

XVth SYMPOSIUM OF THE FRENCH PAIN RESEARCH NETWORK



March 22 & 23, 2019
Brain and Spine Institute (ICM)
Pitié-Salpêtrière Hospital, Paris

Organizing committee:

Yves BOUCHER, Didier BOUHASSIRA, Stéphane MELIK-PARSADANIAN TZ,
Nathan MOREAU, Sophie PEZET, Annabelle REAUX-LE GOAZIGO



The organizing committee would like to thank
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Welcoming address

On behalf of the organizing committee we are happy to welcome you to the XVth symposium of the French Pain Research Network, which will be held on March 22 and 23, 2019 at the Brain and Spine Institute (Institut du Cerveau et de la Moelle Epinière – ICM) within the Pitié-Salpêtrière Hospital, the largest hospital in Europe.

This year we are honored by the participation of members of the Wellcome Trust Pain Consortium. In the spirit of this collaboration, the whole event will be conducted in English and the oral presentation sessions will be moderated by senior investigators from both networks.

We hope you enjoy your stay in Paris and wish you a fruitful and stimulating symposium.

The organizing committee

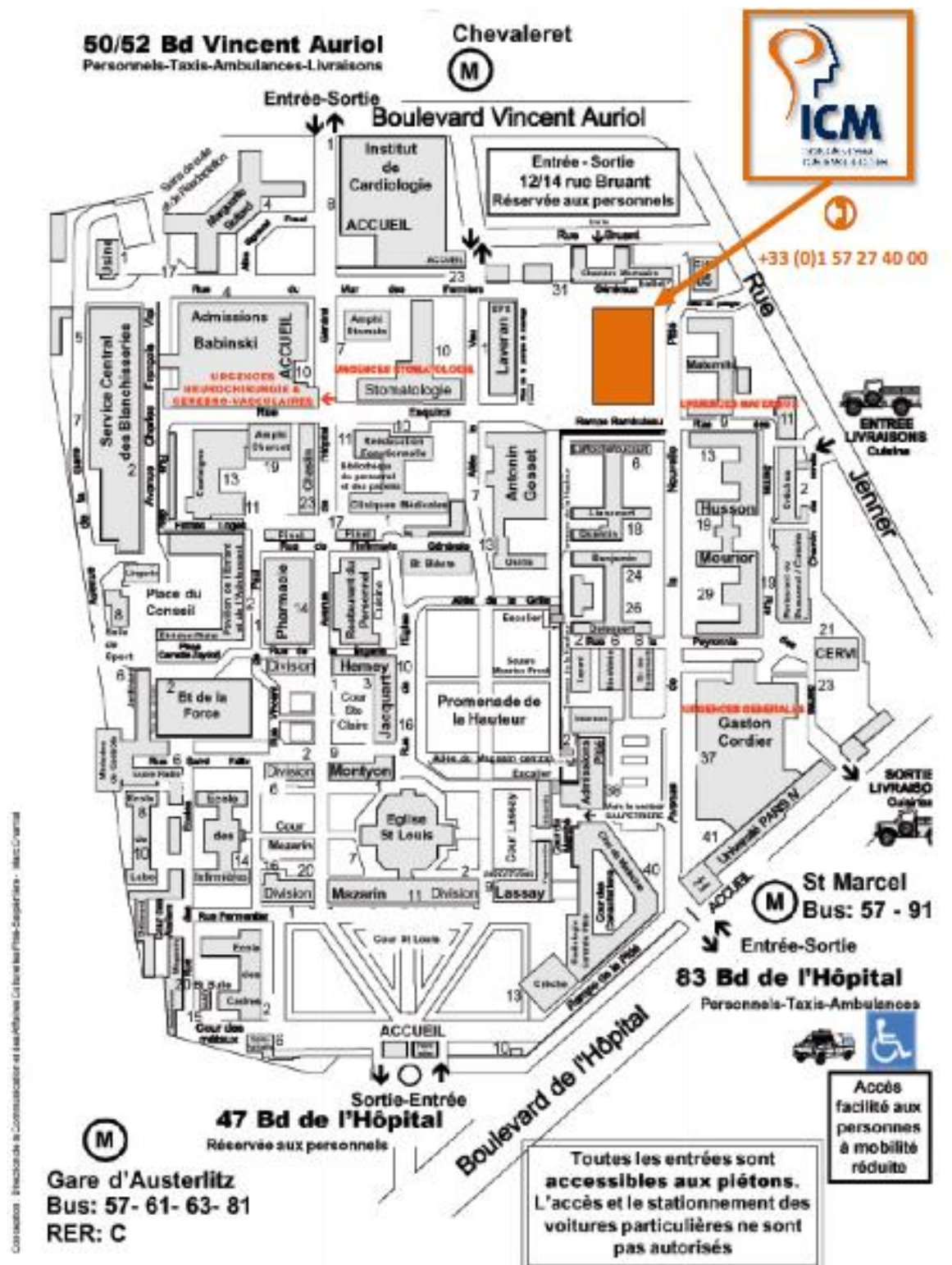
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General information

Venue address:

Institut du Cerveau et de la Moelle Epinière, Hôpital Pitié-Salpêtrière
 47 boulevard de l'hôpital 75013 Paris
 Subway Line 6: Chevaleret



Symposium overview

Friday March 22th, 2019

12:00 – 13:00	Registration
13:00 – 13:45	French Pain Research Network presentation – R. Dallel Wellcome Trust Pain Consortium presentation – S. McMahon ITMO Neurosciences conference – E. Hirsch
13:45 – 14:30	Keynote lecture: « <i>Pain in neurodegenerative disorders</i> » <i>N. Attal</i>
14:30 – 15:30	Spinal mechanisms of pain <u>Speakers:</u> AC. DICKIE, O. DAVIS, E. COURTY
15:30 – 16:30	Group Photo – Coffee break – Networking
16:30 – 18:45	Pain modulation <u>Speakers:</u> F. ABY, O. BOUCHATTA, D. FONTAINE, G. GAZZO, S. LEPETRE-MOUELHI, A. HAFIDI, M. CUMENAL
18:45 – 19:00	Sponsor presentation (Ugo Basile)
19:00	Diner cocktail with musical ambiance (in the ICM lounge)

Saturday March 23th, 2019

09:00 – 10:30	Spinal and trigeminal pain circuitry <u>Speakers:</u> A. FRUQUIERE, M. CANDELAS, D. FAKIH
10:30 – 11:15	Keynote lecture: « <i>Sexually dimorphic influence of the chemokine, colony stimulating factor 1, on spinal cord microglia and neuropathic pain processing</i> » – A. Basbaum (UCSF)
11:15 – 11:45	Coffee break
11:45 – 13:00	Inflammatory pain <u>Speakers:</u> S. PEZET, L. DELAY, C. PETITFILS, M. MEYNIER
13:00 – 14:00	Lunch break – Buffet
14:00 – 15:30	Cellular and molecular mechanisms of pain <u>Speakers:</u> DM. LOPES, M. MEGEMONT, N. PINTO PARDO, A. TASSOU, D. TEWARI
15:30 – 16:00	Awards ceremony (best presentations) and conclusion
16:00 – 17:30	Coffee break and networking
17:30	Event closure



Scientific programme

Saturday march 23, 2019 – Keynote lecture

Sexually dimorphic influence of the chemokine, colony stimulating factor 1, on spinal cord microglia and neuropathic pain processing

Allan Basbaum, UCSF, San Francisco, CA

Recent studies reported that microglia contribute differentially to neuropathic pain in male and female mice. Specifically, depletion of spinal cord microglia in male, but not in female mice, significantly reduced peripheral nerve injury-induced mechanical hypersensitivity in a model of neuropathic pain. To date, however, little is known about the molecular basis underlying these differences. One suggestion is that T cells contribute to the female hypersensitivity, and in their absence, dorsal horn microglia are responsible. In the present study we pursued the question by following up on our previous demonstration that nerve injury induces expression of the chemokine colony-stimulating factor 1 (CSF1) in injured sensory neurons, resulting in spinal cord microglia activation and mechanical hypersensitivity in male mice. Here we show that CSF1 has sex-specific functions in nerve injury-induced microglia activation and injury-induced hypersensitivity. Specifically, sensory neuron depletion of CSF1 prevents nerve injury-induced pain in male, but not female mice. We further demonstrate that intrathecal injections of CSF1, which result in strong microglia activation accompanied by mechanical hypersensitivity in male mice, have much smaller effects on female microglia and do not induce hypersensitivity. Finally, RNASeq analysis from FACS sorted microglia reveals dramatic sex-specific activation profiles in response to CSF1, indicating sex-specific CSF1 signaling to microglia. Importantly, we confirmed the prior observation that depletion of T cells counteracts the nerve injury induced hypersensitivity in female mice. Ongoing studies are examining a possible relationship between T cell activation and the loss of microglia responsiveness in female mice.

Scientific programme

Friday march 22, 2019 - Spinal mechanisms of pain

Substance P & GRP are expressed by distinct populations of excitatory interneuron in the dorsal horn

AC. Dickie¹, AM. Bell¹, N. Iwagaki¹, E. Polgár¹, M. Gutierrez-Mecinas¹, R. Kelly¹, H. Lyon¹, K. Turnbull¹, S. West², A. Etlin³, J. Braz³, M. Watanabe⁴, DLH. Bennett², A. Basbaum³, J. Riddell¹, AJ. Todd¹

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Excitatory interneurons account for the majority of neurons in the superficial dorsal horn, but despite their presumed roles in pain and itch mechanisms, our knowledge about their organisation and function remains limited. We recently identified two populations of excitatory interneuron defined by the expression of gastrin-releasing peptide (GRP) or substance P (SP). The aim of this investigation was to characterise GRP and SP cells, and to determine whether they are functionally distinct populations.

Our findings demonstrate that although there is very limited overlap between SP and GRP cells, they are largely separate populations. They differ in several electrophysiological parameters, including action potential firing patterns and responses to the application of opioids and monoamines. sEPSC and mEPSC frequency was higher in SP cells, suggesting that they receive greater excitatory drive than GRP cells. Analysis of somatodendritic morphology demonstrated that GRP and SP cells were morphologically distinct. Although GRP cells were morphologically heterogeneous, some of these could be classified as central cells. In contrast, many of the SP cells resembled radial cells.

Our findings demonstrate that GRP and SP cells show major differences in their morphological, electrophysiological and pharmacological properties, indicating that GRP and SP cells are functionally distinct, and presumably have different roles in processing somatosensory information.



Scientific programme

Friday march 22, 2019 - Spinal mechanisms of pain

The Role of RorB-expressing Lamina II Interneurons in Gating Nociceptive C-fibre Input

O. Davis, A. Dickie, K. Boyle, M. Mustapa, A. Todd, D. Hughes

Inhibitory interneurons in the spinal dorsal horn play a crucial role in controlling transmission of somatosensory information to the brain. Spinal inhibition is diminished in some chronic pain states, and inhibitory interneurons therefore represent a potential target for therapeutic intervention.

Previous studies have identified a population of inhibitory interneurons in lamina II that express the calcium binding protein calretinin, and shown that these are islet cells. We have identified a subpopulation of these cells that co-express the RAR-related orphan receptor beta (RorB), and this provides means of identifying and manipulating these cells. We have used a combination of anatomical and electrophysiological approaches with genetically modified mice in which fluorescent proteins are expressed in RorB interneurons. We show that the dendritic trees of the RorB cells overlap extensively with central arbors of C-fibre mechano-nociceptors (defined by expression of MrgD) and that they receive synaptic input from CMrgD afferents. We also find that their axons form axo-axonic synapses onto central terminals of type I glomeruli, which originate from these afferents. Peripheral stimulation under terminal anaesthesia revealed that the RorB cells are preferentially activated by noxious mechanical stimulation, rather than noxious chemical (capsaicin) or noxious heat stimulation. We therefore believe these interneurons play a critical role in setting mechanical pain thresholds.

Scientific programme

Friday march 22, 2019 - Spinal mechanisms of pain

Distinct contribution of non-peptidergic C-fibers in spinal and trigeminal formalin induced pain

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Studies in which different nociceptor populations have been deleted revealed remarkably selective behavioral deficits (e.g., heat, mechanical, or chemical pain), demonstrating the existence of behaviorally relevant peripheral-labeled lines for different modalities of pain. Some studies also found distinct functional implications of primary afferents in spinal and trigeminal pain processing. In the present study, we investigated the contribution of non-peptidergic (IB4) fibers in spinal and trigeminal inflammatory pain induced by subcutaneous injection of formalin. To assess their role, we used the ribosomal toxin, saporin, conjugated to the lectin IB4 to specifically ablate nonpeptidergic nociceptive C fibers. Injection of IB4-saporin or unconjugated saporin was performed into the trigeminal mental and infraorbital nerves, or spinal sciatic nerve. Three weeks later, rats received a unilateral subcutaneous injection of formalin into either the lip or the hindpaw. We found that IB4-saporin treatment significantly decreased the duration of hindpaw licking behaviour, but increased the duration of face rubbing behaviour. Collectively, these data show that non-peptidergic C-fibers have a distinct contribution in spinal and trigeminal processing of inflammatory pain.

Scientific programme

Friday march 22, 2019 – Pain modulation

Role of 5HT neurons of the raphe nuclei in spinal nociceptive transmission

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The monoamine serotonin (5HT) exhibits complex modulatory functions in different body areas including pain pathway. 5HT exerts both excitatory and inhibitory influences in pain transmission through peripheral modulation of nociceptive fibers and central modulation of dorsal horn neurons. The respective influence of peripheral or central 5HT is not clear so far. Here, we studied the role of central 5HT in the control of pain transmission in the spinal dorsal horn. We used genetically modified mice (B6.Cg-Tg(Fev-cre)1Esd/J), expressing the CRE recombinase in 5HT neurons. As a preliminary step, we crossed this transgenic mouse to an Ai9 Cre-reporter mouse and confirmed that (i) a majority of Cre positive neurons are localised in the 5HT raphe nuclei, i.e. dorsal raphe (DR), median raphe (MR) and in the rostro-ventral medulla (RVM), and (ii) 82.4% of Cre cells are serotonergic (TPH2 immunostaining positive) in the RVM. Injecting an AAV-FLEX-GFP in the RVM revealed GFP fluorescence in the spinal dorsal horn confirming descending 5HT fibers originating from the RVM. Then, we used optogenetics to activate or silent 5HT neurons in the raphe magnus and we showed that 5HT neurons exert a tonic inhibition of pain transmission in the spinal dorsal horn. This result suggests that (i) descending pathways are constitutively active in controlling pain transmission, and (ii) a decrease of this tonic activity could alter pain transmission in chronic pain syndromes.

Scientific programme

Friday march 22, 2019 – Pain modulation

Descending pain control dysfunction in a mouse model of Attention-Deficit/Hyperactivity Disorder

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Background: Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by impaired attention, impulsivity and hyperactivity. Recent evidence pointed to pain hypersensitivity in ADHD patients.

Aims: We used a neonatal injection of 6-hydroxydopamine with the aims to 1) provide evidence for, and analyze, comorbid pain behavior in this model, and 2) highlight circuits and mechanisms underlying pain alterations in ADHD animal model.

Results: We first validated our ADHD mouse model. Our data then indicated increased pain sensitivity in this ADHD-like model. We further demonstrated in vivo hyperexcitability of the anterior cingulate cortex (ACC) in ADHD-like conditions. In vivo recording of spinal neurons showed increased excitability of the spinal network in response to mechanical stimulations. Finally, pharmacological and optogenetics stimulation of the ACC changed the activity of wide-dynamic range neurons in the spinal cord, and modified pain behavior.

Conclusion: Our data suggested that ACC hyperexcitability after descending pain pathways, therefore increasing spinal neuron excitability. ADHD conditions are thus likely to increase pain sensitivity by changing sensory integration at the spinal level.

Perspective: We will elucidate the descending pathways that are affected by ACC hyperexcitability. We expect to determine overlapping circuits that could be targeted to treat pain and ADHD comorbidities.

Scientific programme

Friday march 22, 2019 – Pain modulation

Occipital nerve stimulation for refractory cluster headache: long-term efficacy and predictive factors

D. Fontaine, A. Leplus, A. Donnet, J. Regis, S. Blond, C. Lucas, S. Raoul, S. Derrey, E. Guegan-Massardier, B. Jarraya, B. Dang-Vu, F. Bourdain, D. Valade, C. Roos, C. Creach', S.C Chabardes, P. Giraud, J. Voirin, J. Bloch, S. Colnat-Coulbois, F. Caire, M. Lanteri-Minet

Chronic occipital nerve stimulation (ONS) has been proposed as a treatment for refractory chronic cluster headache (CCH) patients. Its efficacy has been evaluated only in small short-term series of cases. Our objective was to evaluate the long-term efficacy of ONS in a large series of CCH patients and to identify predictors of response.

We prospectively studied 105 patients with refractory CCH, treated by ONS within the ONS French national observatory (10 participating centers). Efficacy was evaluated by frequency, duration and intensity of CH attacks; quality of life (EQ-5D); functional (HIT-6, MIDAS) and emotional (HAD) impacts; medication consumption. Predictors of the response (defined as attack frequency decrease >50%) were studied by multivariate analysis.

At last follow-up (mean 43.8 months), attack frequency was reduced >50% in 69% of the patients. Median weekly attack frequency decreased from 20 (+/-22) attacks/w to 6 (+/- 12) attacks/w between baseline and last follow-up ($p < 0.001$). Preventive and abortive medical treatments were significantly decreased. Most of the patients (61%) were satisfied (PIGC score). In the whole cohort, functional impact (HIT-6 and MIDAS scores), anxiety (HAD-A) and the health-related quality of life (EQ-5D) significantly improved after ONS, and especially in the group of excellent responders (59%). Significant predictors of response were younger age, attacks strictly unilateral and preoperative low HAD Depression score. Adverse events consisted mainly in infection (6%), Lead migration (12%) and fracture (4,5%), local pain (20%), hardware dysfunction (8%).

Long-term efficacy of ONS in CCH is maintained over time and results in dramatic improvement of quality of life in responders. Complications are numerous, which requires optimization of hardware and surgical techniques. Predictors of good response to ONS were younger age, strictly unilateral attacks and absence of preoperative depression.



Scientific programme

Friday march 22, 2019 – Pain modulation

Can we rescue the consequences of maternal separation on nociception and oxytocin analgesia?

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It is now widely recognized that oxytocin (OT) has potent analgesic properties (1). We recently contributed to better understand how analgesia can be achieved when OT is released in the spinal cord (central action) and in the blood (peripheral action) (2,3). OT is also known to be a key player during early life development. Therefore, we examined the impact of neonatal maternal separation (NMS) on nociception ontogeny and on the efficacy of oxytocinergic analgesia. We already described that NMS is associated with pain hypersensitivity of adult rats (4). We also found that several pain inhibitory controls, including those using OT, are non-functional in rats that had been exposed to NMS. A pharmacological rescue, aimed at restoring OT levels, its downstream signaling or preventing epigenetic changes has been attempted. Together, these results suggest that early life stress durably impairs the adaptive processes of rats submitted to pain and non-painful stress. This might be of importance to better understand the role of OT in other types of neurodevelopmental pathologies.

1. Poisbeau, Grinevich, Charlet (2017) Curr Top Behav Neurosci.
2. Juif & Poisbeau (2013) Pain
3. Juif et al (2013) J Neurosci
4. Juif et al (2016) Eur J Neurosci



Scientific programme

Friday march 22, 2019 – Pain modulation

A new painkiller nanomedicine to by-pass the blood-brain barrier and the use of morphine

S. Lepetre-Mouelhi¹, J. Feng¹, S. Mura¹, P. Couvreur¹, A. Gautier²; M. Hamon², F. Coudoré³

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2. Centre de Psychiatrie et Neurosciences, INSERM UMR894, Univ. Paris Descartes, France

3. Lab. Neuropharmacologie, INSERM UMRS1178, Univ. Paris-Sud, France.

The clinical use of Enkephalin has historically been limited due to pharmacokinetic issues, including restricted plasma stability and blood-brain barrier permeability. This project describes a new enkephalin-based nanomedicine targeting pain, using biocompatible and biodegradable materials for drug delivery and targeting purposes, such as squalene. Here, we show for the first time, that this new nanoformulation prevented rapid plasma degradation of LENK and conferred to the released neuropeptide a significant anti-hyperalgesic effect in a carrageenan-induced paw edema model in rats (Hargreaves test) which lasted longer than after treatment with morphine. Pretreatment with opioid receptor antagonists such as naloxone (brain-permeant) and naloxone methiodide (brain-impermeant) reversed the nanoparticles induced anti-hyperalgesia, indicating that the LENK-SQ NPs acted through peripherally located opioid receptors. Moreover, the biodistribution of these NPs showed a strong accumulation within the inflamed paw as well as in the liver, spleen, and lung, while no signal could be detected in the brain, confirming the peripheral effect of LENK-SQ NPs. Of note, safety of these nanoparticles after intravenous administration was confirmed by normal levels of transaminase and normal histology of vital organs. This study represents a novel nanomedicine approach, allowing the specific delivery of LENK neuropeptide into inflamed tissues for pain control associated with inflammatory events.



Scientific programme

Friday march 22, 2019 – Pain modulation

Nigrostriatal dopaminergic depletion promotes neuropathic pain

A. Hafidi, O. Ouachikh, W. Dieb

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Parkinson's disease (PD) is characterized by pain symptom which may precede or occur simultaneously with the onset of motor disturbances. In order to explore the cellular and molecular mechanisms underlying pain in PD, the rat's model (6-OHDA toxin injection into the SNc) of PD has been used. This produce specifically the degeneration of dopaminergic neurons and dopaminergic depletion within the striatum. Mechanical (von Frey filament) and thermal (acetone) allodynia within the face and hind paw were explored in this model. pERK1/2 expression, which constitutes a molecular pain signature, was significantly higher in insular and cingular cortices and within the medullary dorsal horn 6-OHDA-lesioned rats. Pain chronicity was also investigated using the PKC ζ . There was a high expression of this protein in medullary and spinal dorsal horns of 6-OHDA-lesioned rats when compared to sham. The allodynic behavior could be alleviated by intrathecal or intracisternal administration of bromocriptine which is a D2R agonist. Our data shows that bilateral dopamine depletion promoted neuropathic pain (allodynia). The observed neuropathic pain involved different central structures and probably pain descending inhibitory pathways. The latter is highlighted by an increase in pERK1/2 and PKC ζ expression within medullary and spinal dorsal horns. This elevation of pERK1/2 and PKC ζ expression is probably due to a high excitation or a lower inhibition within medullary and spinal dorsal horns.

Scientific programme

Friday march 22, 2019 – Pain modulation

Research into new molecular targets for the treatment of prostate cancer pain

M. Cumenal, S. Lamoine, L. Prival, L. Boudieu, Y. Aissouni, J. Busserolles

INSERM, UMR 1107, Laboratory of Clinical and Fundamental Pharmacology of Pain, Clermont-Ferrand, France

Introduction: Cancer bone pain is both highly disabling and resistant to standard analgesic treatments. Riluzole, an antiglutamatergic molecule, has both analgesic potential in various pain models and has also shown an antiproliferative effect on several cancer cell lines. The purpose of our study was to evaluate the effect of riluzole on bone pain in prostate cancer (CaP) in vivo and on the proliferation of human CaP cells in vitro.

Methods: We used a murine model of bone cancer pain induced by unilateral injection of human PC3 cells into the tibia of male SCID mice. Seven days after injection, the animals received or not riluzole (60µg/ml) in the drinking water. Pain symptoms and analgesic effect of riluzole were measured on days 14, 21, 28 and 35 post-injection of the tumor using different spontaneous and evoked pain tests. In vitro, we evaluated the effect of riluzole, whether or not combined with docetaxel, on both PC3 and LNCAP cells viability.

Results: We show that animals treated with riluzole show a reduction in mechanical hypersensitivity and spontaneous pain in CaP pain model animals. In addition, in vitro data suggest an antiproliferative effect of riluzole on PC3 and LNCAP cells.

Conclusion: All these data open up prospects for identifying new molecular targets for the treatment of CaP pain and suggest the interest of using antiglutamatergic molecules in this context.



Scientific programme

Saturday march 23, 2019 – Spinal and trigeminal pain circuitry

Chasing the role of T-type channels at multiple levels of neuronal pain circuitry

A. Fruquière¹, M. Candelas¹, B. Bonnetaz¹, F. Aby², S. Fayad³, G. Ourties⁴, P. Giraud¹, M. Landry², P. Fossat², C. Mallet⁴, A. François¹, R. Lambert³, N. Leresche³, S. Laffray¹, PF. Mery¹, E. Bourinet¹

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Many studies have shown that T-type calcium channels, encoded by the Ca_v3.2 isoform, are involved in the pathophysiology of pain in the peripheral nervous system. The impact of Ca_v3.2 channel in the central nervous system remains to be explored. Thanks to a Ca_v3.2-GFP-Lox murine model that we are evaluating i) the localization of Ca_v3.2 positive neurons in the nervous system, and ii) the impact of tissue specific deletion of Ca_v3.2 by the local effect of Cre recombinase encoded by viral vectors at multiple CNS sites on neuropathic pain sensitivity. Using extracellular in vivo recordings of spinal projection neurons, we found that spinal dorsal horn Ca_v3.2 KO decreases the pathologic integration of peripheral nociceptive messages. Cav3.2 ablation in spinal networks abolishes cold and mechanical allodynic nociceptive behaviors, reduces spontaneous pain, and attenuates chronic pain comorbid anxiety. This approach is being developed for supra spinal sites with the ongoing characterization of allodynic effect as well as changes in neuronal excitability in thalamic nuclei. These studies evaluate in parallel the capacity of these local Ca_v3.2 cKO to occlude the analgesic effects of the orally administered potent T-type calcium channel blocker TTA-A2, to evaluate the major site(s) of action of the molecule. The presentation will summarize these results with a focus on spinal cord data.



Scientific programme

Saturday march 23, 2019 – Spinal and trigeminal pain circuitry

Ca_v3.2 T-type calcium channels shape electrical firing in mouse Lamina II neurons

M. Candelas¹, A. Reynders², M. Arango-Lievano¹, C. Neumayer¹, A. Fruquière¹, J. Hamid³, C. Lemmers¹, A. Monteil¹, S. Laffray¹, P. Inquimbert⁴, Y. LeFeuvre⁵, GW. Zamponi³, A. Moqrich², E. Bourinet¹, PF. Méry¹

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The T-type calcium channel, Ca_v3.2, is necessary for acute pain perception, as well as mechanical and cold allodynia in mice. Being found throughout sensory pathways it is a promising target for analgesics. We have detected Ca_v3.2 in ~60% of the lamina II (LII) neurons of the spinal cord, where it was expressed with markers of excitatory and inhibitory neurons, including calretinin and PKCγ. T-type blockers slowed the inhibitory transmission in LII neurons. T-type blockers also abolished low-threshold activated currents, rebound depolarizations, and blunted excitability. The targeted recording of Ca_v3.2+ LII neurons, after intraspinal injection of AAV-DJ-Cav3.2-mcherry, showed that their properties resembled those of the global population. However, Ca_v3.2 ablation in the dorsal horn of Ca_v3.2GFP-Flox KI mice after intraspinal injection of AAV-DJ-Cav3.2-Cre-IRES-mcherry, had drastic effects. Indeed, it 1) blunted the likelihood of transient firing patterns; 2) blunted the likelihood and the amplitude of rebound depolarizations, 3) eliminated action potential pairing, and 4) remodeled the kinetics of the action potentials. In contrast, the properties of Ca_v3.2+ neurons were only marginally modified in Ca_v3.1 knockout mice. Overall, in addition to their previously established roles in the superficial spinal cord and in primary afferent neurons, Ca_v3.2 channel appear to be necessary for specific, significant and multiple controls of LII neuron excitability.

Scientific programme

Saturday march 23, 2019 – Spinal and trigeminal pain circuitry

Chronic dry eye induced corneal hypersensitivity, neuroinflammation and synaptic plasticity

D. Fakh^{1,2}, Z. Zhao*², C. Baudouin^{2,3,4}, S. Melik Parsadaniantz², A. Réaux-Le Goazigo²*

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- 2. Sorbonne University INSERM, CNRS, Institut de la vision, France*
- 3. Quinze-Vingts Hospital, France*
- 4. Versailles University, France*

Dry eye disease (DED) is a multifactorial disease associated with ocular surface inflammation and ocular pain symptoms. The study aims to characterize a novel mouse model of DED obtained by a unilateral excision of the extraorbital lachrymal gland (ELG) and Harderian gland (HG). A drastic reduction of tear production and corneal inflammation were reported at 7, 14 and 21days post-surgery. DED animals developed a mechanical corneal hypersensitivity and a significant increase of the spontaneous ciliary nerve fiber activity. At D21 post-surgery, expression of oxidative (iNOS2 and NOX4), pro-inflammatory (IL-6 and IL-1 β) and astrocyte (GFAP) markers significantly increased in the ipsilateral trigeminal ganglion (TG). DED animals also exhibited higher Iba1, GFAP and activating transcription factor-3 (ATF-3) staining in the ipsilateral TG. At D21, pro-inflammatory cytokines (IL-6, TNF α , IL-1 β and CCL2), iNOS2, neuronal (ATF3 and FOS) and microglia (CD68 and ITGAM) markers were upregulated in the trigeminal brainstem sensory complex (TBSC). In this central structure, we highlighted an increased GFAP and Iba1 immunoreactivity. Consistently, a chronic DE resulted in a central presynaptic plasticity showed by increased piccolo staining in the ipsilateral TBSC. These data highlight neuroinflammatory responses and a central plasticity essential to the development of persistent dry eye pain. This model may be useful to identify new analgesic molecules aimed to alleviate ocular pain.



Scientific programme

Saturday march 23, 2019 – Inflammatory pain

Ultrafast ultrasound imaging of the rat spinal cord

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The aim of this study, performed in collaboration with M. Tanter who developed this technique, was to image haemodynamic variations of the spinal cord in the rat. If such an approach was possible, our goal was twofold: 1) to validate the possibility of imaging haemodynamic spinal variations following nociceptive stimulation and to characterize its sensitivity / selectivity.

Using a surgical approach from the least invasive to the most invasive, we have shown that we can observe with a good spatial resolution (100um) and temporal (0.2 msec-1sec) the vascularization of the spinal cord of the rat, through a laminectomy. The study of spinal functional haemodynamic variations showed a significant increase of the Doppler signal in the ipsilateral dorsal horn to the application of a nociceptive, mechanical or electrical peripheral stimulus. The analysis of the responses induced by the different types of peripheral fibers showed a significant increase exclusively following the stimulation fibers Ad and C. Finally, under conditions of hyperexcitability medullary, following the repeated stimulation of the C fibers in naïve animals, or following peripheral inflammation, the responses to A beta fibers stimulations are exacerbated.

In conclusion, functional imaging is possible and gives very interesting results that are in agreement with the electrophysiological data reproduced by many groups.



Scientific programme

Saturday march 23, 2019 – Inflammatory pain

ACPA autoantibodies induced pain-like behaviour and bone erosion – involvement of ASIC3 channels

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Joint pain is one of the most debilitating symptoms of rheumatoid arthritis (RA). We previously demonstrated that transferring ACPA IgG purified from RA patients to mice induces pain-like behaviour and bone erosion. Thus, we investigated if monoclonal RA-associated antibodies (mAbs) with particular epitope specificities differ in their abilities to induce pain and bone-erosion. 1103:01B02, 1325:01B09, 1276:01D10 and 1276:01G09 (control IgG) mAbs generated from RA patients were injected alone or in combination (B02/B09, B02/D10) in mice. mAbs with particular epitope specificities differ in their abilities to induce pain-like behaviour and bone-erosion with B02/B09 combination having the most potent effect. This effect was not associated with inflammation but administration of specific blockers of osteoclast activity prevented the development of B02/B09 induced mechanical hypersensitivity. The B02/B09 pronociceptive effect was associated with an increase of ASIC3 and TRPV1 expression in DRGs neurones and its effect was inhibited by APETx2 administration and in ASIC3 KO mice. We confirmed that monoclonal ACPA IgG1 subtypes differ in their pronociceptive and bone erosive properties certainly link to their reactivity patterns against citrullinated epitopes on different targets especially those engaging osteoclast activity. As a result of ACPA-induced osteoclast activation, certain factors (e.g. protons and lipids) are released, which sensitize ASIC3, ultimately leading to pain.

Scientific programme

Saturday march 23, 2019 – Inflammatory pain

Bacteria induce the release of proalgesic lipids by epithelial cells

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Irritable bowel syndrome (IBS) is characterized by visceral hypersensitivity associated with gut microbiota dysbiosis. Among the studied pronociceptive mediators, host and bacterial lipid compounds have been described as major regulators of visceral hypersensitivity. The aim of our study was to identify the role of the host and bacterial lipids interplay on visceral hypersensitivity. To differentiate bacterial and host lipids, bacteria were cultivated with ¹³C- glucose as unique source of carbon. ¹³C-labelled bacterial lipids were used to treat polarized epithelial cells. Quantification by mass spectrometry of ¹³C-labelled and unlabeled lipids in the basolateral compartment allowed us to determine epithelial and bacterial lipids potentially in contact with nerve endings. We quantified an increase of bacterial C10-3OH, C12AsnOH and C14AsnOH as well as epithelial 9,10-DiHOME and 12,13-DiHOME in the basolateral compartment. We assessed the ability of these lipids to increase intracellular calcium concentration in primary culture of mouse sensory neurons. Only the 9,10-DiHOME induced calcium flux in sensory neurons. This effect was inhibited by a pretreatment with a TRPV1 antagonist (AMG9810). Our study show that bacterial lipids induced the release of potentially proalgesic lipids by epithelial cells. Lipid metabolism could be the link between dysbiosis and visceral pain in IBS.

Scientific programme

Saturday march 23, 2019 – Inflammatory pain

Role of IL-22 pathway on intestinal disturbances in case of *Citrobacter rodentium* infection

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Introduction: Colonic hypersensitivity (CHS), main feature of Irritable bowel syndrome (IBS), is often associated with comorbidities such as anxiety. Some patients develop such disorders after a gastrointestinal infection, so called post-infectious IBS (PI-IBS). *Citrobacter rodentium* infection in mouse allows us to mimic the pathophysiology of PI-IBS. Interleukin-22 (IL-22) could be involved in intestinal homeostasis by promoting intestinal barrier integrity.

Materials and Methods: C57BL/6 mice were infected with a *C. rodentium* strain. A strain of *Lactococcus lactis* was also used as a vehicle to carry an eukaryotic expression plasmid of the murine IL-22 (L.lactisIL-22) to host intestinal epithelial cells, thus allowing its expression in the colonic mucosa. Pathogen colonization was followed by MacConkey agar plating. Anxiety-like behavior was assessed using the elevated plus maze test and animal well-being performing by PhenoTyper®. CHS was evaluated using colorectal distensions.

Results: Mice infected by *C. rodentium* exhibited CHS and anxiety-like behavior after clearance of the pathogen. Infected mice treated with the L.lactisIL-22 after bacterial clearance exhibited a reduced CHS and anxiety-like behavior in comparison with infected control mice. Data from the PhenoTyper® suggested a well-being improvement for L. lactisIL-22 treated mice.

Conclusion: These data suggest that IL-22 may be a promising therapeutic target for relieving symptoms associated with PI-IBS.



Scientific programme

Saturday march 23, 2019 – Cellular and molecular mechanisms of pain

A role for the splicing regulator Srrm4 in sensory neurons

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The serine/arginine repetitive matrix protein 4 (Srrm4) - a RNA binding protein and pre-mRNA splicing (AS) regulator – has been recently identified and found to be expressed exclusively in neuronal cells, especially during early stages of development. Loss of Srrm4 function leads to a decrease in neurite outgrowth in hippocampal neurons, abnormal cortical layering and defective neurogenesis. Although it is clear Srrm4 play a role in central development, to date it is not clear its contribution for pain and nociception.

Here we set out to investigate whether Srrm4 plays a role in the sensory processing using a mouse model. Our molecular studies show that Srrm4 knockout (KO) is present in the adult DRG neurons, suggesting this gene might play a role in nociception. We show that cultured DRG neurons from Srrm4 KO mice present defected neurite outgrowth. Our data show a skewed distribution in the size of neuronal cell body in Srrm4 KO mice with a small shift in the proportion of non-peptidergic and myelinated fibres in mice lacking the Srrm4 gene. We also show that expression of Srrm4 increases after partial sciatic nerve ligation, and mice lacking the Srrm4 gene show a much higher mechanical threshold after injury. Together, our data suggest that Srrm4 plays a critical role for sensory neuron development and a potential direct involvement of Srrm4 in nociception in the adulthood.

Scientific programme

Saturday march 23, 2019 – Cellular and molecular mechanisms of pain

Astrocytes contribute to the trigeminal central sensitization in rat model of chronic migraine

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Repeated migraine attacks are associated with maladaptive neural plasticity and lead to chronic headache. Sensitization of pain networks play an important role in the transition to the chronic forms of the disorder. It is increasingly recognized that astrocytes are activated in the medullary dorsal horn (MDH) in response to peripheral tissue injury and are involved in central sensitization. Here, we hypothesized that D-Serine, an astroglial neurochemical and NMDA co-agonist, participate in the central sensitization underlying a NO-donor-induced migraine model in the rat. Combining behavioral and in vivo electrophysiological approaches, our study proved the contribution of astrocytes activity in cephalic cutaneous hypersensitivity and neuronal activation within the MDH. Recurrent systemic administration of isosorbide dinitrate (ISDN), a nitric oxide donor, induce a persistent cephalic mechanical allodynia and facilitated C-fiber-electrically-evoked responses of MDH neurons. We demonstrate for first time that intracisternal application of L- α -Aminoadipic acid (gliotoxin) and D-Amino-Acid-Oxidase (D-Serine degrading enzyme) prevented trigeminal sensitization in both approaches, but this preventive effect was abolished by D-serine administration. Interestingly, our results suggest that astrocytes contribute to the trigeminal central sensitization and cephalic cutaneous hypersensitivity that characterize the migraine progression.



Scientific programme

Saturday march 23, 2019 – Cellular and molecular mechanisms of pain

Activation of Cyclin-Dependent Kinase 5 induces facial pain symptoms in adult rats

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Cyclin-dependent kinase 5 (Cdk5), a serine/threonine kinase, activated by the neuron-specific p35 and p25 proteins, plays an important role in neuronal development, and deregulation of its activity leads to neurodegenerative disorders. Cdk5 also regulates nociceptive signaling. Here, we characterized the involvement of Cdk5 in facial inflammatory pain symptoms. We found that intracisternally applied roscovitine, a Cdk5 antagonist, prevents formalin-induced facial rubbing behavior as well as formalin or CFA induced-mechanical allodynia. Roscovitine also inhibits the basal responses of medullary dorsal horn (MDH) WDR neurons to noxious, but not innocuous, stimuli. Interestingly, it prevents the CFA-induced potentiation of the responses of WDR neurons to both innocuous and noxious mechanical stimulations. Finally, both formalin and CFA strongly increase p35 and p25, but not Cdk5, protein levels in the trigeminal ganglion and MDH. This indicates that Cdk5 activity is necessary for selectively C-fiber-MDH neuron transmission, and its activation, following p35 and p25 protein elevation, is involved in the induction of peripheral inflammation-induced spontaneous pain and mechanical hypersensitivity.

Scientific programme

Saturday march 23, 2019 – Cellular and molecular mechanisms of pain

The receptor tyrosine kinase FLT3 regulates morphine-induced analgesia

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Opioids remain the unsurpassed treatment for the management of acute and chronic pain. Despite their potent analgesic effectiveness, their use is limited by detrimental side effects including opioid tolerance (OT) and opioid induced-hyperalgesia (OIH). Both mechanisms counteract opioid analgesia. Interestingly, peripheral μ opioid receptor (MOR) from dorsal root ganglion (DRG) neurons seems to be critical in OT. Since on the one hand neuropathic pain shares some common mechanisms with OIH and on other the hand the peripheral neuronal FLT3 controls the development and the maintenance this type of persistent pain, we considered the role of FLT3 in OIH. We first showed that FLT3 is co-expressed in some MOR-positive DRG neurons. Using Time- Resolved-FRET (TR-FRET), a specific molecular interaction between MOR and FLT3 was observed in HEK293 cells. Chronic morphine application on native primary sensory neurons produced the loss of morphine inhibitory effects on cAMP production, known to regulate OT. Morphine inhibitory effects are restored in chronic morphine treated DRG culture from FLT3 KO mice. At the behavioral level, the deletion of FLT3 expression prevented OIH in animals treated chronically with morphine whereas a single intrathecal injection of FL reduced morphine analgesia and induced sustained OIH. Both OT and MIH were prevented by the FLT3 inhibitor BDT001 administration. Our data identified a cross-talk between FLT3 and MOR in DRG neurons responsible for OT and OIH.

Scientific programme

Saturday march 23, 2019 – Cellular and molecular mechanisms of pain

Mechanisms of IL6-mediated hyperalgesia

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In this study we investigate the role of IL6 in engaging persistent pain mechanisms by in-vivo electrophysiological recordings and behavioural assessment to investigate its involvement in spontaneous pain.

Rats were implanted with osmotic minipumps filled with either saline or IL6 inserted intrathecally, delivering IL-6 or saline for 3 days. Additionally, rats with spinal nerve ligation received either anti-IL-6 antibody tocilizumab, or saline in a single i.p injection. Neural recordings and behavioural testing were carried out at day 3 post-implant/SNL. Behavioural testing continued to day 7.

Intrathecal IL6 induced spontaneous firing of sensory afferents that closely mimicked the spontaneous activity seen following SNL. In IL6-treated rats, spontaneously active fibres were found in $76\pm 8\%$ of strands (mean \pm SEM). By comparison, $87\pm 5\%$ of strands in SNL rats contained ectopically active units. In SNL rats treated with tocilizumab, $68\pm 1\%$ strands were active. In behavioural studies, animals receiving IL6 intrathecally showed significant reduction in paw withdrawal thresholds to mechanical stimulation as compared to controls. Tocilizumab significantly attenuated the post-SNL increase in mechanical sensitivity for up to 7 days after treatment as compared to control.

This study has established the involvement of IL6 in the spontaneous firing of sensory fibres and mechanical hypersensitivity in rats following nerve injury.