Cortical inhibition and decision-making : conflictstudy by transcranial magnetic stimulation

Bastien Ribot, Anne Duron, Michel Goillandeau, Nicolas Langbour, Ignasi Cos, Aymar de Rugy, Thomas Michelet
IMN, CNRS UMR 5293, Université de Bordeaux, France

Adaptive goal-directed behaviors involve monitoring of ongoing actions and performance outcomes, especially in conflict situation. The conflict monitoring theory postulates a leading role to prefrontal cortices such as anterior cingulate cortex (ACC) in this executive control when incompatible choices engender conflict. However, recent behavioral data obtained using a directional Stroop test suggest that purely "motor" cortical regions are also involved in decision-making. Indeed, directional parameters of this test (angular distance between target and distractor) have an impact on behavioral variables like reaction time and are in favor of a conflict-emergence motor hypothesis. An explanation of this data could rely on the reciprocal inhibitions of neuronal populations involved in opposite responses. The purpose of our study consisted in analyzing and quantifying these motor cortical inhibition processes involved in conflict decision-making. Since duration of the silent period (SP) induced by transcranial magnetic stimulation (TMS) in electromyographic recordings is directly correlated with cortical inhibition, 12 participants received TMS during ongoing directional Stroop test and electromyographic records. We expected to obtain a clear modulation of the SP duration directly related to the cognitive and behavioral parameters of the task. However, we partially failed to found such modulation. Methodological concerns are discussed and technical improvements are proposed for future experiments.

Role of mouse-cannabinoid type 1 (CB1) receptors in the motivation for voluntary running

Claire Nguyen, Carolina Muguruza, Francis Chaouloff, Giovanni Marsicano
NCM, INSERM U862, Université de Bordeaux, France

Physical inactivity being a major issue in our modern societies, much work has been devoted to the identification of the motivational processes underlying voluntary physical activity. Based on the relationships between mesocorticolimbic dopamine transmission and the motivation for rewards, the host laboratory is interested in the relationships between the endocannabinoid system (ECS), mesocorticolimbic dopamine transmission, and motivation for running. It has shown that the ECS, through CB1 receptors located on GABAergic neurons in the ventral tegmental area (VTA), might be involved in the motivation to run. Thus, mutant mice lacking CB1 receptors throughout the body or in GABAergic neurons only show a decrease in running, compared to their wild-type littermates, when housed with running wheels. The aim of my project was to analyze the different phases of motivation for voluntary running in a new paradigm called the Runway, allowing to assess the running motivation and not only running performance. Therefore, we were able to evaluate the appetitive ("wanting") and consummatory ("liking") phases of running motivation (by assessing the latencies to reach and to consume the reward, i.e. to begin to run) in constitutive CB1 receptor mutant mice and in conditional mutant mice lacking CB1 receptors on GABAergic neurons. The results suggest the implication of CB1 receptors in both motivational processes, a result that was only partly confirmed in GABA CB1 mutants which displayed only deficits in the "liking" phase of the motivational process. These results open a new route of investigation using operant conditioning in chambers that allow the quantification of the degree to which mice are able to work to access the running wheel.

Role of presenilin and its substrate, the amyloid precursor protein, in neuronal plasticity

Gael Barthet, **Dylan Pommier**, Julie Rumi-Masante, Christophe Mulle
IINS, UMR 5297 CNRS, Université de Bordeaux, France

Rare familial forms of Alzheimer's disease (FAD), characterized by an early onset and genetic inheritance, are caused by mutations of presenilin (PS) gene. PS is the catalytic subunit of the γ-secretase (GS), an intramembrane protease involved in the sequential proteolysis of the amyloid precursor protein (APP). Our bibliographic knowledge of the PS and APP functions suggests that the presenilin/γ-secretase processing of APP and the resulting production of APP intracellular fragment (AICD) regulate activity-dependent transcription and presynaptic plasticity. In this project, the use of new cell-targeted optogenetic tools combined with immunohistochemistry and confocal imaging, have indicated that neuronal activity induced by light seems neither to regulate AICD translocation to the nucleus, nor activity-dependent transcription, contrary to stimulation with KCl. On the basis of the overall results, we question whether these results could be due to limitations from theoptogenetic approach. Then, combining other optogenetic tools and electrophysiology, we obtained indications that absence of AICD is not the molecular event responsible for disruption of presynaptic plasticity observed when presenilin is not present (PS-KO). Completing this study with recordings in condition of β-CTF peptide accumulation would provide a better understanding of the mechanisms underlying the PS-KO phenotype.

Development of the cholinergic phenotype in the appendicular motoneurons of *Xenopus laevis*

Elric Courty, François M. Lambert, Laura Cardoit, Hervé Tostivint, Didier Le Ray
INCIA, UMR 5287 CNRS, Université de Bordeaux, France.
Evolution des Régulations Endocrinniennes, UMR 7221 CNRS, Muséeum National d'Histoire Naturelle, Paris, France.

In vertebrates, motoneurons activate muscles by releasing acetylcholine at the neuromuscular junction. During *Xenopus* development, secondary motoneurons appear in the spinal cord before metamorphosis and rapidly innervate the limb buds that will differentiate during metamorphosis. A recent immunohistological study showed that pre-metamorphosis appendicular motoneurons lacked choline-acetyltransferase (ChAT), the enzyme responsible for acetylcholine synthesis, while they expressed ChAT since the beginning of metamorphosis. The goal of our study was to precise the stage at which ChAT expression started in spinal secondary motoneurons. For this purpose, motoneuron retrograde labeling from limb buds was coupled with ChAT immunohistochemistry between stage 49 (pre-metamorphosis) to stage 58 (during metamorphosis). ChAT-positive appendicular motoneurons were first observed at stage 54, when hind limbs began their growth, although the transcription factor *islet*, characteristic of differentiated motoneurons, could already be detected at stage 49. These data provide new insights in the study of motoneuron development in vertebrates, as well as in the dynamic of cholinergic protein expression.

The impact of Neuropathic Pain on Hypothalamic Orexin Neurons and their signaling at the Spinal Cord level

Kim Le Cann, Sebastian Wilkinson, Christopher Dayas, Brett Graham.
School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia

Pain is an unpleasant sensory and emotional experience associated with tissue damage. This physiological process is essential to protect a threatened part of the body.Some painful sensations, however,persist beyond three months, and are neither beneficial nor helpful in the healing process, termed chronic pain. Neuropathic pain is one example of a complex chronic pain state, initiated or caused by injuries to the sensory pathway. The pain may be spontaneous, and/or amplified following exposure to noxious and innocuous stimuli.Several brain areas have been shown to be involved in the modulation of these painful sensations, and one such brain region is the hypothalamus.In the context of pain, the hypothalamus receives nociceptive information from the spinal cord, a well known sensory information integration center, and transmits appropriate responses in return. A subpopulation of neurons within the lateral hypothalamus, the orexin neurons, has been found to be associated with the modulation of pain processes, and these neurons synthesize two neuropeptides, orexin A and orexin B. In a recent study, administration of orexin A to animals with neuropathic pain attenuated their painful behaviors. Thissuggests that orexin neurons are involved in the modulation of neuropathic pain. However, theaccurate mechanisms remain unclear. In this study, we sought to determine whether neuropathic pain produces changes in the orexin system at the brain and the spinal cord level. To investigate these changes, spared nerve injury (SNI) model was used to induce neuropathic painin mice. Immunohistochemicallabelings showed a decrease in orexin activity in the lateral hypothalamus of SNI mice as compared to sham (control) mice. In addition, whole-cell patch clamp recordings performed at the superficial dorsal horn (SDH) of the spinal cord showed that administration of orexin B changed the spontaneous excitatory postsynaptic currents (sEPSCs) amplitude of two different subpopulations of neurons in SNI mice. Together, these studies showed that neuropathic pain alters the activity of the orexin neurons in the brain and responsiveness of SDH neurons to orexin neuropeptide in the spinal cord.

Role Of inflammation in anxiety and depressive symptoms in patients with rheumatoid arthritis

Lison Huet, Thierry Schaeverbeke, Vincent Rigalleau, Marie Hugo, Lucile Capuron
NutriNeuro, INRA UMR 1286, Université de Bordeaux, France

Rheumatoid arthritis (RA) is the most common chronic inflammatory rheumatism. This autoimmune disease, characterized by an exaggerated inflammatory reaction at the level of the joint synovial membrane leading to joint deformation and destruction, is associated with increased systemic levels of inflammatory markers. In addition, RA is associated with frequent metabolic and neuropsychiatric comorbities. In support of this notion, a large amount of studies indicate a higher prevalence of metabolic disorders including obesity, overweight, metabolic complications together with an increased rate of behavioral symptoms such as anxiety and depression in patients with RA. Recent studies suggest that chronic activation of innate immunity might be involved in the occurrence of these comorbidities. Moreover, it is possible that the coexistence of metabolic disorders participates in the increased risk of neuropsychiatric comorbidities in patients with RA. The objective of this study is to assess the involvement of inflammatory processes in anxiety and depressive symptoms in patients with RA associated or not with metabolic disorders and to evaluate the effect of anti-inflammatory biotherapy (with anti-IL6 and anti-CTLA4) on these symptoms.

A sample of twenty-two patients with RA has been recruited and followed up at two different times, i.e., before the initiation of biotherapy (T0) and at the end of the second month of treatment (T1). Depressive and anxiety symptoms have been assessed respectively with the Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI). Blood samples have been collected for the measurement of circulating inflammatory biomarkers.

At baseline (T0), RA patients with the highest levels of inflammatory markers (IL-6, CRP, leptine, TNF-α, neopterin) exhibit the greatest scores on the STAI and BDI scales. This effect is more pronounced in RA patients afflicted with metabolic comorbidities (overweight/obesity, metabolic disorders). Moreover, 2 months of biotherapy with anti-inflammatory agents is associated with a normalization of the inflammatory profile and neuropsychiatric symptoms, but this effect is more mitigated in patients with metabolic comorbidities.

This study confirmed the role of inflammation in anxiety and depressive symptoms in RA patients and allowed us to determine the influence of metabolic comorbidities on this relationship.

Setting a behavioral task for assessing decision making in urodele

Manon Bonnet-Save, Jean-Marie Cabelguen, Thomas Boraud
IMN, CNRS UMR 5293, Université de Bordeaux, France
NCM, INSERM U862, Université de Bordeaux, France

Decision making takes a crucial role in daily life and can be seriously impaired in neurodegenerative diseases. It implies cortical-basal ganglia loops, but the respective roles of each structure are still debated. Urodeles offer a remarkable opportunity to address this question.
First, whereas the organization of the basal ganglia in these animals is similar to the one of mammals, it has orders of magnitudes fewer neurons[1] and is therefore at a level of complexity that is more tractable in terms of comprehension and modelling. Second, the extensive regenerative capacities of their midbrain networks allow the use of local lesions to investigate their connectivity and functionality. This also makes the urodele a unique experimental model for investigating the mechanisms of decision-making network plasticity after a midbrain injury.
Therefore, we decided to set up a behavioral test of decision making in this animal model. Our task was based on the ability of urodele to discriminate polarized light. The animals were trained[2,3] to associate the location of a shade shelter (urodeles do not like exposure to direct light) with a specific orientation of the polarization axis. We then performed a test to assess if the animals learned this association. This paradigm gave us access to a motor decision making mode. In a second step, we tried to set up a protocol to perform selective lesions of the striatum, the most representative structure (with the accumbens nucleus) of basal ganglia in this animal. It is thought to extend this protocol to the pallium (i.e. the cortex in lower vertebrates) and the thalamus to determine the respective role of each structure. To our knowledge, urodele is the earliest group of limbed vertebrates in which decision-making process has been successfully addressed yet.

Study of the expression of $GABA_A$ receptors subunits in the striatum of mouse model of Huntington's disease

Margot Tertrais, Gilles Courtand, Christophe Halgand, Du Zhuowei, Maurice Garret
INCIA, CNRS UMR 5287, Université de Bordeaux, France

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by degenerative and motor symptoms recapitulated in several transgenic mice models. The striatum appears to be the first structure affected by the mutation in humans and animal models. It is composed of GABAergic projection neurons ("medium spiny neurons", MSN), GABAergic and cholinergic interneurons. GABAergic interneurons can be distinguished by their expression of parvalbumin (PV) or neuronal nitric oxide synthase (nNOS). $GABA_A$ receptors ($RGABA_A$) are a family of pentameric receptors composed of several subunit families. Many studies have shown that the different subunits modulate specific neural networks and may have selective physiological functions. It was found in the laboratory that the expression of the $RGABA_A$ α1, α2 and α3 subunits is modified in R6/1 mice models. As the changes of expression of the subunits could be related to disturbance of GABAergic transmission in HD, it is critical to identify which neuronal populations express the different $RGABA_A$ subtypes. The laboratory has already shown that the α1 subunit is present in MSN and in PV interneurons in the striatum. In R6/1 mice, α1 subunit is increased in MSN and decreased in PV interneurons. We hypothesize that variation of $RGABA_A$ subunit expression in the striatum of R6/1 mice, are specific to certain neuronal populations. Therefore, our goal was to characterize this differential expression in the various neuronal cell types. A quantitative measurement of the α2 and α3 subunits expression has been performed by immunochemistry and image analysis both in 2 and 6 month-old wild type and R6/1 mice. The expression of α2 subunit is increased in MSN and PV interneurons and decreased in nNOS interneurons at 2 months. Due to the absence of apparent motor symptoms, this age is considered presymptomatic. However, our results suggest that molecular alterations are already present very early in the evolution of the disease. At the age of 6 months, the expression of α2 is decreased in MSN and PV interneurons while α3 is increased in PV and ChAT interneurons. These variations of $RGABA_A$ subunits provide information on the control of inhibitory synaptic strength and its changes related to the evolution of HD. These data could be a first step in the identification of new therapeutic targets *via* pharmacological modulation of different subtypes of $RGABA_A$.

Functional contribution of glutamatergic neurons of the medullary reticular formation to motor control in the mouse

Marie Roussel, Maxime Lemieux, Nicolas Josset, Frédéric Bretzner.

The medullary reticular formation (MRF) and its reticulospinal pathway play an important role in motor control. Lesion and coolingof the MRF inhibitsmotor activity and locomotion. Single unit recording studies show that reticulospinal neurons fire phasically during locomotion, and electrical microsimulationsin the MRF evokeelectromyographic (EMG)responses inaxial and limb muscles.While it is well established that the MRF is important to locomotion, little is known about its neurotransmitter phenotype. The MRF being composed of glutamatergic and serotoninergic neurons, standard electrophysiological techniques have not allowed us to decipher the functional contribution of each one of these neuronal populations. Interestingly, glutamatergic neurons of the MRF express selectively the vesicular glutamatergic transporter vGlut2. Using transgenic mice in which a photo-activator (channelrhodospine2) is expressed in glutamatergic MRF neurons under the vGlut2 promoter, we proposedin the current study to investigate the contribution of these neurons to motor control. An optical probe was inserted and secured for chronic photo-stimulation ofthe MRF. Electrodes were also implanted in the tibialis anterior and gastrocnemiuslateralisof both hindlimbsand secured to a connector on the back of the mouse for chronic EMG recordings. After recovery from the surgery, free-walking animals were assessed at rest.Short trains of photo-stimulations(10ms) evoked movements in the ipsilateral neck, forelimb and hindlimb. In addition,photo-stimulations also evoked excitatory EMG responses in ipsilateral hindlimbmuscles and, to a lesser degree, in contralateral muscles. Therefore, photo-stimulations of the glutamatergic MRF can evoke specific motor movements.

Effects of L-DOPA on motor and non-motor symptoms of Parkinson's disease in an alpha-synuclein rat model

Olivier Kerdiles, Frédéric Naudet, Emilie Faggiani., Layna Oubrou, Abdelhamid Benazzouz.
IMN, CNRS UMR 5293, Université de Bordeaux, France

Parkinson's disease (PD) is a neurological disorder characterized by extrapyramidal motor symptoms such as tremor, bradykinesia and muscle rigidity, which are attributed to the degeneration of dopamine neurons in the substantia nigra *pars compacta* (SNc). Furthermore, PD is also characterized by non-motor symptoms such as anxiety, apathy and depression, present in more than 40% of PD patients (Tadaieski*et al.* 2008). The pathophysiology of motor symptoms is well defined, however, the non-motor features remain under-studied in the literature. Injection of levodopa (L-DOPA), a precursor of dopamine is one of the most widespread treatments, which dramatically improves the cardinal motor symptoms. Nevertheless, its functional mechanism of action is still under debate. Several studies led in rats used 6-OHDA induced dopaminergic lesions which is not very representative of the real evolution of PD in humans.

As a little is known about non-motor symptoms of PD, the present study investigated the effects of L-DOPA both on motor and non-motor symptoms. To assess that, the study has been made on a more representative model of PD by injecting α-synuclein in the SNc of rats and doing acute injections of L-DOPA (12mg/kg). The effects of the L-DOPA on motor and non-motor symptoms of PD has been assessed by behavioral tests (Openfield, Forced Swim Test, Elevated Plus Maze). Our results shown that with this α-synuclein model, non-motor symptoms appeared prior to the motor symptoms. Moreover, we demonstrated that L-DOPA improves both motor symptoms and depressive like behavior at later stages of the disease whereas it improves depressive-like behavior at earlier stages. This study also confirmed that α-synuclein model is really relevant to study Parkinson's disease at different stages.

Study of monoamine oxydases' activity in the basal and stimulated metabolism in naïve and L-DOPA treated hemiparkinsonian rats.

Rémi Corne, Morgan Bérard, Philippe De Deurwaerdère
IMN, UMR 5293, Université de Bordeaux, France

L-DOPA is considered as the best medication of Parkinson's disease. Given chronically, the treatment is efficient but causes serious behavioral side effects over time. It has been shown that exogenous L-DOPAinduces oxidative stress and can lead to neurodegeneration. Monoamine oxydases(MAO) likely contribute to these effects as their inhibition prolongs L-DOPA efficacy in the treatment for Parkinsonism. Nonetheless, a change in MAO activity following dopaminergic lesions and chronic L-DOPA treatment has yet to be demonstrated *in vivo*.

The activity of the MAO isoforms A and B was addressed using intracerebral microdialysis in the striatum and the prefrontal cortex of isoflurane-anesthetized rats. 3-methoxytyramine, a substrate of both MAO, wasapplied at different concentrations through the dialysis probes and the product homovanillic acid (HVA) was measured using high pressure liquid chromatography coupled to electrochemical detection.

The application of 3-MT (1-100µM) enhanced in a concentration-dependent manner HVA extracellular levels. After having studied the effects of the MAOA inhibitor clorgyline, and the MAOB inhibitors L-déprényl and F2MPA on monoamine tissue content, we showed *in vivo* that only clorgyline reduced both basal and 3-MT-stimulated HVA extracellular levels in naïve rats. In rats bearing a unilateral lesion of dopaminergic neurons, a rat model of Parkinson's disease, we showed that chronic L-DOPA (3 or 12 mg/kg, twice per day; 3 weeks)was without effect on the conversion of 3-MT to HVA.

Although no effect of chronic L-DOPA was shown on MAO activity, this study provides a model for the assessment of MAO activity *in vivo*. It will permit to further study the function of MAO isoforms in the rat model of Parkinson's disease.

Unraveling the role of p62 in the hypothalamic regulation of energy balance.

Vincent Simon, Nicolas Saucisse, Daniela Cota
NCM, INSERM U862, Université de Bordeaux, France

Obesity is characterized by a disruption of the mechanisms guarantying energy balance. The hypothalamus is a major structure involved in energy balance regulation, especially via anorexigenicpro-opio-melanocortin(POMC)neuronsand the orexigenicagouti-related peptide (Agrp) neurons, of the arcuate nucleus. These neurons directly respond to hormones and nutrients, but the underlying intracellular pathways are still poorly understood.
Recently, the mammalian target of rapamycin (mTOR)pathway has been shown to be involved in the hypothalamic regulation of energy balance. The activation of the mTORpathwayinhibitsautophagy, a ubiquitous process of protein/organelles recycling. Of note, autophagy is essential for POMC function as disruption of autophagy in these neurons causeshyperphagic obesity. The p62 protein in particular linksmTOR and autophagy, as p62 directly activates the mTOR pathway and participates to autophagy. Thus, by connecting mTOR and autophagy, p62 could be involved in energy balance regulation, especially by acting in POMC neurons.
To test this hypothesis, we phenotypically characterized a transgenic mouse model lacking p62 in POMC neurons. Our results demonstrate that these mice have altered mTOR signaling and are hyperphagic, obese, resistant to the anorexigenicaction of leptin and unable to metabolically adapt to a caloric overload. These alterations are associated to morphological changes in POMC neurons that might underlie the observed phenotype.
Thus, our findings have unraveled the importance of p62 in the hypothalamic regulation ofenergy balance, but further studies are needed to extendthe understanding of p62 function in this context.

Role of ROS in THC-induced psychotic-like effects

Yarmo Mackenbach, Arnau Busquets-Garcia, Giovanni Marsicano
NCM, INSERM U862, Université de Bordeaux, France

Schizophrenia and psychosis are severe mental disorders affecting 1-2% of the world population. Evidence strongly suggests that impaired cannabinoid signaling and impaired reactif oxygen species (ROS) signaling in the brain could contribute to the pathophysiology. It is also known that cannabis consumers may develop psychotic-like symptoms. We used the main psychoactive component of cannabis, Δ9-tétrahydrocannabinol (THC), to induce impaired working memory and decreased social interaction in mice, two psychotic-like effects that we tested with behavioral paradigms. The injection of different ROS scavengers demonstrated that ROS are indeed involved in the mechanisms underlying these effects of THC. We then observed through fluorescence microscopy that ROS levels are increased in the hippocampus after injection of THC. Finally, we demonstrated that pregnenolone can block all the psychotic-like effects of THC that we studied. To conclude, this project contributes to the better understanding of the link between psychotic-like symptoms and cannabis consumption, and proposes pregnenolone as a new therapeutic approach to treat psychotic-like states.

Electrophysiological investigation of the spinal sensori-motor networks after a partial spinal cord injury in neonatal rats

Zied Oueghlani, GrégoryBarrière, Gilles Courtand, Christophe Halgand, Laura Cardoit.
INCIA, CNRS UMR 5287, Université de Bordeaux, France

Spinal cord injury (SCI) induces the loss of at least some of the descending pathways controlling the intrinsic spinal networks. Thus such an injury provokes the waste of sensorimotor functions and locomotor activity. Even though no long term and spontaneous recovery has ever been seen in rats spinalized as adults, some studies have shown that recovering complete SCI may occur spontaneously if the SCI strikes early after birth. Thus understanding the recovery mechanisms taking place in animals injured as neonates may be useful to identify the features of recovery that are missing in adults. So far, however the recovery mechanisms are yet to be elucidated. Therefore the aim of our work was to develop a neonatal rat model of incomplete SCI that will offer the possibility to clarify plastic changes occurring after a partial SCI while using the *in vitro* isolated brainstem / spinal cord preparations. All the experiments were conducted on 5 day-old rats that suffer from a partial section of the spinal cord at the mid-thoracic level. We found at first that spinalized animals showed an altered locomotor activity *in vivo* with a loss of coordination between forelimbs and hindlimbs movements. Importantly, the expression of the locomotor movements was, as in adult spinalized animals, dependent upon the extent of the spinal cord lesion. Then, thanks to electrophysiological and pharmacological approaches we evaluated fictive locomotion using the *in vitro* isolated spinal cord preparation. We show that locomotor pattern was still expressed below the lesion at the lumbar level in 52% of spinalized animals in spite of the SCI. Therefore, on these preparations, no statistically significant difference was revealed between spinalized and control animals concerning locomotor parameters (period of locomotor cycles and bursts duration). Finally, we have been interested in the effects that a SCI may have on the spinal sensorimotor functions by studying on the *in vitro* preparation the electrical analog of the stretch reflex. We have shown that the SCI has no significant effect on this reflex pathway. Interestingly the synaptic depression phenomena, well known in control animals, were also found and conserved in spinalized rats. Thus, the model we developed will certainly be very useful in the future to determine the mechanism allowing to maintain the locomotor capacities of the spinal cord networks below a SCI.