Bordeaux

Centre Broca Nouvelle-Aquitaine

13 et 14 mars 2020

XVIème symposium du Réseau Français de Recherche sur la Douleur

université de BORDEAUX

BORDEAUX neurocampus IINS

SOMMAIRE

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A l'occasion de la XVI[ème] édition du colloque Douleur, l'équipe organisatrice a le plaisir de vous accueillir cette année à Bordeaux.

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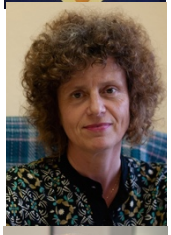

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PROGRAMME

Vendredi 13 mars

9h00 Accueil des participants

9h15 Ouverture du symposium

9h30 -10h45 Communications orales :

Session 1 : Circuits nociceptifs.

1) A pain-related sodium channel CRISPR/Cas9 mouse model for the human Scn9aR185H mutation: establishment and behaviors
   Yaping XUE, Strasbourg.

2) Frequency-dependent short-term synaptic plasticity of GABAergic connections onto lamina II neurons.
   Lou Cathenaut, Strasbourg.

3) Astroglial Kir4.1 ion channel deficit underlies inflammatory mechanical allodynia
   Sarah Mountadem, Clermont.

4) Impact of prenatal stress on visceral sensitivity and intestinal homeostasis in adulthood
   Nicolas Cenac, Toulouse.

5) TrkB-driven differential chloride homeostasis in the spinal dorsal horn locally shapes synaptic metaplasticity and sensitization
   Jimena Perez Sanchez, Marseille.

10h45-11h15 Pause Café**.**

11h15-12h15 Conférence plénière : ***« Neuronal circuits for pain and itch in the spinal dorsal horn »*** **Pr Andrew Todd (Glasgow, UK)**

12h15 Sponsors (20min): **Lucine**, **la maison du cerveau**.

13h-14h30 Déjeuner.

14h30-16h00 Communications orales.

Session 2 : Douleurs Chroniques

1) Peripheral ASIC3 activation by lysophosphatidyl-choline induced chronic joint pain and associated anxiety in mice
   Florian Jacquot, Nice.

2) Gender-independent involvement of P2X4 receptor in chronic pain and associated comorbidities
Damien Gilabert, Montpellier.

3) NGS custom gene panel investigation of genetic predisposition to CPSP
Francesca Puppo, Marseille.

4) Optical modulation of mechanical but not thermal allodynia through peripheral mGlu4 receptors
Cyril Goudet, Montpellier.

5) Loss of Myo1a reveals gene-microbiota interactions in the settlement of chronic pain
Ana Reynders, Marseille.

6) APE1/Ref-1 Redox function is implicated in Pain sensitization mechanism and involved in the global regulation of cellular miRNAs
Amira Zaky, Alexandrie, Egypte.

16h-16h30 Pause Café.

16h30-17h30 : Conférence plénière : « *Uncovering somatosensory coding strategies* » Pr. Yves De Koninck (Québec City, CA)

17h30-18h Actualités du réseau douleur. Pr. R Dallel.

20h00 Dîner de Gala : Café du Port, 1 Quai Deschamps, 33100 Bordeaux

Samedi 14 mars

8h30-9h30 Communications orales

Session 4 : Peptides et douleur :

1) LIT-001 a Nonpeptide Oxytocin Receptor Agonist for a Durable Relief of Inflammatory Pain
Louis Hilfiger, Strasbourg.

2) The RFRP-3/NPFF1R system modulates nociception, opioid analgesia and inflammatory pain in rodents.
Frédéric Simonin, Strasbourg

3) The effect of Relaxin on pain : behavioral and morphological data
Cynthia Alkhoury Abboud. Bordeaux.

4) Oxytocin Acts on Astrocytes in the Central Amygdala to Promote a Positive Emotional State
Damien Kerspern, Strasbourg

9h30-10h30 Conférence plénière : « *Peut-on se passer des morphiniques ?* » Pr. Valeria Martinez, PU-PH (Hôpital Raymond Poincaré, Garches, France)

10h30-11h Pause Café.

11h00-12h00 Communications orales.

Session 5 : Douleur et Parkinson.

1) Involvement of serotonergic descending pathways on pain in a mouse model of parkinsonism
   Zoé Grivet, Bordeaux.

2) Involvement of spinal cord dopamine depletion in the pathophysiology of nociceptive disabilities in a rat model of Parkinson's disease
   Keri Ann Charles, Bordeaux.

3) Noyau subthalamique et douleur, implication sur les symptômes douloureux dans la maladie de Parkinson
   Veronique Coizet, Grenoble.

4) Ablation of the tVTA compensates symptoms, including nociceptive, in a model of Parkinson's disease.
   Michel Barrot, Strasbourg.

12h00 Prix de la communication orale. Conclusions. Déjeuner.

Fin du symposium.

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RESUMES

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Session 1 : Circuits nociceptifs.

A pain-related sodium channel CRISPR/Cas9 mouse model for the human Scn9aR185H mutation: establishment and behaviors

Yaping Xue

Nav1.7 channel is a voltage-gated sodium channel that plays a critical role in the generation and conduction of action potentials and is thus important for electrical signaling by most excitable cells. Recent studies show that in primary sensory neurons, the expression and dynamic regulation of several sodium channel subtypes play important roles in neuropathic pain. A number of SCN9A (encoding Nav1.7) gene point mutations have been found in chronic pain patients with SFN (Small fiber neuropathy). For these inherited neuropathic disorders, gene editing is an important tool for establishing animal models to investigate the role of a mutation in the pathogenesis of disease and provide a rapid avenue for functional drug screening. Clustered regularly interspaced short palindromic repeat/CRISPR-associated 9(CRISPR/Cas9) enables targeted genome engineering. We sought to establish a pain-related sodium channel mouse model Scn9aR185H using CRISPR/Cas9 technology and to characterize this mutation. We have successfully created a pain-related Scn9aR185H knock-in mouse line using the CRISPR-Cas9 technique. The Scn9aR185H mouse line showed no alteration of growth, survival and global health state. Pain sensitivity of the new mutant mouse line was investigated on both genders using behavioral tests of sensitivity to thermal and mechanical stimuli. Our results indicate that the Scn9aR185H mice show a pain phenotype, suggesting that the Scn9aR185H mutation identified in the SFN patients is responsible for their pain symptoms.
This work was Funded by the European Commission H2020 Marie Skłodowska-Curie Actions, ITN Pain-Net (grant no. 721841).

Frequency-dependent short-term synaptic plasticity of GABAergic connections onto lamina II neurons.

Lou Cathenaut

Nociceptive information is conveyed to the spinal cord by primary afferent fibers. In these fibers, intensity of adequate stimuli is encoded as the number of spikes per units of time. The processing of such frequency-encoded information usually involve synapses displaying frequency-selective short-term plasticities. Such plasticities are little known in dorsal horn networks. Our aim is to examine the short-term plasticities expressed by GABAergic inhibitory connections in lamina II of the dorsal horn.
Using spinal cord acute slices prepared from adult transgenic mice expressing Green Fluorescent Protein (GFP) under the GAD65 promoter, we performed patch-clamp recordings of unitary GABAergic synaptic currents evoked by local electrical stimulation.
Our data indicate that short-term plasticity is different in GABAergic connections onto inhibitory and excitatory neurons. These plasticities respectively involve GABAB and A1 receptors.

Astroglial Kir4.1 ion channel deficit underlies inflammatory mechanical allodynia

Sarah Mountadem

Chronic inflammatory pain is a frequent and disabling condition that is significantly maintained by central sensitization, which results in pain hypersensitivity. While microgliosis contributes to the onset of chronic pain, reactive astrocytes and associated chemical mediators play a critical role in chronic pain maintenance and central sensitization.
Inward rectifier potassium channels (Kir4.1) are mainly expressed by astrocytes and are known to be involved in astrocyte cell volume regulation, but also in astrocyte-neuron interactions by controlling the astrocytic resting membrane potential, glutamate uptake and extracellular K+ concentration. Accordingly, it has been shown that dysfunction of these channels is associated with central nervous system pathologies such as epilepsy, Huntington disease or depression. However, the role of central astroglial Kir4.1 in pain has not been clearly investigated. Here, we explore the involvement of astroglial Kir4.1 channels in the maintenance phase of trigeminal inflammatory pain hypersensitivity. By combining biochemical, electrophysiological and behavioral techniques, we show that astrocytes in the medullary dorsal horn (MDH) of inflamed rats have smaller Kir4.1 currents, correlated with a decrease in Kir4.1 protein expression. Moreover, these modifications are associated with an increase in extracellular K+ concentration, leading to neuronal hyperexcitability. Finally, virally-mediated Kir4.1 knockdown, targeted to MDH astrocytes selectively, is sufficient to induce mechanical allodynia and neuronal hyperexcitability.
Together, these findings suggest that astroglial Kir4.1 ion channel deficit underlies inflammatory mechanical allodynia, and thus may offer novel avenues for the treatment of this debilitating symptom.

Impact of prenatal stress on visceral sensitivity and intestinal homeostasis in adulthood

Nicolas Cenac

The prenatal period is a delicate time during which intrauterine exposure to environmental factors may modulate the course of development of the intestinal barrier function and the sensory system, thereby increasing the risk of developing functional disorders. We hypothesized that prenatal stress in mice would predispose adult offspring to visceral hypersensitivity and intestinal homeostasis failure. Prenatal stress was induced by using a restriction stress with bright light during 30 minutes, three times a day, in female mice between day 13 and 18 of gestation. In 8 weeks old offspring, visceral sensitivity to colorectal distention and intestinal paracellular permeability were assessed in male and female. Mucus, tight junction proteins, anti-microbial peptides, chemokines and cytokines were monitored by qPCR and intestinal bioactive lipids by mass spectrometry. MiSeq-based microbial taxonomic analysis was used to determine faecal microbiota composition. Prenatal stress induced visceral hypersensitivity and increased paracellular permeability in the majority of male and female offspring. Only in male, we quantified a decrease in 5-lipoxygenase activity and Tnfα expression and an increase in Ttf3 and Ifnγ expression. Prenatal stress induced in male and female adult offspring a dysbiosis characterized in male by a decrease in intestinal microbiota diversity, an increase in Akkermansia and a decrease in Desulfovibrio and in female by an increase in Akkermansia and a decrease in Lactobacillus animalis. Interestingly, the abundance in Lactobacillus animalis was inversely correlated with visceral hypersensitivity. Prenatal stress is sufficient to induce microbiota dysbiosis, visceral hypersensitivity and increase paracellular permeability in adulthood. Thus, prenatal stress could represent a priming event for the development of functional disorders in adulthood.

TrkB-driven differential chloride homeostasis in the spinal dorsal horn locally shapes synaptic metaplasticity and sensitization

Jimena PEREZ SANCHEZ

GABAA/glycine-mediated neuronal inhibition critically depends on [Cl-]i which is mainly regulated by the K+-Cl- co-transporter 2 (KCC2) in the adult central nervous system (CNS). KCC2 heterogeneity thus affects information processing across different CNS areas. Here, we uncovered a gradient in Cl- extrusion across the superficial dorsal horn (laminae I-II) of the spinal cord (SDH), which remained concealed under low Cl- load. Under high Cl- load or heightened synaptic drive, lower Cl- extrusion capacity was unveiled in lamina I neurons, as expected from the gradient in KCC2 expression observed throughout the SDH. Blocking TrkB receptors attenuated the KCC2 gradient, indicating differential constitutive TrkB activation across laminae. Higher lability of Cl- homeostasis in lamina I resulted in rapidly collapsing inhibition, and a form of activity-dependent synaptic plasticity expressed as runaway, continuous facilitation of excitatory responses. The higher propensity to plasticity in lamina I as compared to lamina II differentially affects sensitization behaviour to thermal and mechanical input. Thus, inconspicuous Cl- heterogeneity across the SDH critically shapes plasticity for specific nociceptive modalities.

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Conférence plénière 1

Neuronal circuits for pain and itch in the spinal dorsal horn

Pr Andrew Todd (Glasgow, UK)

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Session 2 : douleurs chroniques.

Peripheral ASIC3 activation by lysophosphatidyl-choline induced chronic joint pain and associated anxiety in mice

Florian Jacquot

We have shown previously that human painful inflammatory exudates, displaying nonacidic pH, induced a slow constitutive activation of human ASIC3 channels. This effect was mainly driven by lipids, and we identified lysophosphatidylcholine (LPC) and arachidonic acid (AA) as endogenous activators of ASIC3 in the absence of any extracellular acidification. First, we investigated the level of LPC in the synovial fluid of the knee joints in a well characterized cohort of patients suffering from osteoarthritis (OA) versus control patients. Then, our aim was to further assess the pronociceptive in vivo effect of intraarticular LPC in mice and to investigate associated comorbidities, especially anxiety. Finally, we evaluated if ASIC3 channel was involved in these effects using ASIC3 knock out mice. We found a significant increase concentration of LPC especially 16:0 in OA patient compared to control patient confirming the putative pronociceptive role of LPC in joint pain. We further demonstrated that two intraarticular LPC (16:0) injections (10nM) 5 days apart induced significant mechanical and thermal hypersensitivities up to 28 days compared to control mice. This was not associated with peripheral inflammation or bone remodeling using MMP680 and Cathepsin K in vivo fluorescent imaging, respectively, as well as neuronal damage. LPC-injected mice also develop anxiety demonstrating by several anxiety tests (open field, elevated plus maze, hole board, marble burying). Furthermore, pain behaviors and associated anxiety induced by local LPC were significantly reduced in male and female ASIC3 knock-out mice compared to their WT counterpart. Here, we demonstrated that intraarticular LPC induced chronic joint pain behaviors and anxiety related behaviors in an ASIC3 dependent manner in mice. These results suggest that lipids especially LPC 16.0 through ASIC3 activation seems to play a crucial role in the development of chronic joint pain at least in OA and could be a valuable therapeutic target.

Gender-independent involvement of P2X4 receptor in chronic pain and associated comorbidities

Damien Gilabert

Institut de Génomique Fonctionnelle (IGF), University of Montpellier, CNRS, INSERM, Montpellier, France

Purinergic ion-gated channel receptor P2X4 are in part responsible for the hyperexcitability of spinal network during neuropathic pain, with their de novo expression in spinal activated microglia. Their activation by extracellular ATP is followed by an autocrine release of BDNF (Brain derived neurotrophic factor) inducing a down-regulation of the cation-chloride co-transporter KCC2 and an increase of NMDA transmission, underlying hyperalgesia. This pathway is well accepted in male, however, recent studies suggest that female could use a P2X4-independent pathway. However, the mechanisms of this dimorphism remain unclear. Here, we first demonstrate an increase of P2X4 RNA transcripts in male and female spinal cord after neuropathy (SNI model). Using global and conditional P2X4-invalidated mice and pharmacological tools, we demonstrate that P2X4 are required for the development of neuropathy-induced hyperalgesia in males and females mice. Consistent with these results, anxio-depressive comorbidities usually observed in chronic pain are clearly present in WT mice whereas they are absent in males and females KO mice.
Our results suggest that P2X4 are required for neuropathic pain-induced hyperalgesia and associated comorbidities in both male and female mice.

NGS custom gene panel investigation of genetic predisposition to CPSP

Francesca Puppo

Predisposition to Chronic Post-Surgical Pain (CPSP) is multifactorial and includes genetics. Indeed, multiple studies identified associations between several genes and pain. In particular, results coming from mouse model and exploration of public databases indicate Myo1a gene as a plausible risk factor to develop chronic pain. Our working hypothesis is that patients that develop CPSP exhibit enrichment in genetic variants, especially in the Myo1a gene. Thus we designed a custom sequencing panel including 71 genes and collected DNA samples from a cohort of breast cancer/thoracotomy with or w/o pain at 3 and 6 months after surgery (Blanc P. et al. 2019). At present 109 DNA samples have been sequenced. We stratified samples in 2 main groups: patients that develop Chronic Post-Surgical Pain (CPSP), or that had normal post-surgical recovery (No Pain). Age, sex, BMI, pain antecedents, known risk factors and scores resulting from Hospital Anxiety and Depression scale (HAD) and Pain Catastrophic scale (PCS) are also used. We found insertions/deletions, STOP codon creations and amino acid substitutions belonging to Myo1A and other genes of the panel in both CPSP and No Pain patients. Interestingly, 2 patients that carry Myo1A STOP codons, developed CPSP in absence of risk factors. Moreover, 172 out of 1813 variants identified in CPSP patients are significantly more frequent compared to No Pain patients (Khi square test, $P<0,05$) and 4 genes have high density of these variants. Ongoing analysis will allow to establish contribution of Myo1A mutations alone or in combination with the other 70 genes to genetic of chronic pain.

Optical modulation of mechanical but not thermal allodynia through peripheral mGlu4 receptors

Cyril Goudet

Glutamate and its receptors are involved in nociceptive transmission at the periphery. In the present study, we investigated the presence and potential role of mGlu4 receptors at the periphery. To that aim, we chose a photopharmacological approach, using optogluram, a photoswitchable mGlu4 PAM, in combination transdermal light stimulation on acute pain and in mice models of neuropathy or inflammation. We provide the first pharmacological evidences of the presence of mGlu4 receptors at the periphery in mice paw. We demonstrate their ability to modulate specifically mechanical but not thermal allodynia associated to inflammatory or neuropathic pain. However, activation of peripheral mGlu4 receptors by optogluram is not modifying the response to acute mechanical or thermal stimuli in naïve animals. The light-controlled antiallodynic effect of optogluram is lost in mGlu4 KO mice, confirming that mGlu4 receptors are mediating the effect of the drug. Immunohistological and electrophysiological experiments are ongoing to determine more precisely the location and function of peripheral mGlu4 receptors.

Loss of Myo1a reveals gene-microbiota interactions in the settlement of chronic pain

Ana Reynders

Chronic pain is a major health problem. Identifying the risk factors for chronic pain and the mechanisms through which they lead to abnormal pain processing is an important challenge in today's pain research. Mounting evidence demonstrates the critical contribution of intestinal flora to the pathogenesis of several diseases, including those affecting the nervous system. In the pain field, several studies have unraveled the important role of intestinal flora in modulating visceral and chemotherapy pain. However, evidence regarding the contribution of intestinal flora to the pathogenesis of chronic pain outside the gastrointestinal tract remains scarce. We have recently discovered that genetic inactivation of myosin 1a in mouse (Myo1a KO) leads to chronic mechanical hypersensitivity in response to inflammatory, post-operative and neuropathic insults. These data strongly suggest that Myo1a KO mice are predisposed to chronic pain. Using 16S metagenomics sequencing we have unraveled significant differences in several fecal bacterial taxa between Myo1a KO mice and controls. Finally, we have shown that the chronic inflammatory pain phenotype of Myo1a KO mice was abrogated following antibiotic pre-natal treatment. Altogether, these data demonstrate that alterations in gut flora represent an important risk factor for the emergence of peripheral injury-induced chronic pain.

APE1/Ref-1 Redox function is implicated in Pain sensitization mechanism and involved in the global regulation of cellular miRNAs

Amira Zaky

Inflammatory pain is a chronic pathology characterized by maladaptive plasticity that relies on functional reorganization of dorsal horn networks. In the central nervous system, the inappropriate response after neuronal damage triggers oxidative stress and the expression/activation of apurinic/apyrimidinic endonuclease/redox factor (APE1/Ref-1). Until now there has been no data on APE1 expression and function in nervous tissues after induction of chronic pain. Therefore we studied APE1 expression and subcellular distribution in a rat model of persistent inflammatory pain produced by Complete Freund`s Adjuvant (CFA) injection. We demonstrated that despite a global decrease in APE1 expression under inflammatory conditions, APE1 redox function accounts for pain sensitization. Indeed, we blocked selectively APE1-redox activity under inflammatory conditions using (E)-3-[2-(5,6-dimethoxy-3-methyl-1,4-benzoquinonyl)]-2-nonylpropenoic acid (E3330). E3330 treatment causes impairment of APE1 redox activity and mitochondrial localization. In the CFA model, we demonstrated that E3330 exerts potent anti-inflammatory effects through the inhibition of APE1 redox activity and subsequent suppression of down-stream transcription factor activation (e.g. NF-κB) and inflammatory molecule production. Furthermore, we demonstrated the effect of APE1 intracellular distribution on the expression of selected groups of miRNAs following oxidative stress induction in an *in vitro* system. Our results indicate a specific regulatory effect of APE1 sub-cellular distribution and redox functions on a large variety of intracellular miRNAs. Therefore, we hypothesize that APE1/Ref-1 alters the expression and/or the stability of a large number of intracellular miRNA in the spinal cord under chronic pain conditions, thus regulating central pain sensitization. An investigation of the possible involved mechanism is still ongoing.

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Conférence plénière 2 :

Uncovering somatosensory coding strategies

Pr. Yves De Koninck (Québec City, CA)

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Session 3 : Peptides et douleur.

LIT-001 a Nonpeptide Oxytocin Receptor Agonist for a Durable Relief of Inflammatory Pain

Louis Hilfiger

Oxytocin possesses several physiological and social functions, among which an important analgesic effect through its binding to its unique receptor, both in the central nervous system and in the peripheral nociceptive terminal axon in the skin. However, despite its interesting analgesic properties and its current use in clinics to facilitate labor, oxytocin is not used in pain treatment. Indeed, it is rapidly metabolized, with a half-life in the blood circulation estimated at five minutes in humans and rats. Moreover, oxytocin itself suffers from several additional drawbacks: a lack of specificity and an extremely poor oral absorption and distribution. Recently, a non-peptide full agonist of oxytocin receptor (LIT-001) of low molecular weight has been synthesized with reported high stability in rodent and human hepatocytes and a high specificity for the oxytocin receptor with poor levels of off target. Here, we studied the putative analgesic properties LIT-001 in a rat model of inflammatory pain. We found that a single intraperitoneal administration of LIT-001 induced a long-lasting (~5h) reduction in both mechanical and thermal heat hypersensitivities. This was related to its presence in the organism, as measured using mass spectrometry in plasma, cerebrospinal fluid (CSF), brain and urines. Finally, LIT-001 due to its long-lasting analgesic effect could to be a new potential drug candidate for clinical trial due to its low level of off target, paving the way to an original drug development strategy for pain treatment.

The RFRP-3/NPFF1R system modulates nociception, opioid analgesia and inflammatory pain in rodents.

Frédéric Simonin

RF-amide-relatide peptide-3 (RFRP-3) and Neuropeptide FF (NPFF), belong to the family of so-called RF-amide peptides. In mammals, they are involved in the modulation of several functions including metabolism, reproduction and nociception. They target two different G protein-coupled receptor subtypes called Neuropeptide FF1 receptor (NPFF1R alias GPR147 or GnIH receptor) and Neuropeptide FF2 receptor (NPFF2R or GPR74), respectively. However, the respective role of these two receptors is unclear, and the study of their function in vivo is severely limited by the lack of highly selective antagonists. In this work, we describe the identification of small compounds that display high affinity and selectivity for NPFF1R as well as potent antagonist activity in vitro. We then showed that one of them -RF3286- efficiently and selectively blocks RFRP-3 induced hyperalgesia in mouse and LH release in hamster, indicating that this compound is a useful pharmacological tool to study the in vivo functions of NPFF1R and its endogenous ligand RFRP-3. Pharmacological blockade of NPFF1R with RF3286 prevented the development of pain hypersensitivity and analgesic tolerance induced by chronic administration of morphine revealing that NPFF1R/RFRP-3 system is critically involved in neuroadaptation associated with administration of opiates. These results were further confirmed in NPFF1R knockout animals. Moreover, we observed the expression of NPFF1R and RFRP-3 transcripts by fluorescent in situ hybridization in the dorsal horn of spinal cord, indicating that this receptor/peptide system can modulate nociception in part by spinal mechanism. We further observed that cells expressing NPFF1R transcripts were also MOP positives (50%) and DOP positives (20%), suggesting a direct modulatory role of NPFF1R on the action of opioid. Finally, we observed an increase of NPFF1R positive cells in dorsal horn of spinal cord of CFA-treated animals compared to saline controls suggesting a potential role of this system in inflammatory pain. In agreement with these data, we further showed that pharmacological blockade of NPFF1R with RF3286 can efficiently reverse hyperalgesia induced by CFA injection. Altogether, our data allowed us to identify NPFF1R/RFRP-3 as a pronociceptive anti-opioid system and further suggest that antagonists of this receptor might represent interesting therapeutic tools to limit the development of OIH and analgesic tolerance associated with chronic opioid administration as well as hyperalgesia induced by inflammatory pain.

The effect of Relaxin on pain : behavioral and morphological data

Cynthia Alkhoury Abboud

Despite its prevalence and negative impact, chronic pain remains poorly managed. Our previous results indicated a role for the relaxin-3/RXFP3 peptidergic system in pain. Indeed, the intra-cerebroventricular injection of an RXFP-3 agonist reduced mechanical, but not thermal, sensitivity to peripheral stimuli in a mouse model of inflammatory pain (CFA model). Preliminary results also suggested that these effects are mediated by a decrease of spinal neuron firing frequency, thus engaging modulation of descending pain pathways.

We aim at determining the brain areas responsible for relaxin-3/RXFP3 effects on pain. Because of their implication in pain circuits, we mainly focused on the anterior cingulate cortex (ACC) and the basolateral amygdala (BLA). We injected the RXFP3 agonist into the ACC or the BLA of control and CFA mice, and we assessed paw withdrawal threshold after mechanical (Von Frey test) and thermal stimulation (plantar test). In a second step, we used the RNAscope methodology to identify subpopulations of RXFP3 mRNA-containing inhibitory interneurons. Our results demonstrated the heterogeneity of the distribution of RXFP3 mRNA in the various brain areas investigated. RXFP3 mRNA+ cells mostly express somatostatin in the ACC and BLA. RNAscope experiments were then performed to assess possible changes in RXFP3 expression levels and distribution in inflammatory pain conditions.

Altogether, our results demonstrate that the relaxin-3/RXFP3 system has an analgesic effect mediated by brain areas involved in descending controls. Targeting RXFP3 signalling may represent a novel strategy to treat chronic pain through the modulation of descending pathways.

Oxytocin Acts on Astrocytes in the Central Amygdala to Promote a Positive Emotional State

Damien Kerspern

Oxytocin orchestrates social and emotional behaviors through known modulation of neural circuits in brain structures such as the central amygdala (CeA). Given the central role of astrocyte-neuron interactions in neuronal networks functions, we hypothesize that oxytocin effects on CeA neuronal network activity might be relayed by astrocytes. We discovered in the lateral and capsular part of the CeA (CeL) a subpopulation of astrocytes that expresses the oxytocin receptor (OTR). Those astrocytes are capable of direct response to oxytocin receptor activation by long-lasting oscillatory calcium transients, a signal that use GAP junctions to spreads through the CeL astrocytes syncytium. Furthermore, both the inhibition of astrocyte syncytium calcium transients and the specific genetic deletion of astrocyte OTR prevent the OT-induced modulation of CeA neuronal microcircuits. Therefore, the oxytocin-induced activation of CeL astrocytes leads to the modulation of CeA neuronal microcircuits. Finally, we showed that the astrocyte-dependent OT-induced modulation of CeA microcircuits play a pivotal role on modulation of pain- and anxiety related behaviors, not through nociception modulation by itself but through the modulation of anxiety related behavior in neuropathic animal. This suggest an OT-related modulation of the emotional valence of pain, leading to an increase of the sensation of “comfort”. Altogether these data highlight a key role for astrocytes in the oxytocin-induced modulation of central amygdala neuronal network and its behavior corelated.

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Conférence plénière 3

Peut-on se passer des morphiniques ?

Pr. Valeria Martinez, PU-PH (Hôpital Raymond Poincaré, Garches, France).

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Session 4 : Douleur et Parkinson.

Involvement of spinal cord dopamine depletion in the pathophysiology of nociceptive disabilities in a rat model of Parkinson's disease

Zoé Grivet

Parkinson's disease is a neurodegenerative disorder well known for its disabling motor symptoms and also for the wide range of non-motor symptoms, including pain, which is present in more than 80% of cases. However, the pathophysiology of this non-motor disabling symptom remains poorly understood. Using unilateral injection of 6-hydroxydopamine (6-OHDA) in the Medial Forebrain bundle (MFB) of adult male rats, we aimed to investigate how nociceptive integration is altered and how dopaminergic agents can reverse the changes observed after dopamine depletion. Mechanical allodynia and thermal hyperalgesia were evaluated using the von Frey and plantar tests respectively. Behavioral results showed that 6-OHDA injection into the MFB significantly decreased withdrawal threshold and latency of the contralateral hind paw compared to the ipsilateral side and to both sides of sham-operated animals. We next confirmed a spinal alteration of neuronal networks with electrophysiological recordings of wide dynamic range neurons that exhibit an increased response to electrical nociceptive stimuli. Intrathecal injections of dopamine agonists reversed the effect of dopamine depletion on the paw withdrawal threshold and latency of animals and re-established the c-primary sensory neurons mediated response in 6-OHDA animals. Finally, to study the specific role of dopamine projections to the spinal cord in nociceptive hypersensitivity, we used intrathecal injection of 6-OHDA. We found a significant bilateral decrease of paws withdrawal threshold and latency. These results strongly suggest that dopamine projections to the spinal cord are involved in pain hypersensitivity in Parkinson's disease and provide new routes for therapeutic care of pain in the disease.

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Keri-Ann Charles

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Noyau subthalamique et douleur, implication sur les symptômes douloureux dans la maladie de Parkinson

Veronique Coizet

Les symptômes de douleurs sont très fréquents dans la maladie de Parkinson. Les patients décrivent des sensations douloureuses intenses et sans cause apparente. Ce type de douleur est prédominant du côté du corps le plus affecté dans la maladie indiquant que ces symptômes seraient liés à un dysfonctionnement du système nerveux central (1). Curieusement, la stimulation cérébrale profonde du noyau subthalamique apparait être une technique thérapeutique efficace pour soulager ces douleurs (2). Cet effet parait surprenant car le lien entre le noyau subthalamique et douleur reste imprécis. Nous avons ainsi cherché à caractériser les traitements nociceptifs du noyau subthalamique et la connectivité anatomique de ce noyau avec des structures nociceptives (3,4).

A l'aide d'une technique d'électrophysiologie in vivo chez le rat anesthésié, nous avons ainsi montré que le noyau subthalamique présente des réponses neuronales toniques et phasiques complexes suite à des stimuli nociceptifs. De plus, la lésion de cette structure induit des changements des réponses nociceptives des rats, évaluées dans le test comportemental de la « hot plate ». Nous avons également caractérisé le rôle de deux structures nociceptives primaires dans la transmission d'informations nociceptives au noyau subthalamique, le colliculus supérieur et le noyau parabrachial. Nous avons montré que ces deux structures présentaient une projection anatomique directe sur le noyau subthalamique. Enfin, nous avons mis en évidence que cette structure présente des réponses nociceptives anormales dans un modèle de rat Parkinsonien.

L'ensemble de ces résultats indique que le noyau subthalamique est relié à un réseau cérébral nociceptif et impliqué dans le traitement et la modulation d'informations nociceptives. Ce lien pourrait sous-tendre l'effet bénéfique de la stimulation cérébrale sur les symptômes douloureux dans la maladie de Parkinson. Ces travaux ouvrent un nouvel axe de recherche pour explorer le rôle de cette structure dans l'expression des symptômes douloureux chez les patients Parkinsoniens, et dans d'autres maladies neurodégénératives.

Ablation of the tVTA compensates symptoms, including nociceptive, in a model of Parkinson's disease.

Michel Barrot

Parkinson's disease is partly caused by a death of dopamine neurons in the nigrostriatal pathway, its symptoms are both motor and non-motor, including pain and depression. The tail of the ventral tegmental area (tVTA) or rostromedial tegmental nucleus (RMTg) is a major GABAergic brake for substantia nigra pars compacta (SNc) dopamine cells. We tested the impact of tVTA lesion on symptoms in a model of Parkinson's disease. Rats with partial bilateral 6-OHDA SNc lesion displayed motor impairments that were suppressed by a co-lesion of the tVTA. Using a larger set of tests, we show that the SNc lesion also led to lower body weight, lower mechanical and thermal nociceptive thresholds, and a decrease in sucrose preference. Co-lesion of the tVTA compensated body weight, mechanical nociceptive thresholds and anhedonia-like behavior. These data highlight the influence exerted by the tVTA on dopamine systems, modulating for example nociceptive symptoms consecutive to a partial loss of dopamine cells.