), these remote alterations of CMRGlu were more pronounced (-16.8±2.1; -22.1±5.6; -13.1±4.6, respectively in the ipsilateral thalamus, the ipsilateral SN and the contralateral cerebellum). At the chronic stage, there was an increase of CMRGlu in the initially lesioned zone (+37.7±9.9% in the striatum) and a complete resolution of reduced GMRGlu in the remote areas. Transient focal stroke in the marmoset induced several sensorimotor deficits that recovered progressively but only partially. Several significant correlations were found between the subacute diaschisis and the functional deficits assessed at 1 week following the occlusion (e.g. thalamic diaschisis and hemianesthesia R2=0.73). Moreover, subacute diaschisis was also correlated with the chronic deficits assessed at 6 weeks following the occlusion (e.g. thalamic diaschisis and hemianesthesia R2=0.96).
Conclusions: The values of brain glucose consumption in the marmosets are similar to those measured in the human brain. The data also suggest that the severity of functional deficits and the recovery after focal cerebral ischemia could be related to the magnitude of diaschisis
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61 Local delivery of PACAP improves functional recovery after brain stroke in mice

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Despite intense research efforts, cerebral stroke remains the third leading cause of death and the first cause of long term disabilities, in industrialized countries. Several studies highlighted the neuroprotective effect of the neuropeptide Pituitary adenylate cyclase-activating polypeptide (PACAP) in brain ischemia models, based on its neurotrophic, anti-apoptotic and anti-inflammatory properties. However, the clinical use of PACAP is compromised by a very short half-life and the difficulty to reach the ischemic area where the vascularisation is disrupted. In this study, we propose a combined strategy using embryonic stem cell (ES) to locally deliver the neuropeptide PACAP in the infarct area. Thus, we have established a PACAP-over expressing ES cell line (ES-P cells) and assessed the therapeutic potential of the targeted delivery of PACAP by these cells, in a mouse model of focal permanent cerebral ischemia.
Our results show that the local delivery of PACAP improves functional recovery, one and two weeks after brain stroke. More precisely, mice transplanted with ES-P cells present reduced Neurological Severity Score (NSS) and reduced motor coordination deficit in the hole board test, compared to mice transplanted with wild-type ES cells or injected with Saline. Interestingly, the functional benefits are correlated with the modulation of local inflammatory response. Indeed, we report a strong decrease of the neurotoxic, pro-inflammatory TNF-□cytokine production. In parallel, we find a significant increase of the IL-10 anti-inflammatory cytokine and the neuroprotective protein Ym1 levels.
Taken together, these data suggest that the neuropeptide PACAP could exert a local neuroprotective effect by skewing the local inflammatory response toward a neuroprotective phenotype.

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Key-words: Cerebral ischemia, PACAP, Embryonic stem cells, Inflammation.

62 Longitudinal Follow-up of Glutamine and Glucose by MRS 1H single voxel in 20 treated patients with Gliomatosis or Oligodendroglioma

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Magnetic Resonance Spectroscopy (MRS) allows to measure metabolites, and their concentration in a biological tissue. Variations of these metabolites allow characterising some pathology and their processes. The aim of this study is to follow longitudinally by MRI and MRS 1H glutamine and glucose during 5 years in 20 patients with low grade gliomas (gliomatosis or oligodendroglioma) treated. Despite data are not significant, we can observe some results. 3 patients have glutamine and area in tumour side is higher than in contra lateral side. In follow-up, 4 patients have glutamine which decreases under treatments. 2 patients have potentially glucose and area in tumour side is higher than in contra lateral side. In follow-up, 3 patients have potential glucose which decreases under treatments. Glutamine and glucose are two essential nutriments to maintain energy and metabolism of tumoral cells. First results show a tendancy of an increase of glutamine and glucose that could be a biomarker of pathological glial activity. In addition, these metabolites seem to decrease under treatments in patients who have stable clinical parameters. These variations, not present in all patients, could correspond to good answer to treatments and show particular profile and different metabolite evolutions of these glioma. Longitudinal study of these key metbolites of tumoral process by MRS could give additional information in interpretation of spectrum. In relationship with standard classification (Cho, Cr, NAA, lipids, lactate), they could support other prognostic factors in assessment of gliomas and in early therapies assessment.

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63 Complementary information from magnetic resonance imaging and 18F-fluoromisonidazole positron emission tomography in the assessment of the response to an antiangiogenic treatment in a rat brain tumor model

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The aim of the present study was to analyse, using MRI and PET imaging in Day 17 and 24 after inoculation, the effects of an anti-angiogenic treatment (Sunitinib) on tumor growth (C6 glioma), vasculature and hypoxia. Rat received Sunitinib orally from Day17 to Day24 daily (20mg/Kg). T2 and T2* maps were performed using MRI 7 teslas magnet (Bruker) prior and after an intravenous injection of Sinerem (200µmol/kg; Guerbet SA) to compute CBV and VSI maps (1). Hypoxia detection was performed using a microPET imaging (Inveon, Siemens) with injection of 18F-FMISO (600µCi/rat). Our results show the efficiency of the anti-angiogenic treatment with a decrease in tumor volume by 51 % in the Sunitinib group as compared to the Control group ($p<0.01$). Along with this anti-tumor effect, we observe an increase in CBV (Control : 4.6 ± 0.7%; Sunitinib : 5.9 ± 1.03 %; $p<0.05$) and VSI ($\Delta R2^*/\Delta R2$; Control : 1.13 ± 0.13 ; Sunitinib : 1.22 ± 0.14; $p<0.05$) but also a reduction of hypoxia (Mean = Control : 1787 ± 348 nCi/cc, Sunitinib : 1512 ± 134 nCi/cc; Max = Control : 3134 ± 1099 nCi/cc, Sunitinib : 2181 ± 414 nCi/cc; $p<0.05$) detected following the Sunitinib treatment. Using both MRI and PET imaging, we present data demonstrating a vascular normalization following an anti-angiogenic treatment in a rat glioma model. We are currently trying to elucidate mechanisms associated with these vascular effects which may reflect a better vascular supply (high CBV, low hypoxia) paradoxically to a slowdown of tumor growth. Authors thank Guerbet Recherche for providing contrast agents. This work was supported by INCa-Institut Lilly, CNRS, the French Ministère de l'Enseignement Supérieur et de la Recherche and TC2N « Trans Channel Neuroscience Network ».

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64 Immunization with the CD8-specific epitope of myelin oligodendrocyte glycoprotein induces a mild form of experimental autoimmune encephalomyelitis.

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Multiple sclerosis (MS) is an autoimmune, demyelinating and degenerative disease of the central nervous system (CNS). Anti-myelin CD4 T cells are strongly associated with disease development in several animal models of MS such as experimental autoimmune encephalomyelitis (EAE). However, CD8 T cells often outnumber CD4 T cells in the CNS parenchyma of MS patients and recent studies suggest that anti-myelin CD8 T cells may be also implicated. In order to better understand the contribution of pathogenic CD8 T cells, C57Bl/6 female mice were immunized with a recently described epitope of myelin oligodendrocyte protein (MOG37-46) specifically presented by MHC-I to CD8 T cells. Only, one third of the mice immunized with MOG37-46 developed EAE with mild clinical signs. In contrast, all mice immunized with MOG35-55 developed hindlimb paralysis, as expected for this classical EAE model. Proliferation and FACS analysis of T cell reactivity using splenocytes isolated from MOG37-46-immunized mice confirmed that immunization led to the emergence of specific MOG-reactive CD8 T cells in vivo. Yet, the presence of this T cell autoreactivity was not necessary correlated with disease development. Moreover, immunohistochemical analysis of the spinal cord, cerebellum and optic nerve in mice that developed the first clinical sign (clasping reflex or tail weakness) indicates that CD8 T cells infiltrated the white matter of the central nervous system (CNS). Strikingly, the CD8 T cell infiltration was weak compared with CD4 T cells in the same lesions. Further characterization of the CNS lesions in this EAE model is underway. Taken together, these data indicate that a CD8 epitope in the MOG sequence can initiate mild EAE in a subset of mice unlike MOG35-55 that induces CD4 T cell autoreactivity and severe EAE in all mice. The fact that the development of anti-MOG CD8 T cells is not systematically associated with EAE symptoms suggest that anti-myelin CD8 T cells are insufficient to trigger sustained disease in mice. Further work is needed to better delineate the fate of autoreactive CD8 vs. CD4 T cells in the CNS during autoimmune neuroinflammation.

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65 Tissue-type Plasminogen Activator (tPA) promotes long term functional recovery after compressive spinal cord injury in rats

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KThe establishment of glial scar in response to central nervous system injuries creates a dense physical barrier around the injury as well as a chemical barrier due to the release by reactive astrocytes of chondroitin sulfate proteoglycans (CSPG) that are growth inhibitory molecules. This inhibitory environment mainly contributes to the failure of injured axons to regenerate leading to persistent functional impairments. In addition of the liberation of CSPG, reactive astrocytes synthesize and release PAI-1 (Plasminogen Activator Inhibitor type 1), the main inhibitor of tPA, into the extracellular space where it inhibits the proteolytic activity of tPA. Thus we hypothesize that the tPA/PAI-1 axis may have an influence on secondary damages occurring after SCI, in particular on axonal regeneration. In order to determine the role of tPA in the physiopathology of SCI, we use a rat model of spinal cord compression followed the next day by a single injection of tPA directly in the injured spinal cord. Our functional data obtained by using various tests evidence that tPA leads to long term recovery of the bladder and motor functions of the treated rats. In parallel, molecular analysis reveals a decrease in CSPG-neurocan at the lesion site of tPA-treated injured rats compared to untreated injured rats. Taken together, these results demonstrate a beneficial effect of tPA on functional recovery, presumably due to a better axonal regeneration. Thus, the injection of tPA in the injured spinal cord could represent a new original therapeutic strategy to treat spinal cord injuries.

Keywords: Spinal Cord Injury – tissue-type Plasminogen Activator – functional recovery – axonal regeneration

66 Impact of a new class of cyclin dependent kinase inhibitors, on malignant glioma development

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Incidence of primary brain tumors in adult is about 5/100 000 persons/year. The diversity of biomolecular mechanisms involved in glial tumoral cell proliferation and brain tissue invasion explains disease recur in most patients. Indeed, surgery, radiation and chemotherapy exert limited effectiveness in treatment of high grade gliomas.

It is clearly established that cyclin-dependent kinases (CDK) hyperactivity is one of the processes underlying hyperproliferation and tumoral growth. CDK inhibitors (CDKI) present an antiproliferative activity associated to cytotoxic effects. In order to improve selectivity and efficiency of these CDKI, a series of hybrid compounds have been synthesizeda,b. In this study, we tested the ability of these new CDKI to inhibit proliferation of human glioma (astrocytoma, SW1088; glioblastoma, U87) and endothelial cell lines.

Our in vitro approach showed that among 36 compounds tested, 19 were efficient in a concentration range of 10-9 to 10-6 M and two were particularly potent with nanomolar IC50 values. Flow cytometry studies measuring mitochondrial membrane potential, increased that CDKI-A and B exhibited pro-apoptotic activities on SW1088 and U87 cells. Moreover, administrated on glioma cell lines, CDKI-A and B inhibited (50%) the proportion of cells in the S phase of cell cycle whereas the percentage of cells in G2/M phase was stimulated (20%). As one of the characteristic of high grade glioma cells is the capacity to develop a high vasculature to feed the tumor, we tested the impact of CDKI-A and B on the mouse (bEnd3) and the human (HUV-EC-C) endothelial cell lines activities. Our results clearly demonstrate that these compounds drastically blocked the proliferation rate of endothelial cells by arresting cell cycle progression (decrease in S phase) and exhibited pro-apoptotic activities. These last data indicate that the CDKI tested in the present study would represent a potent anti-glioblastoma therapeutic by both inhibiting tumoral glial growth and neoangiogenesis.

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67 Urotensinergic vasoactive peptides as new factors involved in tumor growth and neoangiogenesis of high grade glioma

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Glioblastoma represent in adults the most common primary brain tumors. They are mainly characterized by a tumoral invasiveness of the normal brain tissue, an intense proliferation of endothelial cells (neoangiogenesis), and a high degree of recurrence. The dense vascularization known to be dilated, tortuous, disorganized and leaky, opening a way for specific targeting treatments against tumoral new vessels. Growth factors but also vasoactive neuropeptides have been identified as regulators of recruitment and entrapping of diverse proangiogenic cells, promoting in situ neoangiogenesis. Urotensin II (UII) and its paralog urotensin II-related peptide (URP) are two very potent vasoactive neuropeptides involved in the control of endothelial cell proliferation and migration. Recent data obtained in our team indicate that UII, URP and their receptor UT are expressed in human brain tumors, and that UII exerts potent chemotaxic effects on several human glioblastoma cell lines.
We first investigated the ability of UII and URP to recruit different cell populations known to favor glioma development and neoangiogenesis. We injected in the right flank of C57/Bl6 mice, a synthetic extracellular matrix Matrigel containing urotensinergic peptides UII or URP, vascular endothelial (VEGF) or endothelial (EGF) growth factors (50 ng, each). Our results showed that UII, and in a less extent URP stimulate matrix invasion of macrophages, endothelial cells and vascular smooth muscle cells, as observed for VEGF and EGF. We next investigated the putative role of urotensinergic system on glioma tumorogenesis by using heterotopic xenografts of the human glioblastoma cell line U87 in Nude mice. Tumor growth was followed daily in mice, intratumorally injected with PBS (10 µL), UII (10 µL, 100 ng), the non-peptidic UT inhibitor, urantide (10 µL, 1 µg) or a mixed solution of UII and urantide (10 µL, 100 ng and 1 µg, respectively). We observed that UII drastically accelerated tumor growth, accompanied by an enhanced angiogenesis; as revealed by the dense network of vascular components in UII-treated xenografts. Interestingly, urantide strongly reduced tumor development and significantly prolonged mice survival, and this benefical effect was counteracted by the coadministration with UII. Altogether, these data suggest that UII favors pro-angiogenic cell type recruitment, potentially involved in high grade glioma growth. Therefore, blockade of circulating UII and/or of urotensinergic receptor UT with conventional cytotoxic, radiative and other antiangiogenic therapeutics may improve the efficacy of anti-brain cancer therapies.

68 Characterization of a rat model of global cerebral ischemia: a link between tissue-type plasminogen activator and hippocampal delayed neuronal death?

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Global cerebral ischemia (GCI) is mainly caused by cardiac arrest. With progress in intensive care medicine, more people are expected to be successfully resuscitated. Unfortunately, most of the resuscitated people still suffer from ischemia-related neurological deficits. These deficits are suspected to be triggered by a pathological process known as delayed neuronal death (DND). To fight against this leading cause of morbidity, a good understanding of the mechanisms associated with the DND is needed. To this aim, we have characterized and standardized a GCI model induced by circulatory arrest in rats. In line with recent findings, we investigated the possible involvement of the endogenous serine protease “tissue-type plasminogen activator” (tPA) on delayed neuronal death in the hippocampus.
In this model, the neuronal cell death appears from 3 to 5 days post-GCI in the CA1 layer of the hippocampus. Of note, our study reveals an increase in the fibrinolytic activities of tPA in both plasma and hippocampus 4h post-GCI. In contrast at the different time post-GCI investigated, the transcriptional profile does not show any significant variations. A preliminary study shows that tPA immunoreactivity increases in CA1 hippocampal cells at 24h post-ICG. These results show an increase of tPA activity in plasma and hippocampus before apparition of neuronal death. Further studies (laser microdissection; in situ hybridization; organotypic hippocampal slice cultures …) are however needed to conclude on a link between tPA and DND.

69 Modulation of intracerebral EEG signals from premotor cortical focal dysplasia by thalamic deep brain stimulation: Quantified analysis and mechanisms insights from computational modeling

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Recently, in a patient suffering from partial epilepsy in associated to focal cortical dysplasia in the premotor cortex (PMC), we observed that DBS of the centromedian (CM) thalamic nucleus dramatically reduced the sustained paroxystic activity in the PMC (Pasnicu A. et al. Epilepsia 2010, 51(Suppl. 4) p 24). Visual analysis of depth-EEG recordings showed that some stimulation parameters (frequency and amplitude) could induce a remarkable modulation of the dysplastic pattern whereas others did not affect the recorded signals. This very unique observation led us to quantitatively investigate the mechanisms involved in the modulation of epileptic cortical activity by indirect thalamic stimulation. Our objective is to quantify the time-frequency content of depth-EEG signals recorded in the PMC before, during and after stimulation of the CM, and explain, from a neurophysiological viewpoint, the observed effects and their dependence on stimulation parameters. To quantify the time-frequency content of depth-EEG signals we used the Matching Pursuit method which operates signal decomposition into time-frequency atoms. A feature vector representing the signal energy distribution in predefined frequency bands (delta to gamma) was then built using this decomposition. Feature vectors were computed on segments of depth-EEG activity. They were used to assess the similarities/differences among these segments, as recorded before, during and after stimulation and also for various stimulation parameters. Besides, we introduced a physiologically-relevant computational model of the cortex in order to identify some key parameter configurations allowing for accurate reproduction of observed effects, in particular the suppression of the paroxystic activity in the dysplasia. Signal processing showed a reduction of at least 68% in the dysplastic slow pattern (2-4 Hz) for stimulation at 2, 70, 100 and 150Hz but less than 8% at 50Hz. Moreover, an increase of energy in the β1 band (12-18Hz) was exclusively observed for high frequency stimulations. Computational modeling allowed us to generate hypotheses, at a neuronal population level, on the origin of these stimulation-dependant cortical modulations, as reflected by depth-EEG signals. Main findings include i) the specific behaviour of the principal cells in the CM nucleus in response to stimulation frequency and ii) the subtle role of collateral excitation and feedforward inhibition in the PMC in response to CM input.

70 CEREBROVASCULAR INFLAMMATION IN MICE: HIGHLY SENSITIVE IN VIVO MAGNETIC RESONANCE IMAGING USING MICRO-SIZED PARTICLES OF IRON OXIDE.

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Expression of pro-inflammatory cytokines and cellular adhesion molecules by endothelial cells is an hallmark of numerous neurological pathologies leading to the recruitment of leukocytes that contribute to further brain damages. In order to predict and/or counteract such brain damages, monitoring of the cerebrovascular inflammatory status would be useful. Unfortunately, in vivo attempts to perform molecular magnetic resonance imaging (MRI) of acute cerebrovascular inflammation in clinically relevant experimental models were so far unfruitful. In this study, we aimed at designing contrast agents and imaging protocols that would provide the highest sensitivity to revealed, in vivo, inflammatory processes for common neuropathologies.

We coupled different tools targeting the inflamed endothelium to micro-sized particles of iron oxide and we carefully selected the best targeting moiety in five neuropathological models involving inflammatory processes.

One of our selected contrast agent directed against an adhesion molecule known as Vascular Cell Adhesion Molecule-1 (VCAM-1) allows non-invasive molecular imaging of cerebrovascular inflammation with an outstanding sensitivity concerning intrastriatal injection of Tumor Necrosis Factor (TNF), vascular dementia, ischemic strokes (permanent and transient ischemia) and in a rodent Experimental Autoimmune Encephalomyelitis (EAE) model.

Our study shows that molecular imaging of VCAM-1-targeted paramagnetic microparticles of iron oxide (MPIO) provides sensitive detection of inflammatory areas and interestingly, predicts delayed post-ischemic injuries following acute stroke and presents also a particular interest in the detection of inflammatory status in EAE model which is correlated with functional neuroscore.

We report here the first molecular MRI method allowing highly sensitive detection of acute inflammation in clinically relevant experimental models. Successful translation of this approach to clinical practice could efficiently improve diagnosis accuracy of neurological diseases involving inflammatory processes.

Neuropharmacology

71 Prenatal exposure to ketamine reduces the number of GABAergic interneurons and affects motor behavior of GadGFP mice.

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Introduction: In preterm and full-term neonates, hypoxia-ischemia is the main cause of acquired cerebral palsy. Brain damages are associated with energy deprivation, inflammation and oxidative stress. Among these mechanisms, excitotoxicity, resulting from a massive release of glutamate, constitutes a major deleterious process and several studies reported that glutamate antagonists exert a protective effect against neonate brain lesions. However, a recent manuscript indicates that NMDA antagonists have a injurious action on immature GABAergic interneurons.1 Because ketamine, an anaesthetic routinely used in paediatrics, is a NMDA antagonist, we hypothesized that ketamine could have deleterious effects on cortical immature GABAergic interneurons. The aim of the present study consisted in characterizing the long-term effects of a prenatal ketamine exposure on i) the GABAergic innervation ii) the cortical levels of GABA, GABA transporters GAT-1, GAT-3 and synaptophysin and iii) the motor activity. Materials and Methods: FVB-Tg GadGFP transgenic pregnant mice were anesthetized with ketamine (50 mg/kg) 3 h per day. Ketamine was administrated from GD15 to delivery. Molecular, cellular and behavioural studies were performed at PD45. Results: Morphometric analysis revealed a reduction of the GABAergic dendritic arborisation on layers II-IV in male and female mice exposed to ketamine. At a molecular level, we found an increase of GAT-1 and GAT-3 protein levels in males of the «ketamine» group while no modification on synaptophysin protein levels was found. Quantification of cortical levels of GABA by HPLC showed an increase of GABA amounts in the cortex of males exposed to ketamine while a decrease was found in females. Finally, behavioural studies indicated that the locomotor activity of females from the «ketamine» group was significantly increased. Conclusion: These findings support that prenatal exposure to ketamine affects the cortical GABAergic innervation in both males and females. However, atypic behaviour (hyperlocomotion) was only found in females while an increase of GABA, GAT-1 and GAT-3 levels was only retrieved in males suggesting a gender-dependent compensatory mechanism. This works was supported by the University of Rouen, the French Research Ministry, the Region Haute-Normandie, IFRMP23, Inserm and FEDER.. 1Desfeux et al., Cereb. Cortex, 2010

72 Possible evidence of cerebral endomorphins in anxiety and depressive symptoms

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The identification of biological markers is extremely important way of improving patient's care in psychiatry and contributes to developing new treatment options for anxiety and depression. However, with certain exceptions, truly sensitive and specific markers have not still emerged. Several studies have reported opioid systems dysfunction in pathogenesis of anxiety and depression. Recently we have demonstrated that endomorphin-1 (EM-1) and endomorphin-2 (EM-2), two specific endogenous ligands of μ-opioid receptors, displayed antidepressant-like effect in rodents. It has also been reported that these peptides exert anxiolytic-like action. Based on these data, we have quantified, with both HPLC and mass spectrometry methods, the brain EM-1 and EM-2 levels, in two animal models selected according to their divergences in anxiety and depression tests.
Using different behavioral paradigms, we observed important inter-individual variation of animal in vulnerability to anxiety and depression. From these observations, among the whole population of bred CD1 mice (Charles River, France), we selected : in anxiety tests, high-anxiety-related behavior "anxious, A", low-anxiety-related behavior "non anxious, NA" and intermediate-anxiety-related behavior "intermediate, IA" mice; and in depression, high-helpless-related behavior "helpless, H", low-helpless-related behavior "non helpless, NH" and intermediate-helpless-related behavior "intermediate, IH" mice.
Compared with NA and IA mice, A mice display lower basal brain EM-1 and EM-2 levels, quantified in both HPLC and mass spectrometry methods. Similarly, H mice display lower basal brain EM-1 and EM-2 levels than NH and IH mice.
These results provide the first evidence of cerebral deficiency of both EM-1 and EM-2 levels in physio-pathology of anxiety and depression. Endomorphins could be used as potential biomarkers to diagnose anxiety and depressive disorders.

Keywords: Endomorphin-1, Endomorphin-2, Anxiety, Depression, Animal model
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73 THE EFFECT OF GAMMA HYDROXYBUTYRIC ACID (GHB) AND BACLOFEN ON FOOD AND WATER INTAKE IN RATS

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Gamma hydroxybutyric acid (GHB) is a metabolite of GABA and has been found in binding studies to have agonist characteristics at GABAB receptors (Mathivet et al., 1997 Eur. J. Pharmacol., 321, 67 – 75). We have previously demonstrated that the GABAB receptor agonist baclofen increases food intake in non-deprived rats and decreases water intake in water-deprived rats (see Ebenezer and Patel, 2011. Eur. J. Pharmacol., 653, 58 – 62). It was therefore of interest to examine the effects of baclofen and GHB on food and water intake in rats. Experiment A. Non-deprived male Wistar rats (n=8 in each experiment; b.wt. 300 – 370g) were injected i.p. with either (i) saline or baclofen (1 – 4 mg kg-1) (Expt. 1A), or (ii) saline or GHB (0.5 – 8 mg kg-1) (Expt. 2A), or (iii) saline or GHB (16 – 64 mg kg-1) (Expt. 3A) immediately before being placed singly in separate experimental cages with free access to food and water and cumulative food intake measured. Experiment B. Male Wistar rats (n=8 in each experiment) that had been deprived of water for 16h were injected i.p. with either (i) saline or baclofen (1 – 4 mg kg-1) (Expt. 1B), or (ii) saline or GHB (8 – 32 mg-1) (Expt. 2B) immediately before being placed singly in separate experimental cages with access to water and water intake measured. In all experiments, a repeated measures design was used with each rat receiving all treatments; 3 - 4 days separated successive drug trials. The data were analysed by ANOVA and the Newman-Keuls post-hoc test. Analyses of the results from Experiment A showed that baclofen (1 – 4 mg kg-1) produced a dose-related increase in food intake. However, neither the lower doses (0.5 – 8 mg kg-1) nor the higher doses (16 – 64 mg kg-1) of GHB had any effects on food intake in non-deprived rats. The results from Experiment B showed that baclofen (1 – 4 mg kg-1) significantly decreased water intake in water-deprived rats in a dose-related manner. By contrast, GHB (8 – 32 mg kg-1) had no effects on water intake in water-deprived rats In agreement with previous observations, the results of the present study confirm that the GABAB receptor agonist baclofen increases food intake in non-deprived animals and decreases water-intake in thirsty animals. By contrast, GHB had no effects on food or water intake in rats. These observations suggest that GHB does not stimulate GABAB receptors in vivo to elicit effects on ingestive behaviours.

74 Evaluation of Sunitinib's side effects in physiological conditions in the adult rat.

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It is well known that tumoral vascularization development is a key event for tumor growth. Because VEGF (vascular endothelial growth factor) is a key actor in this phenomenon, molecules targeting this factor or its receptor have been developed as cancer therapies. Sunitinib is an oral multitargeted tyrosine kinase inhibitor with both anti-angiogenic and antitumor activities due to inhibition of various receptor tyrosine kinases, including VEGFR. Sunitinib has been approved for the treatment of renal carcinoma, gastrointestinal stromal tumors, and more recently, sunitinib has been introduced for brain tumors. However, this agent can induce side effects in patients such as hypertension, fatigue, behavioural or mnesic troubles. These cognitive effects might be due to the cerebral role of endogenous VEGF as this angiogenic factor displays neurotrophic and neurogenic effects that can modulate the mnesic and emotional state of patients1. In this context, the aim of this work was to study the impact of a sunitinib treatment on the normal brain. For this purpose, we administered sunitinib to healthy rats (20mg/kg) and evaluated the effect of this treatment on performances at different sensorimotor and cognitive tests such as: spatial (Morris water maze) and emotional (passive avoidance test) long term memory, working memory (spontaneous alternation test) anxiety like behaviour (elevated plus maze), depressive like behaviour (forced swimming test) and sensorimotor capacities (grip test). Moreover, magnetic resonance imaging (MRI) and positons emission tomography (PET) studies were done to evaluate the potential modifications of the cerebral vascularization as well as metabolism. Our results showed no functional perturbation of brain vascularization and metabolism when the rats were treated with sunitinib. In addition, no difference was observed between treated and control groups in behavioural and cognitive tests. However, at this dose, the treatment causes an important weight-loss associated with a low recuperation rate after an acute stress. Our results suggest that in our experimental conditions, sunitinib displays no apparent toxicity on healthy rat brain function.

1 Ruiz de Almodovar et al. (2009) Physiological Reviews 89(2):607-48.

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75 Design and evaluation of the agonistic and antagonistic activities of peptidic analogs of 26RFa

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26RFa, a neuropeptide of the RFamide family, is the endogenous ligand of a former orphan GPCR named GPR103. Both 26RFa and GPR103 are highly expressed in hypothalamic nuclei involved in the control of feeding behavior. Indeed, intracerebroventricular injection of 26RFa or 26RFa(20-26), the strictly conserved C-terminal heptapeptide (GGFSFRF-NH2), stimulates food intake in rodents. In vitro, 26RFa increases [Ca2+]i in GPR103-transfected cells with an EC50 of 10.2 ± 1.1 nM, while 26RFa(20-26) is about 75 times less potent than 26RFa. In order to develop potent and physiologically stable ligands of GPR103 with low molecular weight, we have removed or replaced each amino acid of 26RFa(20-26) by different residues. The peptides [desPhe22]-, [Tic22]-, [Pcp24]-, [Pcp26]-, and [pNO2Phe26]26RFa(20-26) were 2- to 5-fold more potent than 26RFa(20-26). Substitution of Phe22 by a tBuPhe, or Phe26 by a D-Trp led to compounds that inhibited 26RFa-evoked [Ca2+]i increase with an IC50 in the micromolar range. Similarly, asymmetric di-methylation of Arg25 generated the most potent analog, [ADMA25]26RFa(20-26), that reduced the agonistic effect of 26RFa (10-6 M) with an IC50 of 5.1 ± 1.1 µM. These three peptides are the first compounds exhibiting antagonistic activity for GPR103 described so far, even though their potencies are still low. In contrast, mono-methylation of Arg25 enhanced by 11-fold the agonistic activity of 26RFa(20-26). Thus, arginine 25 is a strategic residue for the development of both GPR103 agonists and antagonists with low molecular weight. Of note, [Arg(Me)25]26RFa(20-26) and [ADMA25]26RFa(20-26) are the most potent agonist and antagonist of GPR103, respectively, described so far. These results constitute an important step towards the development of new GPR103 analogs that could prove useful for the treatment of feeding disorders.

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76 Co-modulation of serotonin 5-HT4 and 5-HT6 receptors improves recognition memory and increases hippocampus activation.

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Rationale and objectives: Efficiency of current treatments for neurodegenerative disease, is under huge debate mainly because it often consists in a single targeted drug, having only symptomatic effects. Time is now to find curative treatments. Alzheimer disease, for instance, is multifactorial and new treatments have to consider other targets than the classical acetylcholine and glutamate systems. Serotonin system and particularly 5-HT4 and 5-HT6 serotonergic receptors (5-HT4R and 5-HT6R) are among potent targets of interest. Both are located in brain structures involved in memory processes. Neurochemical and behavioural studies have showed that activation of 5-HT4R and blockade of 5-HT6R improve memory processes. Accordingly, a therapeutic approach combining a simultaneous modulation of these two receptors could be an interesting and innovative strategy in the treatment of memory disorders associated with different physiopathological situations. Herein, we investigated in mice the potent interest of associated 5-HT4R activation and 5-HT6R blockade on episodic-like memory and on c-Fos expression, a marker of neuronal activation.
Methods: Effects of an acute administration of RS 67333 (1mg/kg), a 5-HT4R agonist, and/or of SB-271046 (20 mg/kg), a 5-HT6R antagonist on NMRI mice were evaluated in the novel object recognition test in mice. Thereafter, effects of these two ligands on c-Fos expression in the hippocampus were assessed on mice exposed or not to the behavioural test.
Results: 5-HT4R activation combined to 5-HT6R blockade improved episodic-like memory performances in mice, more than if each ligand is administered alone. Concerning immunohistochemical studies, c-Fos expression tended to be increased in the CA1 field of hippocampus by the two ligands, and particularly by their co-administration.
Conclusion: Although further experiments are needed (analysis of pharmacologic-induced modulation c-Fos expression in other structures are in current investigation), those findings showed that the co-modulation of 5-HT4R and 5-HT6R improves recognition memory and increases neuronal activation. This combination could represent a novel therapeutic approach in the treatment of Alzheimer's disease.

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Key words : episodic-like memory ; c-Fos ; mice ; Alzheimer

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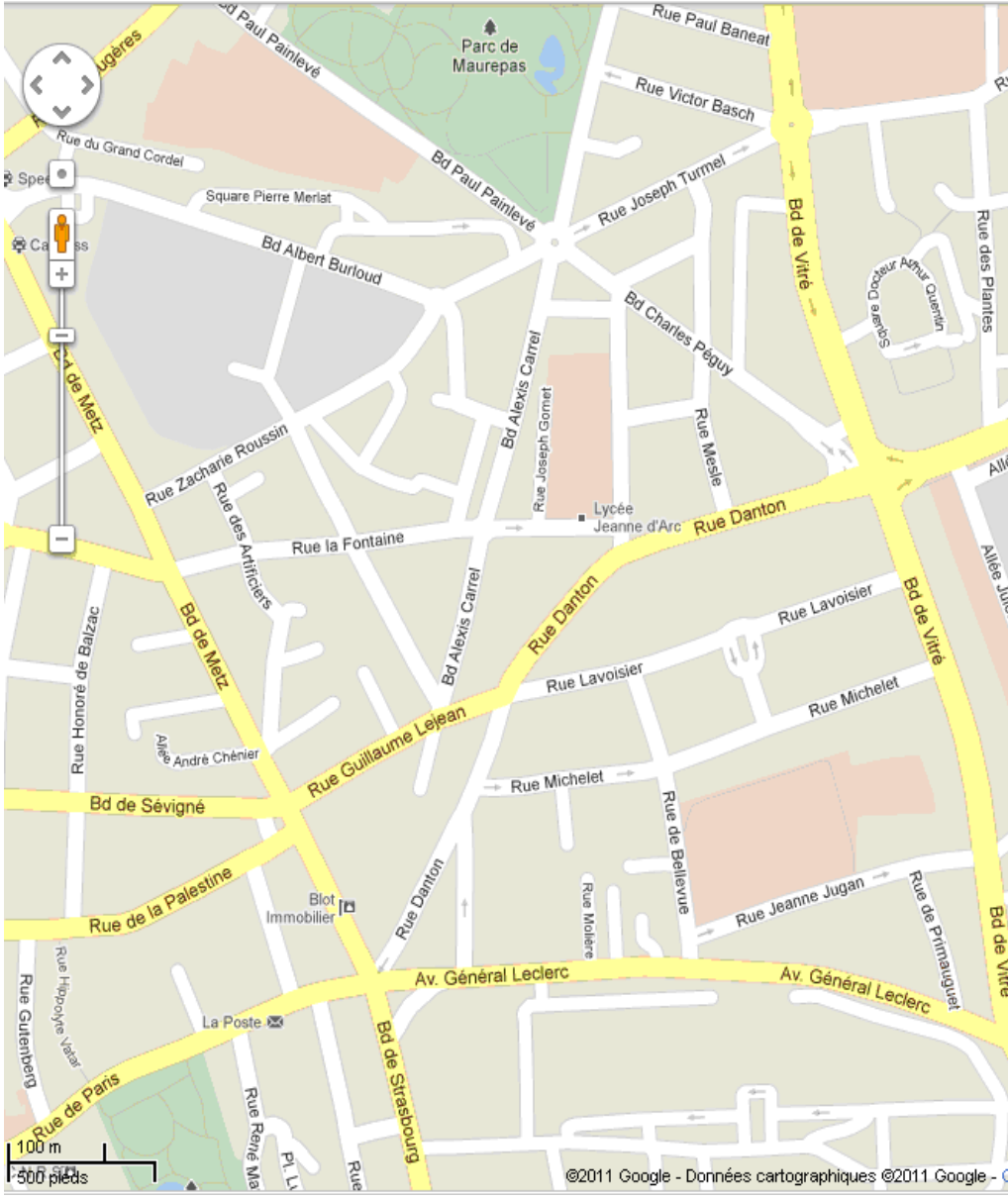


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