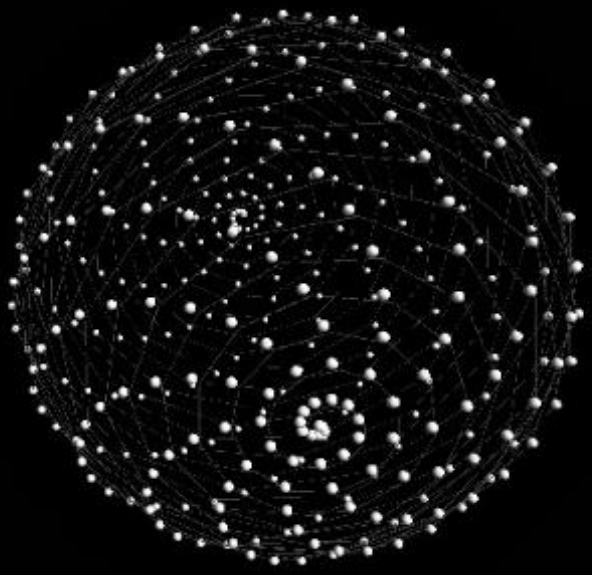
Livret de résumés
Abstract booklet



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Présentations blitz

Blitz presentations

1 - Creation of a brain-penetrant peptide-neurotensin(8-13) conjugate exerting analgesic activities after systemic administration

Jérôme Côté[1,2], Michel Demeule[3], Nicolas Beaudet[1], Anthony Regina[3], karine Belleville[1,2], Alain Larocque[3], Jean-Michel Longpré[1,2], Jean Lachowicz[3], Jean-Paul Castaigne[3], Philippe Sarret[1,2]

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Neuropeptides play a crucial role in brain functions, and peptide receptors hold great promise in advancing structure-based drug discovery for the treatment of brain disorders. However, one of the major remaining challenges in the development of peptides as potential drugs is to achieve therapeutic brain concentrations after systemic delivery, the blood-brain barrier (BBB) preventing entry of molecules from cerebral capillaries into surrounding brain tissue. In the present study, we conjugated the biologically active neurotensin fragment (NT(8-13)), which produces strong analgesia when injected directly into the brain to the Angiopep-2 peptide (An2), a proprietary 19–amino acid peptide that crosses the BBB by LRP1 receptor-mediated transcytosis. The brain uptake of this new chemical entity, An2-NT(8-13), was first determined using positron emission tomography coupled to computed tomography (PET/CT) imaging. For this purpose, we acquired dynamic PET scans over 60 min followed by a CT scan and quantified brain distribution of ^{64}Cu-radiolabeled An2-NT(8-13) with or without pre-blockage of LRP1 receptors with an excess of unlabeled An2. These experiments showed that An2-NT(8-13) accumulates more efficiently in the brain when pre-blockage is not performed, thus demonstrating transcytosis through a LRP1-dependent mechanism. We next investigated whether the An2-NT(8-13) conjugate exhibited potent analgesic activity in different pain models. In rats, An2-NT(8-13) administered intravenously (i.v.) attenuates the stereotypical nociceptive behaviors observed following intraplantar injection of formalin into the right hind paw (formalin tonic pain model). At a dose of 0.05 mg/kg, An2-NT(8-13) was also effective in reversing the pain behaviors induced by chronic constriction injury of the sciatic nerve (neuropathic pain). Finally, we found that i.v. An2-NT(8-13) significantly reversed the allodynic state induced by the femoral inoculation of MRMT-1 rat breast cancer cells (bone cancer pain). Altogether, these results demonstrate that the An2-NT(8-13) derivative penetrates the BBB efficiently after systemic administration and mediates relief of chronic pain, thus supporting the potential of An2-NT(8-13) as a first-in-class NT-based chronic pain therapeutic.

2 - An α2,3 GABA(A) receptor synaptic switch is associated with the KCC2 deficit in neuropathic pain: therapeutic implications.

Louis-Etienne Lorenzo[1,2,3], Antoine G. Godin[1,4], Dominic Boudreau[1], Nicolas Doyon[1], Karine Bachand[1], Francesco Ferrini[5], Alfredo Ribeiro-da-Silva[2,3], Yves de Koninck[1,6]

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[6]Université Laval

Loss of spinal inhibition has long been hypothesized to underlie pain hypersensitivity after peripheral nerve injury (PNI). But how GABA(A)and glycine receptor-mediated inhibition is modified has remained elusive. Here, we show a reduction in number of inhibitory synapses and of the K+-Cl- transporter KCC2 in the spinal dorsal horn after PNI. In contrast, this is accompanied by an increase in GABA(A)R(but not GlyR) synaptic expression and a selective switch towards α2,3 GABA(A)R subunits. Consistently, GABAAR mIPSC decay kinetic was prolonged, enhancing Cl- load. BDNF administration replicated the synaptic GABAAR plasticity and blocking TrkB reversed the PNI-induced changes identifying BDNF-TrkB signaling as both necessary and sufficient to explain both synaptic GABA(A)R and KCC2 plasticity. Yet, KCC2 hypofunction mitigates the efficacy of benzodiazepine-induced analgesia. Rescuing Cl- transport with the KCC2-enhancer CLP257 potentiated analgesia by the α2,3-GABA(A)Rpreferring L838,417 benzodiazepine. These findings point to a double strategy for analgesia: targeting the proper GABAAR subtypes while restoring Cl- homeostasis.

3 - Changes in DNA methylation in the prefrontal cortex in chronic neuropathic pain: effect of S-Adenosylmethionine (SAM) on pain behaviors and co-morbidities

Stephanie Gregoire[1], Renaud Massart[2], Magali Millecamps[1], Sergiy Dymov[2], Sonia Do Carmo[2], A. Claudio Cuello[2], Moshe Szyf[2], Laura Stone[1]

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Despite considerable advances in understanding mechanisms involved in chronic pain, it remains difficult to treat effectively. Co-morbid conditions including anxiety, depression and cognitive impairment further impact quality of life. Chronic pain is associated with reversible changes in brain anatomy and function and with long-term alterations in gene expression. One mechanism underlying stable, long-term programming of gene expression is DNA methylation, a dynamic process that responds to environment and experiences throughout the life cycle. We previously reported a decrease in global DNA methylation in the prefrontal cortex (PFC) in a rodent model of chronic neuropathic pain (Spared Nerve Injury, SNI). However, neither the identity of individual differentially regulated genes nor the effect of pharmacologically increasing global methylation on pain, mood and cognitive have been examined.

In the current study, DNA methylation was first explored in SNI and control animals in rat PFC nine months following nerve injury. Genome-wide DNA methylation analysis revealed difference in the DNA methylation landscape in the brain at over 12,000 individual genes. DNA methylation of numerous individual genes the PFC showed robust correlation with pain sensitivity (von Frey test), including genes involved in the DNA methylation machinery (e.g. DNA methyltransferases, DNMTs). The expression levels of the enzymes that catalyze the DNA methylation reaction, DNMT3a and DNMT3b, showed an increase and a decrease in SNI, respectively. This first study supports the plausibility of DNA methylation involvement in chronic pain.

In the second study, the effect of modulating DNA methylation on pain-related behaviors was assessed. Animals with nerve injury and sham surgery controls were treated with the methyl donor S- adenosylmethionine (SAM). SAM is used by the DNMTs to transfer a methyl group to cytosine bases in DNA. Three months following nerve injury, SAM or saline solution was given orally for four months and the effect of SAM on sensitivity to mechanical and cold stimuli, anxiety/depressive-like behaviors, pain avoidance and cognitive ability were measured. SAM attenuated SNI-induced mechanical hypersensitivity and pain avoidance in SNI mice. SAM had no effect on cold sensitivity and on anxiety/depressive-like behaviors but reversed the SNI-induced cognitive impairment in the novel object recognition test.

In summary, chronic neuropathic pain results in changes in the DNA methylation of thousands of individual genes in the rodent PFC and the repeated administration of a methyl donor attenuated sensory and cognitive symptoms associated with nerve injury.

Funding: Supported by a grant from Pfizer Canada to LSS and MS and a Louise and Alan Edwards Foundation Postdoctoral Fellowship to SG.

4 - Figuring out how CCL2 and CCR2 contribute to chronic pain by using RNAi technology.

Marc-Andre Dansereau[1], Ashley Jacobi[2], Scott Rose[2], Mark Behlke[2], Jean-Michel Longpré[1], Philippe Sarret[1]

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Activation of the chemokine receptor CCR2 in the spinal cord is a key component of chronic pain. Many studies reported analgesic properties of various strategies aiming to prevent its activation. However, these have not yet yield any clinically useful therapy so far. It suggests that the mechanisms by which CCR2 activation contributes to hypernociceptive states are more complex than anticipated and require a deeper understanding in order to take advantage of. As such, we used dicer substrate small interfering RNAs (DsiRNAs) targeting CCL2 or CCR2 in the dorsal root ganglia (DRGs) in a rat model of chronic inflammatory pain induced by complete Freund's adjuvant (CFA). When injected pre-emptively, both DsiRNA prevented CFA-induced mechanical hypersensitivity for 5 days. When injected on days 1 and 2 post-CFA, CCR2 DsiRNA was similarly effective, while CCL2 DsiRNA analgesia was gone by day 5. Interestingly, animals receiving CCR2 or CCL2 DsiRNA on day 1 and 2 post-CFA showed reduced mechanical hypersensitivity on day 14, while having similar hypersensitivity to untreated CFA animals on day 7, 10 and 21. This suggests a dynamic biphasic role of DRG-derived CCL2 and CCR2 in the context of chronic inflammatory pain.

5 - Interrogating the role of peripheral opioid receptors using an optogenetic approach

Hélène Beaudry[1,2], Ihab Daou[1,2], Ariel R Ase[1,2], Alfredo Ribeiro-da-Silva[1,2], Philippe Séguéla[1,2]

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Opioids are the most potent analgesics currently available, but their use is often accompanied by un-wanted side effects such as nausea, constipation, respiratory depression and sedation. To overcome this limitation, peripheral administration of opioids represents an interesting alternative. Opioids bind and activate 3 receptor subtypes, namely mu (MOPR), delta (DOPR) and kappa (KOPR) expressed in peripheral tissues such as sensory primary afferents. Until recently, DOPR and MOPR were thought to be co-expressed in both peptidergic and non-peptidergic nociceptors in most species. In the mouse howev-er, opioid receptors have been shown to be segregated in primary afferent neurons, with DOPR and MOPR expressed in non-peptidergic (MrgprD-positive) and peptidergic (TRPV1-positive) neurons re-spectively. In addition to these distinct expression patterns, it has been reported that MOPR activation exclusively relieves thermal pain whereas DOPR activation decreases mechanical pain, which casts a doubt on many past studies.
To enable control of specific genetically-identified neurons in vivo, optogenetics involves the expression of light-sensitive channels (e.g. ChR2) that transduce pulses of light into action potential trains. Using this approach, precisely timed depolarization can be achieved in freely moving mammals, with high cel-lular and spatial selectivity. In the present study, we investigated the role of peripheral MOPR and DOPR in 3 optogenetic mouse lines. We compared the analgesic effect of deltorphin II and DAMGO, respective agonists of DOPR and MOPR, on light-induced pain behaviors in Nav1.8-ChR2, TRPV1-ChR2 and MrgprD-ChR2 mice. Unexpectedly, our results show that intradermal delivery of opioids did not affect ChR2-mediated pain behaviors at doses that decreased mechanical hypersensitivity in a neuropathic (SNI) model. In line with in vivo data, bath application of DAMGO did not modify light-induced action potentials in dissociated DRG neurons from Nav1.8-ChR2 mice. These results may re-flect a different opioid pharmacology between artificial light-activated vs natural stimuli-activated no-ciceptors and we are investigating this hypothesis.
HB is a recipient of FRQS and Arthritis Society of Canada postdoctoral fellowships. ID holds a FRQS doctoral studentship. ARS and PS acknowledge funding from CIHR, LAEF and QPRN.

Présentations Orales

Oral presentations

1 - Assessment of Postoperative Movement-Evoked Pain After Spinal Fusion Surgery in Adolescents during Hospitalization: Preliminary Results

Diana-Luk Ye[1,3], Sheila Bote[2,3], My-Linh Ma[1,2], Neil Saran[2,3], Jean A. Ouellet[2,3], Catherine Ferland[2,3]

[1]McGill University
[2]McGill University Health Centre
[3]McGill Scoliosis and Spine Group

Background: Pediatric patients undergoing spinal fusion surgery are at a high risk of suffering significant pain because of the invasive nature of surgical procedures. Effective early pain management is critical to avoid the development of chronic postsurgical pain. The impact of early mobilization in the acute postoperative period is primordial to manage efficiently postoperative pain in order to improve recovery following a spinal fusion surgery.

Methods: Fifty adolescents diagnosed with Adolescent Idiopathic Scoliosis (AIS) and scheduled to undergo spinal fusion surgery at the Shriners Hospital for Children – Canada were enrolled. Each patient's medical chart was retrospectively studied for their self-reported pain and medication intake every hour for the first fifty postoperative hours – postoperative days 1 (POD1) and 2 (POD2). Mobilization activity (sitting and walking) and duration of performed activity were reviewed.

Results: Although more pain was reported when mobilizing, mobilization caused transient spike in pain (POD1 sitting $p=0.001$, POD2 sitting $p<0.0001$, POD2 walking $p=0.002$), but returned to baseline intensity within 10 minutes of activity (POD1&2 sitting $p<0.0001$, POD1 walking $p=0.009$, POD2 walking $p=0.02$). Patients remembered greater pain intensity than the actual pain perceived (POD1 $p<0.0001$, POD2 $p=0.003$) and overestimated their anticipated postoperative pain (POD1 $p<0.0001$, POD2 $p=0.0002$). Patients without pain before surgery mobilized faster.

Future Direction: Continued long-term follow-ups on patients and improved postoperative functional assessment are being developed to investigate and quantify if patients with the least mobilization in the acute postoperative period are at increased risk to develop chronic postsurgical pain.

2 - Etude descriptive des caractéristiques cliniques des cervicalgies chroniques

Olivier LAM[1]

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Background :Neck pain is one of the most common reasons to consult a health professional[1], such as physical therapy (PT). In rehabilitation, better outcomes are obtained when treatments are driven by non-specific diagnosis, such as when patients are classified according to their clinical presentation. The mechanical diagnosis and therapy (MDT)[4], although it has been used extensively, does not fully incorporate the concepts of central sensitization (CS), a crucial part of a biopsychosocial model. Identifying clinical characteristics of patients with chronic neck pain is crucial as it is the first step towards improving diagnosis and treatments given by physical therapists. Objectives: 1) Determine the prevalence of each MDT subgroups in a population of patients presenting chronic cervical pain (>6 months). 2) Estimate the prevalence of CS in a sample of patients with chronic neck pain. 3) Explore associations between patients' clinical and psychosocial characteristics. 4) Explore association between the McKenzie chronic pain subgroup and CS.

Methods: A cross-sectional observational study using survey data. 100 patients with chronic neck pain (>6 months) with or without upper-extremity symptoms will be recruited with a convenience sampling method. Physical therapists from Canada with a certificate or diploma in MDT will be recruited through the McKenzie Institute provider lists from the McKenzie Institute website. These therapists will recruit potential participants. Variables: (1) MDT classification: MDT evaluation form (2) Central sensitization: the Central Sensitization Inventory Questionnaire (CSI)[16], a valid and reliable tool. (4) Psychosocial barriers: measured with the Tampa Scale of Kinesiophobia (TSK) and the Pain Catastrophizing Scale (PCS), two validated tools that have been used in previous studies[5]. Procedures: Therapists will evaluate consecutive admissible patients. They will give to the potential participants a card with an alphanumeric identification number on which a link towards the online questionnaire is written. Therapist will complete the MDT evaluation form (which they already routinely do). The evaluation form will be anonymous and identified with the alphanumeric identification number of the patient. Therapists will then send the MDT evaluation form to the researcher. The patients will complete the DN4, CSI, TSK, PCS and sociodemographic information online. Ethical consideration: All the data will be kept anonymous. At no time the researcher will have access to patient’s name or personal information other than the survey data. Data analysis: Using SPSS, we will calculate descriptive statistics to estimate the prevalence of each MDT subgroups, CEN, DP and CS. Association between patient biological characteristics and psychosocial characteristics will be evaluated via the chi-squared test, the ANOVA test and linear regressions.

3 - Remembering pain: when the superior temporal gyrus clouds the past

Francis Houde[1,3], Marie-Philippe Harvey[1,3], Marylie Martel[1,3], Vincent Auclair[2,3], Kevin Whittingstall[2,3], Philippe Goffaux[2,3], Guillaume Léonard[1,3]

[1]Centre de recherche sur le vieillissement
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[3]Université de Sherbrooke

INTRODUCTION: Pain perception is a complex process. How the brain remembers painful experiences is even more complicated. In fact, many studies have shown that recall of past pain is rarely precise, a phenomenon called mnemonic pain bias. The importance of understanding how pain memories are formed is crucial considering that individuals who exaggerate past pain are more susceptible to develop persistent pain. In a previous electroencephalography study, we showed that superior temporal gyrus (STG) activity at encoding predicted pain recall. More specifically, we noted that late STG activity (recorded 392 ms after administration of a painful electrical stimulation) was related to mnemonic pain bias, with individuals with greater STG activity showing greater exaggeration of past pain. The aim of this study was to determine if the pain-evoked activity in the STG observed during encoding is causally implicated in mnemonic pain bias.

METHODS: In this randomised study, we used single-pulse transcranial magnetic stimulation (TMS) to transiently disrupt (virtual lesion paradigm) the activity of the STG at encoding. Participants were either assigned to the sham (n = 21) or real (n = 21) TMS group. TMS was delivered on the STG using the Brainsight neuronavigation system 392 ms after administration of the painful paradigm. Pain intensity and unpleasantness were assessed using visual analog scale (VAS; 0-10) immediately after the painful event (electric stimulation of the sural nerve) and at recall, 2 months later. The accuracy of the pain recall was determined by calculating the difference between the pain recall and the pain experienced (positive score = exaggeration; negative score = underestimation).

RESULTS: Mean mnemonic pain intensity bias was similar in both groups (delta score = -0.3 for sham group and = 0.0 for experimental group; $p = 0.831$). However, mean mnemonic pain unpleasantness bias was significantly lower in the real TMS group (sham = 1.0; real = -0.4; $p < 0.05$) suggesting that the STG affects how we remember the affective component of a painful event.

CONCLUSIONS: Pain perception can be subdivided into two major components, the sensory discriminative aspect (location and intensity), which is mainly processed by the somatosensory cortex, and the affective component of pain (pain unpleasantness), which mostly relies on the limbic system. Both the intensity and the pain unpleasantness exaggeration have been proposed as a factor predisposing to the chronification of pain. Since the disruption of the STG with TMS was associated with subsequent pain underestimation, our results provide the first evidence that the STG plays a causal role in the affective component of the mnemonic pain bias. Given the fact that exaggerated past pain is closely linked to the development of persistent pain, this study suggests that non-invasive brain stimulation, targeting the STG, could be a promising avenue for individuals at risk of developing chronic pain.

4 - Decreased opioid receptor availability in rat brain three months after peripheral nerve injury

Scott Thompson[1], Mark H Pitcher[2], Farid Tarum[2], Gang Niu[2], Xiaoyuan Chen[2], Dale O. Kiesewetter[2], Petra Schweinhardt[1], M. Catherine Bushnell[2]

[1]McGill University
[2]National Institute of Health

Brain imaging studies using positron emission tomography (PET) have demonstrated that the opioid system is different in patients with chronic pain than in healthy individuals. Nevertheless, it is not known if altered opioid receptor availability is caused by the chronic pain or if it is a predisposing factor for chronic pain. We investigated this issue using a longitudinal preclinical model of neuropathic pain. Forty-six male Sprague-Dawley rats underwent spared nerve injury (SNI, n = 24)) or sham surgery (n = 22) on the left hind limb. Sensory and behavioral tests were performed pre- and post-surgery. Three months post-surgery, rats underwent PET brain imaging using [18F]-fluoroethyl-diprenorphine ([18F]-FDPN), a tracer with comparable affinity for mu, kappa, and delta opioid receptors. A 30-min scan was performed under sevoflurane anesthesia on a Siemens Inveon preclinical PET scanner 30 minutes after tracer injection. Using SPM8 and minc brain imaging tools, PET data were aligned to a common space, coregistered to an anatomical MRI of a size-matched rat and normalized to the cerebellum, a region devoid of opioid receptors. Three months post-surgery, SNI operated rats were hypersensitive to both cold (acetone test) and mechanical stimuli (Dynamic Plantar Aesthesiometer), and showed reduced sucrose preference (anhedonia) compared to controls. SNI rats also showed reduced opioid receptor availability in motor cortex (M1/M2), insula and caudate/putamen. Further, sucrose preference positively correlated with opioid-receptor availability in the caudate-putamen for SNI rats, but not for controls. These findings support reports of reduced opioid availability in chronic pain patients and show that such effects may be caused by the chronic pain condition rather than being a predisposing factor. Further, they show that reduced opioid availability may underlie altered affect sometimes observed with chronic pain.

5 - La morphologie et la fonction des muscles du plancher pelvien chez les femmes survivantes d'un cancer de l'endomètre souffrant de dyspareunie

Marie-Pierre Cyr[1,2], Chantal Dumoulin[3,4], Paul Bessette[5], Walter Gotlieb[6], Mélanie Morin[1,2]

[1]Université de Sherbrooke
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[3]Université de Montréal
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[5]CHUS
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Contexte : Le cancer de l'endomètre est le cancer gynécologique le plus fréquent. Suite aux traitements oncologiques, les femmes survivantes peuvent présenter des problématiques uro-gynécologiques débiliantes. Parmi celles-ci, la douleur lors des relations sexuelles (dyspareunie) est prédominante avec une prévalence s'élevant à plus de 63%. Il a été établi auprès d'une population non-oncologique que la dyspareunie est associée à des altérations des muscles du plancher pelvien. Or, à l'heure actuelle, aucune étude n'a investigué les altérations au niveau de la morphologie et la fonction des muscles du plancher pelvien en lien avec la dyspareunie chez les survivantes d'un cancer de l'endomètre.

Objectif : L'objectif est de comparer la morphologie et la fonction des muscles du plancher pelvien des femmes survivantes d'un cancer de l'endomètre atteintes de dyspareunie et des femmes asymptomatiques (sans douleur) ayant subi une hystérectomie pour raisons bénignes.

Méthodologie : Un total de 60 femmes ayant eu une hystérectomie (30 survivantes avec dyspareunie et 30 asymptomatiques contrôles) sera recruté pour participer à cette étude comparative bi-centrique (Sherbrooke et Montréal). Les participantes seront appariées selon l'âge et le nombre d'accouchements. Les femmes survivantes devront avoir complété leurs traitements oncologiques et souffrir de dyspareunie depuis au moins trois mois. Les femmes asymptomatiques devront avoir reçu l'hystérectomie également depuis au moins trois mois. Les participantes seront évaluées par une physiothérapeute expérimentée en douleurs gynécologiques à l'insu quant au groupe d'appartenance. L'échographie transpérinéale 3D/4D sera utilisée pour mesurer la morphologie au repos et à la contraction (position du col de la vessie par rapport au pubis, angle anorectal, angle des muscles du plancher pelvien et dimensions du hiatus urogénital). L'évaluation dynamométrique permettra de mesurer le fonction des muscles du plancher pelvien (tonus, force de contraction, vitesse de contraction, nombre de contractions rapides et endurance). Des tests t seront utilisés pour comparer les deux groupes sur ces variables musculaires (α=0,05).

Pertinence et retombées anticipées : Cette étude répond à un besoin d'évaluer l'implication des muscles du plancher pelvien dans la dyspareunie chez les femmes survivantes d'un cancer de l'endomètre. Ces résultats pourront être utilisés en vue de guider le développement des traitements en physiothérapie.

6 - Attenuation of Opioid Analgesia in T-cell Deficient Mice

sarah rosen[1], Boram Ham[2], Michael Haichin[1], Ilana Walters[1], Jeffrey Mogil[1]

[1]McGill University

[2]McGill

It is now known that neurons are not the only cell type involved in pain processing, which is now known to involve Schwann cells, satellite cells, and cells of the immune system, such as microglia, macrophages, and T-cells. Many pain researchers have adopted the use of T-cell deficient mice in their experimental methods to elucidate the role of T-cells in neuropathic pain (Fitzgerald et al., 2009; Zuo et al., 2013), and T cells have been shown to release endogenous opioids (Dietrich et al., 2011). While it is well known that opioids have varying effects on the immune system, very little attention has been given to how the immune system may affect opioid regulation. We now have evidence that T-cell deficient mice (CD-1 nude and Rag1 null mutant) exhibit pronounced deficiencies in morphine analgesia, measured using the tail withdrawal or formalin test. We also observe a sex difference in morphine analgesia in the nude mice, which appears to be dose dependent; the female nude mice need a much higher dose of morphine to exhibit analgesia than the male nude mice. Furthermore, T-cell deficient mice do not exhibit stress-induced analgesia after restraint. These results suggest that T-cells play a role in opioid-mediated analgesia, in a sex dependent manner. Current experiments are investigating the mechanism behind this phenomenon.

Affiches

Posters

1 - A conditional knock-in to study the role, function and regulation of the delta opioid receptor

Khaled Abdallah[1], Kimberly Fontes[1,2], Véronique Blais[1], Louis Gendron[1]

[1]Faculté de médecine et des sciences de la santé, Département de pharmacologie et physiologie, Université de Sherbrooke

[2]Bishop's University

Delta opioid receptors (DOPrs), unlike mu, have limited adverse effects and therefore, represent an interesting therapeutic target for the treatment of chronic pain and the emotional disturbances that often accompany it. Our research presents a novel mouse design with which we will be able to specifically target the DOPr and further study the receptor in very specific neuron populations. We created Flag-DOPr-Stop (knock-out) mice with the tagged DOPr in the N-terminal position preceded by a translational stop cassette. By introducing a Cre recombinase to the KO mouse to remove the stop cassette, we generated a mouse that conditionally re-expresses Flag-DOPr. The objective of this part of the study is to characterize the Flag-DOPr conditional knock-in (KI) mouse to ensure that the Flag-DOPr behaves like its endogenous counterpart, both in expression and functionality. Results from the binding assays with deltorphin II (selective delta agonist) show that Flag-DOPr displays pharmacological properties similar to DOPr. KO mice show no residual binding for DOPr agonists. To determine receptor expression patterns, autoradiography was performed on brain and spinal cord slices using 125I-deltorphin I as a ligand. Results show that Flag-DOPr expression follows the same pattern of expression as the wildtype DOPr, with the greatest concentration and distribution of delta receptors in the rostral regions of the brain. G protein coupling efficiency has been tested using [35S]–GTPγS binding assays to ensure the DOPr retains its cellular functionality; WT and KI mice follow the same activation trends and KO mice show no activation. Behavioural tests, using SNC80 (selective DOPr agonist), suggest that the locomotive effects of delta agonists on Flag-DOPr function similarly to DOPr. Wildtype mice experience analgesia during the Hargreaves test after the i.t. administration of deltorphin II and our data also demonstrates that the DOPr agonist has no effective analgesia in our KO mice. Therefore, this research will not only allow us to characterize a new animal model to study the role of the DOPr but also its regulation. Thus, Flag-DOPr can be re-expressed and studied in specific subpopulations of primary afferents.

2 - Assessment of Postoperative Movement-Evoked Pain After Spinal Fusion Surgery in Adolescents during Hospitalization: Preliminary Results

Diana-Luk Ye[1,3], Sheila Bote[2,3], My-Linh Ma[1,2], Neil Saran[2,3], Jean A. Ouellet[2,3], Catherine Ferland[2,3]

[1]McGill University

[2]McGill University Health Centre

[3]McGill Scoliosis and Spine Group

Background: Pediatric patients undergoing spinal fusion surgery are at a high risk of suffering significant pain because of the invasive nature of surgical procedures. Effective early pain management is critical to avoid the development of chronic postsurgical pain. The impact of early mobilization in the acute postoperative period is primordial to manage efficiently postoperative pain in order to improve recovery following a spinal fusion surgery.

Methods: Fifty adolescents diagnosed with Adolescent Idiopathic Scoliosis (AIS) and scheduled to undergo spinal fusion surgery at the Shriners Hospital for Children – Canada were enrolled. Each patient's medical chart was retrospectively studied for their self-reported pain and medication intake every hour for the first fifty postoperative hours – postoperative days 1 (POD1) and 2 (POD2). Mobilization activity (sitting and walking) and duration of performed activity were reviewed.

Results: Although more pain was reported when mobilizing, mobilization caused transient spike in pain (POD1 sitting $p=0.001$, POD2 sitting $p<0.0001$, POD2 walking $p=0.002$), but returned to baseline intensity within 10 minutes of activity (POD1&2 sitting $p<0.0001$, POD1 walking $p=0.009$, POD2 walking $p=0.02$). Patients remembered greater pain intensity than the actual pain perceived (POD1 $p<0.0001$, POD2 $p=0.003$) and overestimated their anticipated postoperative pain (POD1 $p<0.0001$, POD2 $p=0.0002$). Patients without pain before surgery mobilized faster.

Future Direction: Continued long-term follow-ups on patients and improved postoperative functional assessment are being developed to investigate and quantify if patients with the least mobilization in the acute postoperative period are at increased risk to develop chronic postsurgical pain.

3 - Attenuation of Opioid Analgesia in T-cell Deficient Mice

sarah rosen[1], Boram Ham[2], Michael Haichin[1], Ilana Walters[1], Jeffrey Mogil[1]

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It is now known that neurons are not the only cell type involved in pain processing, which is now known to involve Schwann cells, satellite cells, and cells of the immune system, such as microglia, macrophages, and T-cells. Many pain researchers have adopted the use of T-cell deficient mice in their experimental methods to elucidate the role of T-cells in neuropathic pain (Fitzgerald et al., 2009; Zuo et al., 2013), and T cells have been shown to release endogenous opioids (Dietrich et al., 2011). While it is well known that opioids have varying effects on the immune system, very little attention has been given to how the immune system may affect opioid regulation. We now have evidence that T-cell deficient mice (CD-1 nude and Rag1 null mutant) exhibit pronounced deficiencies in morphine analgesia, measured using the tail withdrawal or formalin test. We also observe a sex difference in morphine analgesia in the nude mice, which appears to be dose dependent; the female nude mice need a much higher dose of morphine to exhibit analgesia than the male nude mice. Furthermore, T-cell deficient mice do not exhibit stress-induced analgesia after restraint. These results suggest that T-cells play a role in opioid-mediated analgesia, in a sex dependent manner. Current experiments are investigating the mechanism behind this phenomenon.

4 - β-arrestin-2-biased apelin receptor agonists as novel potent analgesics

Élie Besserer-Offroy[1,2], Mylene Lafrance[1,2], Mona Ouirzane[1,2], Alexandre Murza[1,2], Jean-Michel Longpré[1,2], Richard Leduc[1,2], Éric Marsault[1,2], Philippe Sarret[1,2]

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Apelin is the endogenous ligand of the class A G-protein coupled receptor APJ. We and others have recently demonstrated that spinal or supraspinal delivery of apelin-13 exerts potent analgesic effects in both acute and tonic pain paradigms. Accordingly, APJ is highly expressed in brain regions involved in the regulation of pain transmission and modulation. The present study was aimed to decode by which signaling pathways the apelin peptide exerts its antinociceptive activity. For this purpose, we synthesized a series of apelin-13 analogs exhibiting unnatural amino acids in position 12 and 13. Pro^{12} and Phe^{13} were respectively substituted by aminoisobutyric acid (Aib) and by the aromatic residues 1-Naphtylalanine (Nal) or 2-Nal. The antinociceptive activities of these newly synthesized compounds were evaluated in the experimental model of formalin-induced tonic pain in Sprague- Dawley rats. We also determined, in vitro, their ability to trigger different signaling pathways such as engagement of G-proteins $G\alpha_{i1}$, recruitment of β-arrestins 1 and 2, and inhibition of cAMP production. We then quantified bias using the Black and Leff operational model by calculating the transduction ratio for each pathway in order to identify potential biased APJ agonists. Our results revealed that all the tested compounds were less active than apelin-13 to inhibit forskolin-induced cAMP production with EC_{50} 10- to 20-fold higher than the native peptide. However, only three analogs were 2- to 8-fold more potent than apelin-13 to recruit β-arrestin 2. Interestingly, these compounds were also the ones to reverse pain behaviors elicited by intraplantar formalin. These analogs present a unique and conserved biased signaling profile towards β-arrestin 2 recruitment over inhibition of cAMP production with significant bias factors ranging from 35- to 83-fold. Taken together, our results demonstrate that incorporation of unnatural amino acids at the C-terminal end of the apelin peptide impacts on the APJ receptor signaling signature and that β-arrestin 2 biased APJ agonists elicit potent analgesia. These results also emphasize the involvement of the apelinergic system in central modulation of pain and represent valuable information for future development of analgesics.

5 - Characterization of Behavioral and Histopathological Changes in a MIA Model of Osteoarthritis of the Rat Ankle Joint

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Osteoarthritis (OA) is a complex disease of the whole joint, and in humans it commonly manifests itself as mechanical hypersensitivity in the knee, ankle, hip and shoulder joints. To this day, there is no satisfying method of relieving osteoarthritic pain, which affects over 3 million Canadian adults. Mechanisms of osteoarthritic pain have been studied in the rat knee joint by intra-articular injections of the chondrocyte glycolytic inhibitor Mono-Iodoacetate (MIA). MIA-induced pain indicates that this model is clinically relevant and will continue to be useful for the development of better therapeutic strategies. The present study characterizes the behavioral changes and histopathological changes of cartilage necrosis and degeneration that occur in a new model of MIA-induced OA in the rat ankle joint.

In a dose-response experiment, we determined that a dose of 2.4 mg of MIA in 40 µl of saline administered via intra-articular injection in the right ankle joint generated significant mechanical hypersensitivity starting at 4 weeks post-injection as detected by the Von Frey Filament test. Additionally, the mechanical hypersensitivity was accompanied by cold allodynia starting at 5 weeks post-injection as tested by the Acetone Test. Cold allodynia had not been previously reported in the rat osteoarthritic knee joint. Safranin histological staining of the cartilage and grading in accordance with the Osteoarthritis Research Society International (OARSI) pathology assessment showed significant cartilage loss matching the pathologic features of the clinical disease. We predict that the proposed model will more closely replicate the clinical symptoms than the existing knee joint model.

6 - Creation of a brain-penetrant peptide-neurotensin(8-13) conjugate exerting analgesic activities after systemic administration

Jérôme Côté[1,2], Michel Demeule[3], Nicolas Beaudet[1], Anthony Regina[3], karine Belleville[1,2], Alain Larocque[3], Jean-Michel Longpré[1,2], Jean Lachowicz[3], Jean-Paul Castaigne[3], Philippe Sarret[1,2]

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Neuropeptides play a crucial role in brain functions, and peptide receptors hold great promise in advancing structure-based drug discovery for the treatment of brain disorders. However, one of the major remaining challenges in the development of peptides as potential drugs is to achieve therapeutic brain concentrations after systemic delivery, the blood-brain barrier (BBB) preventing entry of molecules from cerebral capillaries into surrounding brain tissue. In the present study, we conjugated the biologically active neurotensin fragment (NT(8-13)), which produces strong analgesia when injected directly into the brain to the Angiopep-2 peptide (An2), a proprietary 19–amino acid peptide that crosses the BBB by LRP1 receptor-mediated transcytosis. The brain uptake of this new chemical entity, An2-NT(8-13), was first determined using positron emission tomography coupled to computed tomography (PET/CT) imaging. For this purpose, we acquired dynamic PET scans over 60 min followed by a CT scan and quantified brain distribution of ^{64}Cu-radiolabeled An2-NT(8-13) with or without pre-blockage of LRP1 receptors with an excess of unlabeled An2. These experiments showed that An2-NT(8-13) accumulates more efficiently in the brain when pre-blockage is not performed, thus demonstrating transcytosis through a LRP1-dependent mechanism. We next investigated whether the An2-NT(8-13) conjugate exhibited potent analgesic activity in different pain models. In rats, An2-NT(8-13) administered intravenously (i.v.) attenuates the stereotypical nociceptive behaviors observed following intraplantar injection of formalin into the right hind paw (formalin tonic pain model). At a dose of 0.05 mg/kg, An2-NT(8-13) was also effective in reversing the pain behaviors induced by chronic constriction injury of the sciatic nerve (neuropathic pain). Finally, we found that i.v. An2-NT(8-13) significantly reversed the allodynic state induced by the femoral inoculation of MRMT-1 rat breast cancer cells (bone cancer pain). Altogether, these results demonstrate that the An2-NT(8-13) derivative penetrates the BBB efficiently after systemic administration and mediates relief of chronic pain, thus supporting the potential of An2-NT(8-13) as a first-in-class NT-based chronic pain therapeutic.

7 - Efficacy of External Cold and Vibration Stimulation on Pain and Anxiety Management in Children Undergoing Needle-Related Procedures: A Proposal.

Ariane Ballard[1,2], Sylvie Le May[1,2], Christelle Khadra[1,2], Sylvie Charette[2], Evelyne Doyon-Trottier[2,3], Jessie Laflamme[3], Céline Pinard[3]

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Objective : To assess the efficacy of a new device (Buzzy®) combining the effect of cold and vibration on procedural pain and anxiety levels in children undergoing needle-related procedures.

Methods : Design : Non-inferiority randomized clinical trial. Setting : Emergency Department (ED) of a pediatric university health center in Montreal. Sample : Inclusion criteria : Children : 1) from 4-17 years old, 2) who visit the ED and require a needle-related procedure, 2) who can understand and speak French or English, 3) who are accompanied by at least one parent who can understand, speak, read and write in French or English. Exclusion criteria : Children : 1) with a diagnosed cognitive deficit, 2) who do not have the ability to self-report their pain level, 3) who are taking analgesics on a daily basis for chronic pain. Sample size : For a non-inferiority margin of 1.0 on the Numerical Rating Pain Scale (0-10), a standard deviation of 2.0, an alpha of 0,025 and a power of 90%, the total estimated sample size is 172 participants (86 participants by groups). Allocation and Randomization : Participants will be allocated to one of the study groups according to a randomized list using a 1 :1 allocation. Randomization will be stratified by age group (4-7, 8-12, 13-17) and block randomization (with block sizes of 4) will be used for each stratum. Experimental group : Before the needle-related procedure, the Buzzy® device, based on the gate control theory and the descending noxious inhibitory control, will be applied 5 cm over the insertion site and will be maintained throughout the painful procedure. Control group : 30 minutes before the needle-related procedure, a topical anaesthetic (Maxilene®) will be applied over the insertion site. Study time periods : Measurements will be taken at five time-points: before randomization (T-0), 5 min. before the needle-related procedure (T-1), immediately after the needle-related procedure (T-2), 15 min. after the needle-related procedure (T-3), and 24 hours after the needle-related procedure (T-4). Outcomes and Measures : Procedural pain will be measured using the Faces Pain Scale-Revised (4-7 years old) and the Numerical Rating Pain Scale (8-17 years old) (T-1, T-2). Procedural anxiety will be measured using the Procedural Behaviour Check List (T-1, T-2). Memory of pain will be measured using the Numerical Rating Pain Scale (8-17 years old) (T-4). Satisfaction of children, parents and nurses will be measured using questionnaires previously

developed by authors (T-4). Data analysis plan : Multivariate analysis will be performed to compare both groups at all study time periods. Sub-group analyses will be performed for each age stratum (4-7, 8-12, 13-17). ANCOVA will be used for covariates. Descriptive analyses will be used for sample characteristics and report on satisfaction.

Relevance : This study will be the first Canadian RCT to verify the efficacy of the Buzzy® device for procedural pain management in children.

8 - Exploration du 5e résidu de la Leu-enképhaline en vu de développer de nouveaux analogues ayant pour cible le récepteur delta

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[5]Département de médecine nucléaire et de radiobiologie

La morphine est un opioïde analgésique couramment utilisé quant au traitement de la douleur chronique. Il produit son effet via l'activation du récepteur opioïdergique mu (MOPr). Ce dernier présente différents effets secondaires dont la constipation, la dépendance physique, la dépression respiratoire, la myosis et l'euphorie. Or, à long terme, ces effets secondaires peuvent s'avérer problématiques en plus de nuire au style de vie des patients. De récentes études suggèrent le récepteur opioïdergique delta (DOPr) comme nouvelle cible quant au traitement de la douleur chronique. Son activation présenterait moins d'effets secondaires que l'activation du récepteur mu. Par contre, les récepteurs mu et delta ont pour ligands naturels les enképhalines. La met-enképhaline (Tyr-Gly-Gly-Phe-Met) présente une meilleure sélectivité pour MOPr tandis que la leu-enképhaline (Tyr-Gly-Gly-Phe-Leu) se lie préférentiellement à DOPr, ce, via une intéraction hydrophobe entre le récepteur le ligand endogène. La leu-enképhaline possède d'excellentes propriétés pharmacodynamiques, mais hélas un mauvais profil pharmacocinétique. Le principal but de cette étude est donc l'obtention et la caractérisation de différents analogues de la leu-enképhaline afin d'améliorer la sélectif pour le récepteur delta en plus d'optimiser l'hydrophobicité et la résistance enzymatique du pentapeptide. Pour ce faire, la leucine a été substituée par des acides aminés non naturels comportant des chaines latérales hydrophobes. L'activité biologique de chacun des composés a été par la suite testée in vitro dans des cellules transfectées avec DOPr via des immunobuvardages de type Western.

9 - Factors Associated with Acute and Chronic Painful Temporomandibular Disorders: Preliminary results

Sherif Elsaraj[1], Omar Sabsoob[1], Ana Velly[1], zovinar der khatchadourian[1], Mervyn Gornitsky[1], Richard Hovey[1]

[1]McGill University

Introduction:
Temporomandibular Disorders (TMD) are a type of orofacial pain which affect the muscles of mastication and/or the temporomandibular joint. Painful TMD is estimated to ensue in 5-19% of the general population. It is considered as the second most commonly occurring musculoskeletal disorder after chronic low-back pain.

Objective:
The aims of this study are to assess the association between putative risk factors and both (i) clinically significant pain on Grade Chronic Pain Scale (GCPS II-IV), and (ii) chronic temporomandibular disorders (TMD)pain (pain ≥ 6 months).

Methods:
Participants were recruited from the Jewish General Hospital (JGH) and McGill University undergraduate dental clinics. The recruitment process involved: (1)signing the consent form, (2)patients' interview (10-20 minutes), and (3) saliva collection(± 5 mL).

Results:
Total recruited participants were 43 patients;24 patients from the JGH, and 19 from McGill University.
Aim 1: Results show that 24 patients (53% females) had clinically significant pain (GCPS II-IV). The non-clinically significant pain(GCPS I) group consisted of 19 patients (47% females). The pain intensity was significantly different in both groups (Mean GCPSI = 34.6 and Mean GCPS II-IV = 65, $P < 0.05$). As well, the comorbidities were significantly different in both groups (Mean GCPS I = 2.1 and Mean GCPS II-IV = 3.3, $P < 0.05$).
Aim 2: Results show that 13 patients (24% females) had acute TMD pain. However, 30 patients (76% females) had chronic TMD pain.The pain intensity was significantly different between the groups (Mean acute = 55.6 and Mean chronic = 49.8, $P < 0.05$). Also, the comorbidities were significantly different (Mean acute = 2.3 and Mean chronic = 2.9, $P < 0.05$). Finally, acute pain patients were significantly

10 - Factors Associated with Acute and Chronic Painful Temporomandibular Disorders: Preliminary results

Omar Sabsoob[1], Mervyn Gornitsky [1], Sherif Elsaraj[1], Zovinar Der Khatchadourian [1], Richard Hovey [1], Ana Velly[1]

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11 - Figuring out how CCL2 and CCR2 contribute to chronic pain by using RNAi technology.

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[2]Integrated DNA Technologies Inc.

Activation of the chemokine receptor CCR2 in the spinal cord is a key component of chronic pain. Many studies reported analgesic properties of various strategies aiming to prevent its activation. However, these have not yet yield any clinically useful therapy so far. It suggests that the mechanisms by which CCR2 activation contributes to hypernociceptive states are more complex than anticipated and require a deeper understanding in order to take advantage of. As such, we used dicer substrate small interfering RNAs (DsiRNAs) targeting CCL2 or CCR2 in the dorsal root ganglia (DRGs) in a rat model of chronic inflammatory pain induced by complete Freund's adjuvant (CFA). When injected pre-emptively, both DsiRNA prevented CFA-induced mechanical hypersensitivity for 5 days. When injected on days 1 and 2 post-CFA, CCR2 DsiRNA was similarly effective, while CCL2 DsiRNA analgesia was gone by day 5. Interestingly, animals receiving CCR2 or CCL2 DsiRNA on day 1 and 2 post-CFA showed reduced mechanical hypersensitivity on day 14, while having similar hypersensitivity to untreated CFA animals on day 7, 10 and 21. This suggests a dynamic biphasic role of DRG-derived CCL2 and CCR2 in the context of chronic inflammatory pain.

12 - Optimisation de la sélectivité de dérivés peptidiques endogènes envers le récepteur opioïde delta pour le traitement de la douleur

Jean-Louis Beaudeau[1], Véronique Blais[2], Brigitte Guérin[3], Louis Gendron[2], Yves Dory[1]

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[3]Faculté de Médecine et des Sciences de la santé, département de Radiologie et Médecine Nucléaire, Université de Sherbrooke

La douleur est un phénomène auquel chacun a déjà été confronté. Pour certains patients, celle-ci peut revêtir une importance majeure en diminuant fortement leur qualité de vie. Il est donc capital de développer des traitements efficaces tout en limitant au maximum les effets secondaires. Parmi les différents sous-types de récepteurs opioïdes, le récepteur delta (DOPR) semble offrir le meilleur compromis entre analgésie et effets secondaires. Ainsi, le développement d'agonistes ciblant sélectivement celui-ci semble très prometteur. Les travaux menés au laboratoire jusqu'à présent sur des dérivés de la Leu-enképhaline, nous ont permis d'obtenir une bonne compréhension du binding de ces derniers dans le récepteur et de leur conformation active. La présente étude s'intéresse à l'occupation d'une poche du récepteur jusqu'alors non étudiée, et de son effet sur l'activité et la sélectivité envers DOPR. Aidé par la modélisation moléculaire, plusieurs candidats dérivés de la Leu-enképhaline et de la Deltorphine II ont été conçus, puis synthétisés, avant d'être testés biologiquement.

13 - Interrogating the role of peripheral opioid receptors using an optogenetic approach

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Opioids are the most potent analgesics currently available, but their use is often accompanied by un-wanted side effects such as nausea, constipation, respiratory depression and sedation. To overcome this limitation, peripheral administration of opioids represents an interesting alternative. Opioids bind and activate 3 receptor subtypes, namely mu (MOPR), delta (DOPR) and kappa (KOPR) expressed in peripheral tissues such as sensory primary afferents. Until recently, DOPR and MOPR were thought to be co-expressed in both peptidergic and non-peptidergic nociceptors in most species. In the mouse howev-er, opioid receptors have been shown to be segregated in primary afferent neurons, with DOPR and MOPR expressed in non-peptidergic (MrgprD-positive) and peptidergic (TRPV1-positive) neurons re-spectively. In addition to these distinct expression patterns, it has been reported that MOPR activation exclusively relieves thermal pain whereas DOPR activation decreases mechanical pain, which casts a doubt on many past studies.
To enable control of specific genetically-identified neurons in vivo, optogenetics involves the expression of light-sensitive channels (e.g. ChR2) that transduce pulses of light into action potential trains. Using this approach, precisely timed depolarization can be achieved in freely moving mammals, with high cel-lular and spatial selectivity. In the present study, we investigated the role of peripheral MOPR and DOPR in 3 optogenetic mouse lines. We compared the analgesic effect of deltorphin II and DAMGO, respective agonists of DOPR and MOPR, on light-induced pain behaviors in Nav1.8-ChR2, TRPV1-ChR2 and MrgprD-ChR2 mice. Unexpectedly, our results show that intradermal delivery of opioids did not affect ChR2-mediated pain behaviors at doses that decreased mechanical hypersensitivity in a neuropathic (SNI) model. In line with in vivo data, bath application of DAMGO did not modify light-induced action potentials in dissociated DRG neurons from Nav1.8-ChR2 mice. These results may re-flect a different opioid pharmacology between artificial light-activated vs natural stimuli-activated no-ciceptors and we are investigating this hypothesis.
HB is a recipient of FRQS and Arthritis Society of Canada postdoctoral fellowships. ID holds a FRQS doctoral studentship. ARS and PS acknowledge funding from CIHR, LAEF and QPRN.

14 - Plasticité cérébrale et syndrome douloureux régional complexe : une étude randomisée contrôlée en neurostimulation

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INTRODUCTION. Cinq ans après le début des symptômes et malgré les interventions conventionnelles, 62% des personnes souffrant du syndrome douloureux régional complexe (SDRC) demeurent limités dans leurs activités de la vie quotidienne et/ou domestique (Geertzen et al., 1998). Les mécanismes sous-jacents seraient liés à une modification synaptique et anatomique des réseaux neuronaux impliqués dans le traitement et l'intégration de l'information sensorielle et dans la planification motrice (Eisenberg et al., 2005; Kirveskari, Vartiainen, Gockel, & Forss, 2010; Krause, Förderreuther, & Straube, 2006; Pleger et al., 2006; Turton, McCabe, Harris, & Filipovic, 2007; Vartiainen, Kirveskari, & Forss, 2008; Walton, Dubois, & Llinás, 2010). Influencer le fonctionnement de ces circuits serait donc une avenue pour traiter la douleur et les limitations reliées au SDRC. Les stimulations magnétiques répétées en périphérie (rPMS) ont déjà montré en accident vasculaire cérébral, déficience motrice cérébrale et douleur lombaire chronique qu'elles pouvaient influencer le fonctionnement cérébral en optimisant l'intégration et la planification sensorimotrices, en diminuant la douleur et améliorant la fonction (Beaulieu & Schneider, 2015; Flamand & Schneider, 2014; Massé-Alarie, Flamand, Moffet, & Schneider, 2013; Allen Demers & Schneider, en préparation).

L'objectif de l'étude est donc de mesurer l'effet des rPMS sur le SDRC au niveau du fonctionnement moteur cérébral, de la fonction et des symptômes.

MÉTHODOLOGIE. Seize adultes (>18 ans) avec un diagnostic de SDRC de type I (sans lésion nerveuse, validé par les critères de Budapest) seront recrutés à leur congé des centres anti-douleur des centres hospitaliers de l'Université Laval, de l'Hôtel-Dieu de Québec et de Lévis et comparés à un groupe de seize adultes en santé (appariés pour le sexe et l'âge). Les adultes avec SDRC seront randomisés aléatoirement par bloc en deux groupes de n=8 participants : groupe expérimental (recevant les rPMS) et groupe placebo (recevant des stimulations placebo). Le protocole comprend 5 séances (S) identiques pour les deux groupes, excepté pour le type de traitement reçu (rPMS ou placebo) : S1 avec mesures pré et post traitement, S2 traitement seulement, S3 traitement seulement, S4 avec mesures pré et post traitement, et suivi à S5 (une semaine après S4). Les mesures neurophysiologiques (stimulations magnétiques

transcrâniennes, non-invasives et indolores) permettront de mieux comprendre l'impact des rPMS sur les circuits cérébraux; les mesures cliniques et les questionnaires standardisés permettront de noter les changements cliniques et de symptômes des participants. Les tests d'ANOVA avec tests post-hoc et les corrélations permettront de vérifier les hypothèses à l'étude.

15 - Selective CCR2 chemokine receptor antagonists as potential treatment of bone cancer pain

Élora Midavaine[1], David Barrière[1], Jean-Michel Longpré[1], Philippe Sarret[1]

[1]Université de Sherbrooke

Many types of cancers have a propensity to metastasize to the bone microenvironment. In 75-90% of patients coping with metastatic cancer, tumor-induced bone remodeling results in moderate to severe pain, which significantly compromises the patient's quality of life. Unfortunately, current pain relieving treatments, primarily relying on opioid analgesics, become ineffective over time and the most severe type of pain, called breakthrough pain, often remains uncontrolled. Treatments for painful osseous metastases thus require designing new therapies for improving pain control. In recent years, the CCL2/CCR2 chemokine system has gained a prominent place in the field of spinal nociceptive processing, notably in the genesis and maintenance of metastatic breast cancer-induced bone pain. Likewise, the CCL2/CCR2 axis was shown to play a central role in the bone-tumor ecosystem, establishing a fine dialogue between invasive breast carcinoma cells, infiltrating inflammatory cells, and osteoclast/osteoblast bone resident cells. The project was thus aimed at investigating the efficacy of targeting the CCL2/CCR2 axis in both acute and bone cancer pain models. First, we tested the efficacy of the CCR2 antagonist, RS 504393 (25 µg/rat, intrathecally) to alleviate mechanical allodynia in a CCL2-induced acute pain model. We observed that RS 504393 produces a significant long-duration reversal of the mechanical allodynia induced by CCL2. The breast cancer bone metastasis pain model, consisting in injecting MRMT-1 carcinoma cells (endogenously expressing CCL2/CCR2) into the female rat femoral medullary cavity, was then used to evaluate the role of CCL2 in bone pain facilitation. Cancer-induced bone destruction was observed using histological, PET and MR medical imaging. Bone matrix pathological destruction was explained by an osteoclast/osteoblast imbalance in cancer-bearing animals. In the spinal cord dorsal horn, bone cancer progression induced major glial cells activation, as observed by an increased in GFAP and Iba1 immunostainings. Moreover, pERK and pp38 stainings were observed over neuronal and glial cell population in cancer-bearing rats, respectively. CCL2 immunolabeling further revealed that CCL2 is anterogradely transported along the sciatic nerve, probably contributing to the mechanical sensitivity. Punctual spinal injection of RS 504393 significantly reduced tactile allodynia in tumor-bearing rats achieving up to 48% of pain relief. In conclusion, blocking CCL2/CCR2 axis represents a promising avenue to control bone cancer pain and improve the quality of life of patients dealing with bone metastases.

16 - Site-selective modifications of the neurotensin hexapeptide fragment lead to the generation of highly active and metabolically stable NT(8-13) analogs

Melanie Vivancos[1], Élie Besserer-Offroy1[1], Rebecca Brouillette1[1], Adeline René2[2], Roberto Fanelli2[2], Mylène Lafrance1[1], Pascal Tétreault1[1], Jasmin Colerette-Tremblay1[1], Jean-Michel Longpré1[1], Jean Martin[2], Florine Cavelier[2], Philippe Sarret[1]

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The field of peptide-based therapeutics and diagnostics is experiencing renewed interest during the last decade, when compared to small molecule therapies. However, despite their excellent safety, tolerability and efficacy profiles, naturally occurring peptides are often not directly suitable for use as future therapeutics because they have intrinsic weaknesses, including poor physicochemical stability, and a short circulating plasma half-life. In the present study, we developed several strategies to increase the chemical stability and reduce the enzymatic degradation of the endogenous neurotensin hexapeptide fragment (NT(8-13)). To this aim, we synthesized a series of NT(8-13) analogs harboring site-specifically modified unnatural amino acids and reduced amide bonds. Pro10 and Leu13 were respectively substituted by silaproline (Sip) and (trimethylsilyl)alanine (TMSAla) unnatural amino acid residues and a reduced amide bond was incorporated between the Lys8-Lys9 amino acid pair. Our results revealed that these new NT(8-13) analogs bind with high affinity to both NTS1 and NTS2 receptors and exhibited improved plasma stability, with half-life exceeding 1 hour. We also determined, in vitro, their ability to trigger different signaling pathways linked to NTS1 activation. To this end, we used a BRET-based biosensor assay to monitor β□arrestins □□□□□□□□ment and G-protein engagement. A few of these compounds were found to be more effective in promoting β□arrestin and G-protein activation than the native NT peptide. We also demonstrated that these newly synthetized analogs were highly potent in reversing carbachol-induced ileal smooth muscle contractions in isolated organ bath. In in vivo assays, some of these NT(8-13) derivatives were shown to exert pronounced and sustained hypotensive and/or hypothermic actions. We finally tested their ability to reduce the pain sensations in the acute thermal tail-flick test and in the formalin-induced tonic pain model. Our results revealed that all the tested compounds were more potent than NT(8-13) to produce analgesic responses in the tail-flick assay and to abolish the formalin-evoked spontaneous nociceptive behaviors. Altogether, these results demonstrated that the chemically modified NT(8-13) analogs exhibit improved therapeutic profiles and may represent a promising avenue to regulate the physiological functions of the neurotensinergic system.

17 - T-cell dependent reversal of allodynia in pregnant mice

sarah rosen[1], Boram Ham[2], michael hachin[1], ilana walters[2], Jeffrey Mogil[1]

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It has been consistently reported in the clinic that many female chronic pain sufferers have an attenuation of symptoms during pregnancy, but there have been few studies investigating the mechanism behind this phenomenon. It has been shown that rats have an increased acute pain tolerance during pregnancy, due to an increase in opioid receptors in the spinal cord. However, these past studies did not consider that neurons are not the only cell type involved in pain processing, which is now known to involve cells of the immune system, such as microglia, macrophages, and T-cells. Recently, we found that females are likely using adaptive immune cells (T-cells) to achieve pain hypersensitivity after injury, while males use microglia. A possible explanation for this sex difference is that females have a stronger adaptive immune system and more circulating T-cells than males, thus males and females use different immune cells as a mean to the same end. However, during pregnancy, due to changes in sex hormone levels and protection of the fetus, females mount a dampened adaptive immune response. We wished to investigate the implications of this for microglia-dependence of mechanical allodynia in pregnant mice and attenuation of chronic pain during pregnancy.

Here, we show that CD-1 early pregnant mice first switch to a microglia-mediated mechanism of pain signaling. Then, in late pregnancy, CD-1 females have complete reversal of neuropathic and inflammatory mechanical allodynia, while nude pregnant mice do not. This strongly suggests that T-cells are playing a role in these effects. However, the anti-allodynic effects of late pregnancy appear to also be opioid mediated, as naloxone administration reinstates allodynia. Due to the fact that naïve nude mice also exhibit a blunted response to opioid analgesia, we can conclude that T-cells are playing a role in opioid-mediated analgesia. Further experiments are aimed at determining how T-cells affect opioid analgesia produced by pregnancy, as well as morphine administration.

18 - Temporal changes in inflammatory infiltrate in the rat upper dermis following intra-articular CFA injection

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A reported 16.5% of Canadians suffer from arthritis, a disease in which chronic pain is the most common symptom. Pain relief is an important goal, but, as relatively little is known about the mechanisms that drive this pain, the results obtained are suboptimal. Any hope for the development of more effective pain relief depends on a more complete understanding of the processes underlying the pain in arthritis. To this end, we use animal models of arthritis to investigate the changes in the nerves and tissues around the arthritis joints and how these changes drive pain. The importance of studying changes in the skin adjacent to the joints in arthritis is rooted in the phenomenon of neurogenic inflammation. In a model of regional poly-arthritis we have shown de novo sympathetic fibre sprouting into the upper dermis of the skin over inflamed joints only when obvious signs of arthritis were present. In a model of mono-arthritis we observed this same occurrence in addition to increased sympathethic innervation of the inflamed joint. We demonstrated the relevance of these fibres by pharmacological inhibition with guanathedine; this led to an attenuation of pain-related behavior. Here, we have investigated the time-course of inflammatory infiltration of the upper dermis after intra-articular injection of Complete Freund's Adjuvant (CFA). Animals were tested for mechanical allodynia by von Frey fibre and cold allodynia by acetone test at baseline, and at weekly intervals up to 4 weeks post-injection. At 1 week, 2 weeks and 4 weeks post-injection animals were sacrificed, the joints and hind paw skin from ipsilateral and contralateral paw was collected and processed for immunohistochemistry. Macrophages were detected using an anti-body against CD68+ and mast cell infiltration was visualised by staining with toluidine blue solution at pH2.3. Comparing ipsilateral paw to contralateral paw, mechanical and cold allodynia were present at all time-points post-injection. At 2 weeks post-CFA, there was significantly more mast cell infiltration in the upper dermis in the ipsilateral skin compared to contralateral. This increase was no longer present at 4 weeks post-CFA. We expect that at 1 week this infiltration will be even more marked. No macrophages were present in the upper dermis of any conditions studied. In the synovium, we expect macrophage and mast cell infiltration to be present from 1 week post-CFA and increase over time. These studies show that at different time points following intra-articular injection of CFA there are likely different mechanisms underlying pain behavior. This knowledge is important to fully exploit animal models of pain in arthritis for the gain of mechanistic insight and for the development of novel treatments. This work was carried out thanks to funding awarded to Dr Ribeiro-da-Silva

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19 - The associations between low back pain intensity variability, patterns of activity and work performance

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Objectives: The goals of this pilot study were to examine (a) the association between pain-related patterns of activity (avoiding, overdoing, pacing) and pain intensity variability and (b) the association between pain intensity variability and work productivity (absenteeism and presenteeism) among chronic low back pain (CLBP) patients.

Methods: A total of 52 CLBP patients completed baseline questionnaires, an electronic pain diary 4 times daily for 7 days and follow-up questionnaires six weeks later. Results were analysed using Generalized Estimating Equations (GEE) and multivariate general linear model.

Results: Results of the GEE model showed a significant interaction between time and pacing ($p = 0.006$) in predicting fluctuations in pain intensity over a 7-day period. More specifically, patients who used pacing strategy more reported greater pain variability compared to patients who used pacing to a lesser degree. In addition, patients with high levels of pain variability (30% of patients with mean absolute difference between worst and least pain intensity scores ≥ 2 / 10) also reported lower work-related presenteeism compared to patients with low levels of pain variability.

Discussion: Results showed that almost one third of CLBP patients experience high levels of within-day pain intensity variations. Patients resorting to pacing strategies (as opposed to overdoing or avoiding) experienced the most pain intensity variations suggesting that this activity pattern might be a reaction to increases in pain intensity levels as opposed to an active pain management strategy. Furthermore, results showed that pain variability plays an important role in pain-related interference, notably in terms of work performance.

20 - Treatment of glossopharyngeal neuralgia by gamma knife radiosurgery: Case report and review of the literature

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Introduction: La névralgie glossopharyngienne (NGP) est un syndrome douloureux facial rare dans la distribution du nerf crânien IX, et parfois X, caractérisée par une douleur sévère paroxystique unilatérale. Le traitement standard des cas réfractaire à la médication consiste en une décompression microvasculaire (DMV) qui peut comporter certain risque opératoire. Une autre option est l'utilisation du traitement par gamma knife (GK).

Problématique: L'utilisation du GK est bien acceptée dans le traitement de la névralgie du trijumeau (NT) mais peu de littérature existe dans le cas de la NGP. Il existe jusqu'à ce jour 21 cas rapporté de NGP traité par GK. Nous présentons ici le premier cas au Canada de NGP réfractaire traité par GK.

Case report: Il s'agit d'un homme de 43 ans avec une histoire de NGP avec augmentation récente de l'intensité de la douleur et la fréquence des crises réfractaire à la médication. L'option de traitement par DMV a été proposé au patient mais ce dernier a refusé la chirurgie et a été ensuite référé en GK. Le patient a reçu un traitement centré sur le méat glossopharyngien du foramen jugulaire avec une dose de 80Gy. Aucune complication n'a été noté post traitement.

Discussion : Pour ce qui est du traitement par DMV, on rapporte dans la littérature des taux de déficit du NC X allant jusqu'à 5.5% et 19% si effectué avec rhizotomie. Incluant notre patient, il y a 22 cas de NGP rapporté dans la littérature avec un suivi allant jusqu'à 32 mois. La dose reçue était de 80Gy ou plus pour 18 des 22 patients. Seulement 2 de ces 18 patients n'ont eu aucun changement de la douleur et ont opté pour un traitement par DMV. Parmi les patients ayant reçu une dose de 80Gy ou plus, 89% ont eu une résolution complète ou quasi complète de leur douleur. Trois patients ont reçu une dose inférieure à 80Gy et ont tous nécessité un deuxième type de traitement. Aucun effet secondaire majeur n'a été noté parmi les 22 patients.

Conclusion (Implications ?) : Le traitement de la NGP par GK semble être un traitement efficace et sécuritaire pour des doses de 80Gy. Il est a noté que la planification de la cible de traitement est plus délicate que dans la NT vu la proximité des NC X-XI et de la veine jugulaire. Une étude a plus long-terme sur une plus grande cohorte est nécessaire.

21 - Validation of the French version of the UNESP-Botucatu multidimensional composite pain scale for assessing postoperative pain in cats

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The purpose of this study was to validate the French version of the UNESP-Botucatu multidimensional composite pain scale (MCPS) to assess postoperative pain in cats.

The English scale was translated to French with subsequent back-translation. Videos of 27 cats in the preoperative and postoperative period (before and after rescue analgesia and 24 hours post-surgery) were randomized. Three French-speaking individuals (a DVM student, a PhD candidate and a board-certified veterinary behaviourist) with training on scale use completed the video analysis. Validity and reliability tests were performed. The cut-off point to discriminate the need for rescue analgesia was determined by the Receiver operating characteristic curve.

Three domains were identified by factor analysis. The Cronbach's alpha coefficient was excellent for 'psychomotor change' and 'protection of the painful area' (0.94 and 0.90, respectively) and moderate for 'physiological variables' (0.61). Relevant changes in pain scores at clinically distinct time points (e.g., post-surgery, post-analgesic therapy), confirmed the construct validity and responsiveness (Wilcoxon test, $p < 0.001$). Good to very good agreement between blinded observers and 'gold standard' evaluation supported criterion validity. Inter- and intra-rater reliability for each scale item were good to very good. The optimal cut-off point identified was > 7 (scale range 0 – 30 points), with a sensitivity of 97.8% (95% CI: 92.2 – 99.7%), and specificity of 99.1% (95% CI: 96.9 – 99.9%).

The French version of the UNESP-Botucatu-MCPS is a valid, reliable and responsive instrument for assessing acute pain in cats undergoing ovariohysterectomy when used by individuals with different experience. The instrument has high discriminatory ability with a cut-off similar to the English version.

22 - Vesicular glutamate transporter isoforms in the central boutons of synaptic glomeruli in the dorsal horn of the rat spinal cord

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In glutamatergic neurons, including primary afferents, vesicular glutamate transporter (VGLUT) molecules are present in the membrane of synaptic vesicles and mediate the transport of glutamate into these vesicles. In this study, antibodies against three isoforms of vesicular glutamate transporters (VGLUT1-3) were used to investigate the synaptic arrangements of spinal primary afferents by means of confocal and electron microscopy in the dorsal horn of rat spinal cord. Previous studies have shown that, in rodents, VGLUT1, VGLUT2 and VGLUT3 are expressed by different populations of primary afferents. Briefly, VGLUT1 immunoreactivity was detected in non-nociceptive myelinated primary afferents, VGLUT2 in nociceptive small diameter afferents and VGLUT3 in mechanoreceptive unmyelinated afferents. By confococal microscopy, we observed VGLUT1 immunoreactivity in laminae III-V, which corresponds to the area of termination of non-nociceptive myelinated primary afferents. At the ultrastructural level, VGLUT1 immunoreactivity was detected mostly in the central element of Type II synaptic glomeruli. VGLUT2 immunoreactivity was abundant throughout the spinal cord, in agreement with the concept that it is the isoform expressed in spinal cord excitatory interneurons. At the EM level, VGLUT2 immunoreactivity occurred in a high number of boutons establishing axodendritic synapses. However, it also occurred in the central boutons of Type Ia glomeruli which are thought to be the central termination of nociceptive non-peptidergic primary afferents. Lastly, VGLUT3 immunoreactivity was restricted to the deeper part of inner laminae II which is an area known to be innervated by mechanoreceptive C fibers. At the EM level, VGLUT3 immunoreactivity was detected in boutons establishing axodendritic synapses and in some central elements of synaptic glomeruli. Our data supports the differential expression of VGLUT isoforms in different populations of primary afferents.