MONTREAL CONFERENCE ON PAIN CIRCUITS

Friday, August 2, 2019

7:30 Continental breakfast in the IRCM lobby

8:15 Introductory remarks

NEUROPATHIC PAIN

Session chair: Helen Lai

8:30 Allan Basbaum: *Sexually dimorphic influence of the chemokine, colony stimulating factor 1, on spinal cord microglia and neuropathic pain processing*

9:30 Annemarie Dedek (Hildebrand Lab): *STEP61 is the linker between loss of inhibition and NMDAR potentiation in rat and human models of pathological pain*

9:45 Francesco Ferrini: *Uneven chloride homeostasis across the spinal dorsal horn shapes synaptic plasticity and nociceptive behavior*

10:00 Coffee break

10:30 Ana Reynders (Moqrich Lab): *From acute to chronic pain: a critical role for an atypical Myosin*

11:00 Wendy Imlach: *Targeting spinal adenosine signalling in neuropathic pain*

11:30 Emmanuel Bourinet: *Spinal Cav3.2 T-type calcium channels modulate sensory and affective symptoms of neuropathic pain*

12:00 Lunch and poster viewing

SPINAL CIRCUITS I

Session chair: Sung Han

13:30 Ariel Levine: *Spinal Cord Biology at Single Cell Resolution*

14:00 Andrew Todd: *Neuronal circuits for pain and itch processing in the dorsal horn*

14:30 Carole Torsney: *Sex and injury dependent plasticity of spinal nociceptive drive*

15:00 Hugues Petitjean (Sharif Naeini Lab): *Characterization of the role of dorsal horn calretinin-expressing interneurons to the processing of pain inputs*

15:15 Greg Weir (Bennett Lab): *Chemogenetic control of primary afferents*

15:30 Coffee break

16:00 David Hughes: *Defining a spinal microcircuit that gates myelinated afferent input*

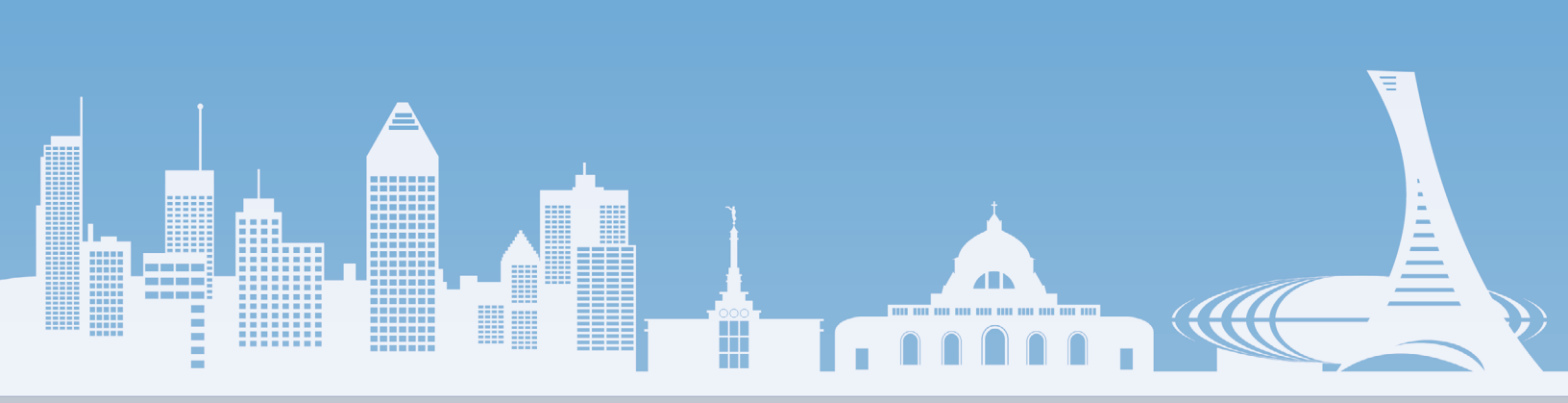
16:30 Helen Lai: *Elucidating the logic of somatosensory-motor circuits*

17:00 Stephanie Koch (Fitzgerald Lab): *Touch, pain and reflexes: the spinal circuits that allow us to interact with our environment*

17:30 Yves de Koninck: *Mapping afferent coding strategies*

18:00 Plenary discussion (Allan Basbaum and Andrew Todd)

18:30 Wine and cheese reception: networking and poster viewing in the IRCM lobby



MONTREAL CONFERENCE ON PAIN CIRCUITS

Saturday, August 3, 2019

7:30 Continental breakfast in the IRCM lobby

ASCENDING AND DESCENDING PATHWAYS

Session chair: Nicholas Betley

8:30 Nicholas Betley: *A neural circuit for the suppression of pain*

9:00 Noémie Frézel (Zeilhofer Lab): *Characterization of direct descending projections from the somatosensory cortex to the spinal dorsal horn*

9:15 Qiufu Ma: *The exteroceptive versus interoceptive dimensions of pain*

9:45 Brian Roome (Kania Lab): *Phox2a defines a developmental origin of the anterolateral system and is required for supraspinal nociception*

10:00 Seungwon Choi (Ginty Lab): *Genetic dissection of ascending spinal pathways for affective touch and pain*

10:30 Coffee break

11:00 Sung Han: *Elucidating Neural Circuits That Transmit Affective-Motivational Pain Signals to the Amygdala*

11:15 Fan Wang: *Identification of a central pain suppression circuit in the amygdala*

11:45 Sonia Paixao (Klein Lab): *Identification of spinal neurons contributing to the dorsal column projection mediating fine touch and corrective motor movements*

12:00 Lunch + poster viewing

SPINAL CIRCUITS II

Session chair: Carole Torsney

13:00 Maria Fitzgerald: *Developing pain circuits: how do we learn about pain*

13:30 Martyn Goulding: *The Touchy Subject of Mechanical Itch*

14:00 Ulrich Zeilhofer: *How gastrin-releasing peptide (GRP) opens the spinal gate for itch*

14:30 Mark Hoon: *Processing itch signals*

15:00 Coffee break

15:30 Brett Graham: *Optogenetic analysis of excitatory interneuron function and plasticity in spinal sensory processing circuits*

16:00 Allen C. Dickie (Todd Lab): *Electrophysiological and morphological characterisation of excitatory interneurons in the dorsal horn of the spinal cord*

16:15 Arnab Barik (Chesler Lab): *A brainstem-spinal circuit controlling nocifensive behavior*

16:45 Olivia Davis (Hughes Lab): *The role of RorB-expressing lamina II interneurons in gating nociceptive C-fiber input*

17:00 Wenqin Luo: *Development of a mouse pain scale using sub-second behavior mapping and statistical modeling*

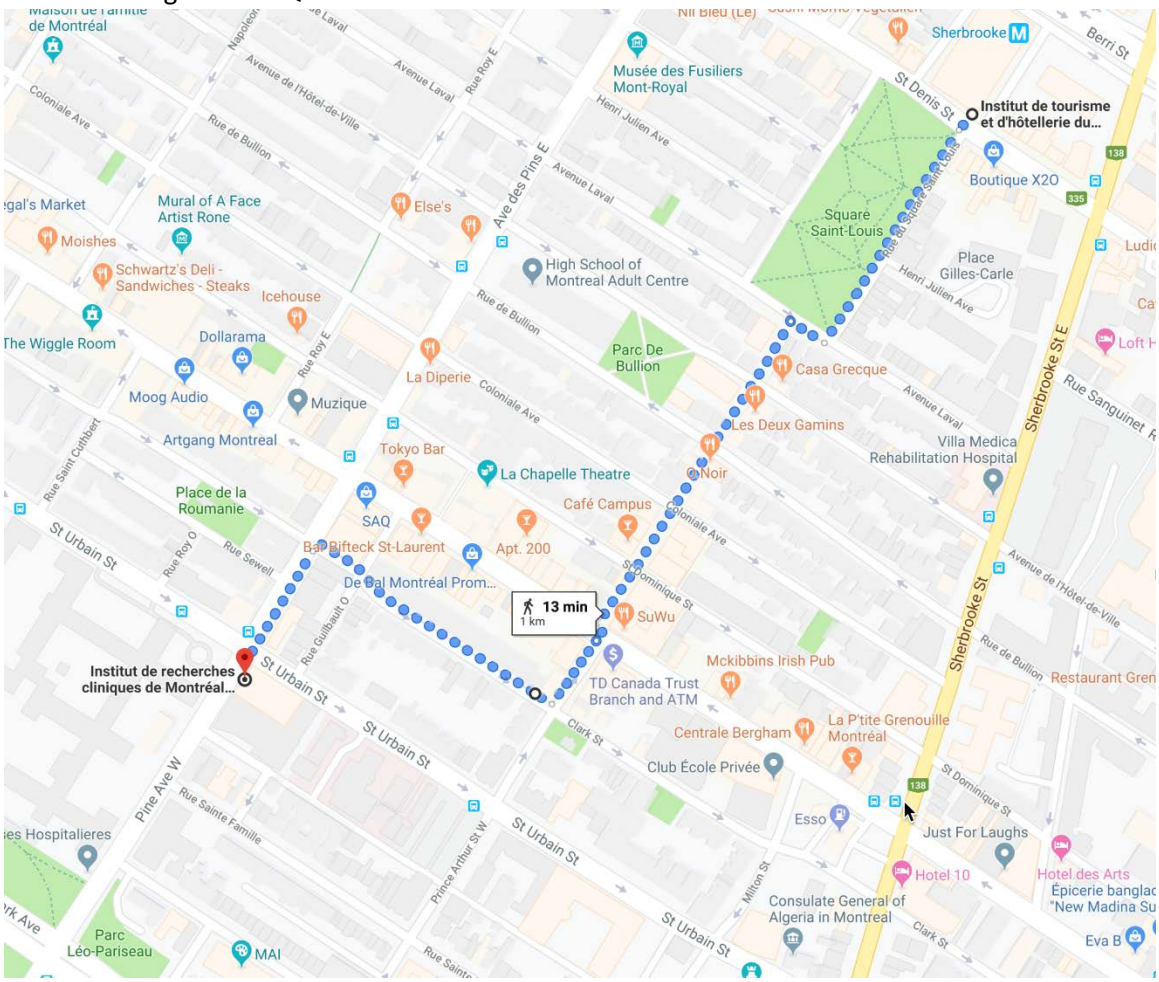
17:30 Jeffrey Mogil: *I made my fancy-schmancy mouse. What do I do with it*

18:00 Plenary discussion and concluding remarks (Yves De Koninck and Wenqin Luo)

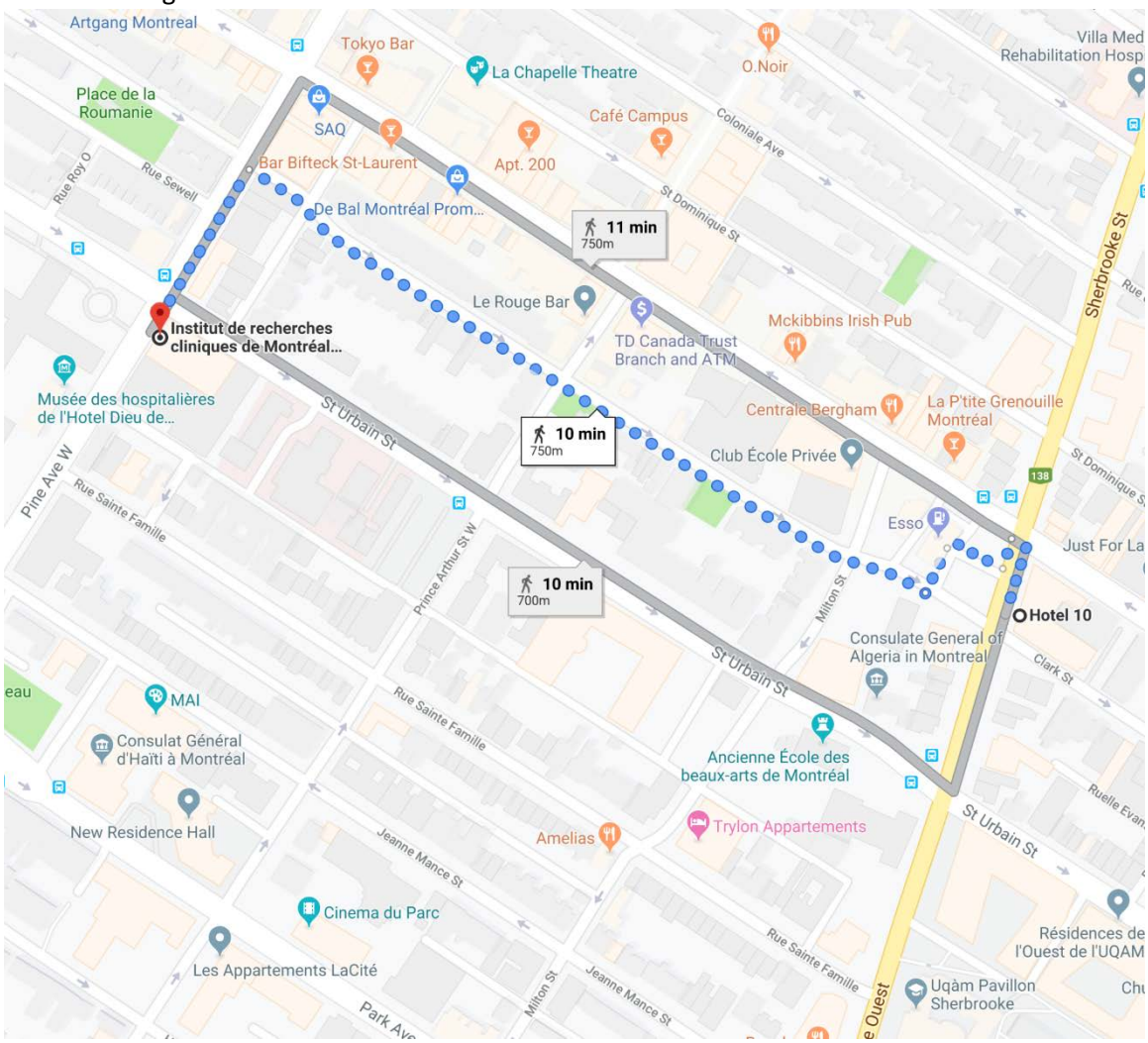
18:30 Closing cocktail in the IRCM lobby

19:30 Gala dinner (Salle St-Louis, ITHQ)

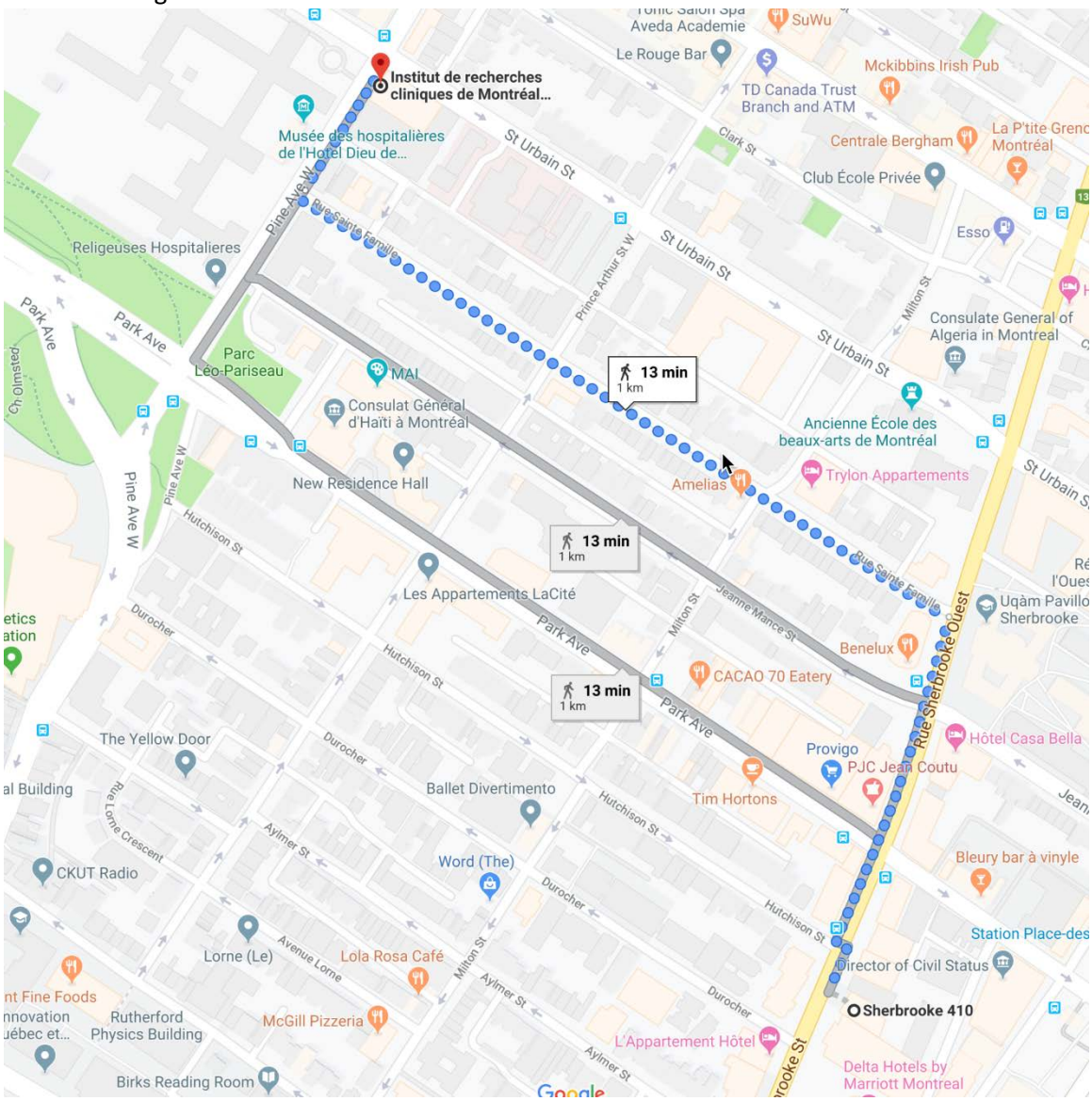
Directions to get to ITHQ from IRCM



Directions to get to Hotel10 from IRCM



Directions to get to McGill Residence La Citadelle from IRCM



Abstracts list

16 - Gastrin-Releasing Peptide Neurons in Itch Processing Circuits of the Spinal Cord
Gioele W. Albisetti

33 - A neural circuit for the suppression of pain and itch
Amber Alhadeff

28 - Developmental access to the principal spinothalamic neuron population of the lumbar spinal cord
Farin B. Bourojeni

50 - Cyclic AMP-dependent transcription factor 3 (ATF3) expression in proprioceptors correlates with consistent and irreversible pain in a MIA model of osteoarthritis in the rat ankle joint
Valérie Bourassa

3 - C fibre driven modulation of oscillatory activity in the young and adult rat somatosensory cortex
Pishan Chang

31 - Neurobiological determinants of opioid-mediated conditioned analgesia
Chulmin Cho

5 - The role of RorB-expressing lamina II interneurons in gating nociceptive C-fiber input
Olivia Davis

27 - STEP61 is the linker between loss of inhibition and NMDAR potentiation in rat and human models of pathological pain
Annemarie Dedek

18 - Electrophysiological and morphological characterisation of excitatory interneurons in the dorsal horn of the spinal cord
Allen C. Dickie

39 - PVN oxytocinergic projections to rostral agranular insular cortex: a possible role in nociception modulation
Mohammed Gamal-Eltrabily

45 - Role of CCK-8 and its receptors (CCK1 and CCK2) on persistent pain and morphine analgesia following chronic inflammation and peripheral nerve injury in mice.
David W. Ferreira

47 - Uneven chloride homeostasis across the spinal dorsal horn shapes synaptic plasticity and nociceptive behavior
Francesco Ferrini

15 - Characterization of direct descending projections from the somatosensory cortex to the spinal dorsal horn.
Noémie Frezel

14 - A functional topographic map for cutaneous somatosensory reflexes
Graziana Gatto

26 - Role of m6A RNA in CFA-induced inflammatory pain
Mayank Gautam

12 - Parvalbumin+ interneurons contribute to dorsal horn oscillations in vitro
MA Gradwell

36 - Neuropeptide Y attenuates the development of tolerance to morphine following intrathecal Co-administration in rats
Shivani Gupta

43 - Elucidating Neural Circuits That Transmit Affective-Motivational Pain Signals to the Amygdala
Sung Han

1 - Separate pathways driving sustained pain versus reflexive-defensive reactions to external threats
Tianwen Huang

10 - Pruriceptive afferents and their interneuron partners in the mouse spinal cord
Noboru Iwagaki

35 - Somatostatin modulates pain at both central and peripheral level in rats: A behavioural study
Rahul Kumar

46 - Conditional knockout of Prdm12 from sensory neurons in mice recapitulates aspects of human congenital insensitivity to pain
Mark Landy

41 - Inhibitory synapse loss through a complement-microglia pathway in models of joint pain
Samantha Locke

34 - COOLING REVERSES PATHOLOGICAL BIFURCATIONS TO SPONTANEOUS FIRING CAUSED BY MILD TRAUMATIC INJURY.
André Longtin

9 - Preventing the collapse of augmented α2,3-GABAA-dependent inhibition rescues benzodiazepine-mediated analgesia in neuropathic pain
Louis-Etienne Lorenzo

49 - CCR2-induced neuroimmune communication drives bone cancer pain and breast tumor growth
Élora Midavaine

38 - Lionfish venom elicits pain predominantly through the activation of non-peptidergic nociceptors
Stephanie Mouchbahani-Constance

21 - Characterization of the role of dorsal horn calretinin-expressing interneurons to the processing of pain inputs
Hugues Petitjean

17 - Characterization of neuronal circuits processing somatosensory information in the spinal cord
Sofia Pimpinella

44 - TrkB receptors engage different signaling cascades regulating respectively KCC2 function, trafficking and degradation.
Isabel Plasencia Fernandez

48 - Selective melatonin MT2 receptor ligands induce an opioid-mediated antiallodynic effect by activating the opioid system in the descending brainstem antinociceptive pathway
Luca Posa

40 - Cold-Response is Reduced in the African Naked Mole-Rat
Sandra J. Poulson

37 - Distribution of delta and mu receptors in rat dorsal root ganglia neurons
Béatrice Quirion

20 - Studying the Role of Dcc in the Development of Nociceptive Topognosis
Shima Rastegar-Pouyani

25 - Phox2a defines a developmental origin of the anterolateral system and is required for supraspinal nociception.
R. Brian Roome

22 - Phox2a defines a developmental origin of the anterolateral system and is required for supraspinal nociception.
Charleen Salesse

30 - Cellular and circuit mechanisms underlying sensorimotor processing in the spinal cord
Anupama Sathyamurthy

8 - Multi-modality of a Single Afferent Subset Transmitting Itch or Pain is Achieved by Differential Recruitment of TRP Channels
Behrang Sharif

32 - Microglia-mediated removal of perineuronal nets contributes to the development of hypersensitivity in neuropathic pain models
Shannon Tansley

7 - Methylglyoxal, a glycolytic metabolite associated with painful diabetic neuropathy, alters C-fibre activity-dependent slowing and induces thermal hyperalgesia in a sex-dependent manner.
Atanaska Velichkova

13 - Different coding strategies for heat and cold by primary sensory neurons
Feng Wang

6 - Chemogenetic control of primary afferents
Greg Weir

19 - The role of mTORC2 in the spinal cord in the development of chronic pain
Calvin Wong

42 - A complement-microglia pathway drives spinal inhibitory synapse loss in neuropathic pain
Noosha Yousefpour

16 - Gastrin-Releasing Peptide Neurons in Itch Processing Circuits of the Spinal Cord

Gioele W. Albisetti[1,2], Martina Pagani[1,2], Nandhini Sivakumar[1], Evgenia Platonova[3], Ladina Hösli[1,2], Mirko Santello[1], Jean-Marc Fritschy[1], Helge C. Johannssen[1], Hendrik Wildner[1], Hanns Ulrich Zeilhofer[1,2,4,5]

[1]Institute of Pharmacology and Toxicology, University of Zurich, [2]Neuroscience Center Zurich, [3]Center for Microscopy and Image Analysis, University of Zurich, [4]Drug Discovery Network Zurich, [5]Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich

Itch, also known as pruritus, is an irritating cutaneous sensation that induces a scratching response. In the spinal cord, gastrin-releasing peptide (GRP) is well-known to induce itch through the activation of GRP receptor (GRPR). On the other hand, the role of spinal cord interneurons expressing GRP (GRP neurons) has remained controversial. This study aimed at a more comprehensive picture about the function of GRP neurons in itch and to gain new insight into the mechanisms that drive GRP-mediated itch signaling. Our results identify spinal GRP neurons as a subset of excitatory interneurons located in superficial dorsal horn. Confocal microscopy revealed that spinal GRP neurons receive direct synaptic inputs from MrgprA3-positive pruriceptors and in turn project directly to GRPR-expressing neurons (GRPR neurons). Chemogenetic activation of GRP neurons in freely behaving mice elicited itch-like behavior whereas toxin-mediated ablation of GRP neurons reduced itch sensitivity. By contrast, activation or ablation of GRP neurons did not affect responses to painful stimuli. Mechanisms of GRP release have been studied in spinal cord slices. We found that repetitive burst-like stimulation of GRP neurons elicited progressive depolarization of GRPR neurons and fostered suprathreshold activation while stimulation at regular intervals evoked only subthreshold EPSPs and no progressive depolarization. Accordingly, burst-like optogenetic stimulation of GRP neurons in vivo evoked itch-like behavior, whereas repetitive stimulations at regular intervals failed to induce behavioral responses. Thus, our results support a critical role of dorsal horn GRP neurons in itch, but not in pain transmission and reveal a new mechanism by which GRP neuropeptide opens the spinal gate for itch.

33 - A neural circuit for the suppression of pain and itch

Amber Alhadeff[1], Onyoo Park[1], Michelle Klima[1], Elen Hernandez[1], Sophie Phillips[1], J Nicholas Betley[1]

[1]University of Pennsylvania

How does hunger affect somatosensory perception? Here, we demonstrate that food deprivation inhibits behavioral responses to both pain and itch stimuli, and uncover a neural circuit for the suppression of these responses. We discovered that hunger attenuates behavioral responses to inflammatory pain and itch without altering responses to acute noxious stimuli. These effects are correlated with the level of food deprivation, as the animals with the greatest weight loss show the greatest suppression of pain and itch. The inhibition of pain and itch is centrally controlled, as activity in hunger-sensitive hypothalamic Agouti-Related Protein (AgRP)-expressing neurons specifically abrogates these responses. Systematic analysis of AgRP neuron projection subpopulations revealed that the neural processing of hunger and noxious somatosensory inputs converge in the hindbrain parabrachial nucleus (PBN). Strikingly, activity in AgRPàPBN neurons blocked the behavioral response to inflammatory pain and itch as effectively as hunger or analgesics. These effects of hunger are mediated by Neuropeptide Y (NPY) signaling in the PBN. By investigating the intersection between hunger and somatosensation, we have identified a neural circuit that mediates competing survival needs and uncovered NPY Y1 receptor signaling in the PBN as a target for pain and itch suppression.

28 - Developmental access to the principal spinothalamic neuron population of the lumbar spinal cord

Farin B. Bourojeni[1,2], Martyn Goulding[3], Artur Kania[1,2]

[1]Institut de recherches cliniques de Montréal, [2]McGill University, [3]Salk Institute for Biological Sciences

Nociception relies on the integration of both somatosensory and emotive inputs in the brain. Somatosensation is established as a multi-level organisation. It relies on the activation of peripheral sensory neurons that synapse onto second-order spinal neurons that project to various brain targets, including the lateral thalamus. These third-order thalamic neurons then relay this sensory input onto the cortex for the integration of an appropriate reactionary motor response. Although, the spinothalamic (ST) neurons are central to this organisation, little is known about their developmental organisation and relative contribution to nociception.

We demonstrate that ST neurons arise from multiple developmental lineages and migrate to populate distinct domains of the spinal cord. In particular, at the hindlimb level, ST neurons are predominantly derived from the V3 cardinal group marked by the expression of the Sim1 transcription factor, as well as the dI5 group expressing Lmx1b. While Lmx1b ST neurons are located in the superficial dorsal horn (DH) and lateral spinal nucleus, Sim1 ST neurons give rise to the deep DH ST population. While the central endings of primary nociceptors form appositions on Lmx1b ST neurons, Sim1 ST neurons coincide with proprioceptive inputs. Furthermore, Lmx1b and Sim1 ST neurons display regional innervation biases in the thalamus. Collectively, we propose that the developmental origin of spinothalamic neurons suggests their differential functional roles in the relay of nociceptive, tactile and proprioceptive inputs for thalamic integration.

50 - Cyclic AMP-dependent transcription factor 3 (ATF3) expression in proprioceptors correlates with consistent and irreversible pain in a MIA model of osteoarthritis in the rat ankle joint

Valérie Bourassa[1,3], Noosha Yousefpour[2,3], Haley Deamond[2,3], Valérie Cabana[2], Alfredo Ribeiro-da-Silva[2,3]

[1]Integrated Program in Neuroscience, McGill University, [2]Department of Pharmacology and Therapeutics, McGill University, [3]Alan Edwards Center for Research on Pain, McGill University

There are many clinical and pre-clinical lines of evidence suggesting a neuropathic component to osteoarthritis (OA) pain. Here we studied the possible contribution of primary afferent damage following joint degeneration to this neuropathic component by investigating the expression of Activating Transcription Factor 3 (ATF3), a marker of neuronal stress, in various cell populations of dorsal root ganglia (DRG).

Male Sprague Dawley rats received an intra-articular injection of 2.4 mg of sodium mono-iodoacetate (MIA) in 40 µl saline in the tibio-talar joint (ankle joint), and were perfused at 1, 2, 3, 4, 5, and 6-week time-points. DRGs (L4-L6) were extracted and immunostained with anti-ATF3, anti-calcitonin gene-related peptide (CGRP), anti-neurofilament 200 (NF200), and anti-parvalbumin (PV) antibodies, as well as stained with fluorescent isolectin GS-IB_4 and Nissl.

Our characterization of ATF3 expression in dorsal root ganglia shows a biphasic pattern, with a large expression of ATF3 following MIA injection at week 1, which subsides completely by week 3. Interestingly, significant ATF3 expression returns at week 5, principally in large-diameter cell bodies, which is the onset of the development of pain hypersensitivity in this model. Indeed, at this time point, 50 % of ATF3-positive cells also colocalize with PV, a marker of proprioceptors, while proprioceptors make up approximately 25% of cells in the rat DRG. This suggests that Aβ mechanoreceptors innervating the articular joint in OA pain are mostly affected following joint degeneration, which drives interest in understanding the contribution of lamina III-V that receives input from these fibers, in the persistence of OA pain.

Acknowledgments: Valérie Bourassa and Noosha Yousefpour are recipients of Louise and Alan Edwards Foundation Doctoral Studentships. Haley Deamond is a recipient of studentship from the McGill University Faculty of Medicine. This work was funded by Canadian Institutes of Health Research Operating Grant MOP-136903.

3 - C fibre driven modulation of oscillatory activity in the young and adult rat somatosensory cortex

Pishan Chang[1], Lorenzo Fabrizi[1], Maria Fitzgerald[1]

[1]Dept. Neuroscience, Physiology & Pharmacology, Medawar Building University College London, Gower St., London WC1E 6BT

Primary somatosensory cortical (S1) neurons are activated by noxious stimulation from a very young age, however the contribution and postnatal emergence of nociceptive afferent C fibre inputs to this activity is unclear. Here we record S1 local field potentials (LFPs) in lightly anaesthetised rats, aged 7, 14, 21 and 30 days, following electrical and mechanical innocuous and noxious hindpaw stimulation. Electrical stimulation at low and high intensity allowed selective activation of peripheral A fibres and C fibres at each age. While stimulus evoked LFPs could be recorded in S1 at all ages, only time-frequency analysis revealed significant differences between A and C fibre evoked activity, and then only from postnatal day (P) 21, suggesting delayed postnatal development of C fibre driven cortical activity. To verify this, peripheral C fibres were selectively silenced with intraplantar QX-314 in 0.1% capsaicin, validated by reduced nociceptive reflexes and behavioural heat sensitivity. C fibre silencing led to significant, long duration (1000ms) reductions in activation energy following high intensity stimulation in P21 and adult rats, but not in younger animals. Thus, we have identified a distinct pattern of oscillatory activity in adult S1 cortex associated with C fibre nociceptive input, which is not observed until rats are 3 weeks old. This data casts new light on the role of S1 cortex in pain processing and reveals its slow postnatal maturation.

31 - Neurobiological determinants of opioid-mediated conditioned analgesia

Chulmin Cho[1], Vassilia Michailidis[1], Areej Fatima[1], Hyun Been Park[1], Batul Presswala[1], Natalia Dziekonski[1], Loren J Martin[1]

[1]University of Toronto

Aim of investigation: Chronic pain can be interpreted as a maladaptive pain memory that is susceptible to alterations through learning that includes conditioning. In support, conditioned analgesia represents a phenomenon where an inert treatment induces pain relief. The objective of the study was to identify the neural pathways of the central nervous system responsible for conditioned analgesia using a mouse model of chronic neuropathic pain.

Methods: Mechanical pain thresholds were measured using von Frey filaments in 6-8wk old male CD-1 mice, before and following spared nerve injury (SNI). The SNI mice then underwent a four-day conditioning phase where contextual (Plexiglas cubicles) and tactile (intraperitoneal injection) stimuli were coupled with an unconditioned drug stimulus (morphine, 10mg/kg). Following the conditioning period (i.e. test day), the SNI mice were administered either saline or one of the opioid receptor antagonists – naloxone (non-specific opioid receptor antagonist, 10mg/kg), CTOP (m-opioid receptor antagonist, 1mg/kg), naltrindole (d-opioid receptor antagonist, 5mg/kg) or nor-binaltorphimine (k-opioid receptor antagonist, 30mg/kg). Following behavioral testing, neuronal activity was mapped in the spinal cord and brain by probing for c-fos expression, using immunoblotting and immunohistochemistry.

Results: After pharmacological conditioning, saline administration was analgesic comparable to that of morphine, which was reversed by naloxone. Furthermore, systemic administration of antagonists for opioid receptor subtypes m, d and k demonstrated that inhibition of m-opioid receptor (MOR), but not d- or k-opioid receptors, blocked saline-induced analgesia, suggesting that opioid-mediated placebo analgesia occurs via MOR. Upon immunoblotting, the observed conditioned analgesia was negatively correlated with c-fos expression, a marker of neuronal activity, in the dorsal horn of the spinal cord. Furthermore, immunohistochemical analyses revealed significant changes in c-fos expression in pain processing regions of the brain, including the anterior cingulate cortex, insula and hypothalamus.

Conclusions: Here, we demonstrate a novel animal model of conditioned analgesia within the context of chronic neuropathic pain. The changes in neuronal activity in the brain and spinal cord are similar to those observed in humans, warranting further investigations in order to elucidate the underlying neural basis for conditioned analgesia.

5 - The role of RorB-expressing lamina II interneurons in gating nociceptive C-fiber input

Olivia Davis[1], Allen Dickie[1], Kieran Boyle[1], Marami Mustapa[1], Andrew Bell[1], Kelly Smith[2], Brett Graham[2], Andrew Todd[1], David I Hughes[1]

[1]University of Glasgow, [2]University of Newcastle, Australia

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 Mas-related G-protein coupled receptor D) and that they receive extensive synaptic input from these afferents. We also find that their axons form axo-axonic synapses onto central terminals of type I glomeruli, which originate from C^{MrgD} afferents. Peripheral stimulation under terminal anaesthesia revealed that 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.

27 - STEP61 is the linker between loss of inhibition and NMDAR potentiation in rat and human models of pathological pain

Annemarie Dedek[1,2], Jian Xu[3], Chaya Kandegedara[1,2], Eve C. Tsai[2,4,5], Paul J. Lombroso[3], Mike E. Hildebrand[1,2]

[1]Carleton University, Neuroscience, Ottawa, Ontario, Canada, [2]Ottawa Hospital Research Institute, Department of Neuroscience, Ottawa, Ontario, Canada, [3]The Child Study Centre, Yale University School of Medicine, New Haven, Connecticut, USA, [4]University of Ottawa, Department of Neuroscience, Ottawa ON, Canada, [5]University of Ottawa Division of Neurosurgery, Faculty of Medicin e, Ottawa Ontario, Canada

Chronic pain arises when there is an imbalance between excitation and inhibition of neurons in the spinal superficial dorsal horn. The mechanisms underlying this imbalance remain unclear in rodent models, and unexplored in human tissue. We have recently shown that in nerve-injured rats, BDNF-mediated disinhibition gates potentiation of GluN2B-containing NMDARs through Fyn kinase activation at lamina I dorsal horn synapses. We will explore whether loss of an associated phosphatase, $STEP_{61}$, mediates this pathological coupling in lamina I neurons of rodents and humans. To investigate mechanisms of human spinal pain signalling, we have, for the first time, functionally characterized synaptic NMDAR responses in human lamina I neurons. We paired patch-clamp electrophysiological recordings with pharmacology, behaviour, and biochemical approaches. We used an ex vivo BDNF spinal pathology model in rodent and human tissue. To model chronic inflammatory pain, we administered anin vivo injection of CFA into the rodent hindpaw. Human tissue was collected from organ donors 1-4 hours post-mortem. In humans and rats, we observed a decrease in $STEP_{61}$ and an increase in pGluN2B and pFyn at lamina I synapses. Downregulation of $STEP_{61}$ was both necessary and sufficient to prime subsequent phosphorylation and potentiation of synaptic NMDARs by BDNF. Preliminary data suggests that GluN2B-containing NMDARs dominate synaptic NMDAR responses in human lamina I neurons.$STEP_{61}$ is the molecular brake that is lost to drive the potentiation of excitatory NMDAR responses following BDNF-mediated disinhibition at lamina I synapses of rodents and humans. Like rats, GluN2B-containing NMDARs dominate human lamina I synaptic responses.

18 - Electrophysiological and morphological characterisation of excitatory interneurons in the dorsal horn of the spinal cord

Allen C. Dickie[1], Andrew M. Bell[1], Noboru Iwagaki[1], Erika Polgár[1], Maria Gutierrez-Mecinas[1], Rosalind Kelly[1], Heather Lyon[1], Kirsten Turnbull[1], Steve West[2], Alexander Etlin[3], Joao Braz[3], Masahiko Watanabe[4], David L.H. Bennett[2], Allan Basbaum[3], John Riddell[1], Junichi Hachisuka[1], Andrew J. Todd[1]

[1]1. Spinal Cord Group, Institute of Neuroscience and Psychology, University of Glasgow, [2]2. The Nuffield Department of Clinical Neurosciences, University of Oxford, [3]3. Department of Anatomy, University of California, San Francisco, [4]4. Department of Anatomy, Hokkaido University School of Medicine

Excitatory interneurons (eINs) account for the majority of neurons in the superficial dorsal horn, but despite their presumed roles in pain and itch, our knowledge of their organisation and function is limited. We have shown that eINs can be defined based on the expression of neurochemical markers, which broadly agrees with recent transcriptomic studies.

We have used anatomical and electrophysiological techniques to characterise eINs that express Substance P (SP), Gastrin-releasing peptide (GRP), or the GRP receptor (GRPR), to determine whether they are functionally distinct. Transgenic mouse lines used to identify these eINs include; GRP::eGFP (GRP), $Tac1^{Cre}$ (SP) and $GRPR^{CreERT2}$;Ai9 (GRPR). $Tac1^{Cre}$ mice were intraspinally injected with AAV.Flex.tdTom or AAV.Flex.eGFP.

$Tac1_{Cre}$;GRP::eGFP mice spinal injected with AAV.Flex.tdTom demonstrated minimal overlap between GFP+ (GRP) and tdTom+ (SP) cells, showing that they are distinct populations. This was further confirmed with in situ hybridisation. We similarly found that GRPR cells are a distinct group.

Patch-clamp electrophysiology, in spinal cord slices, was used to study these eINs. Action potential firing patterns in GRP cells were predominantly transient or single spike, this differed from SP cells, which mostly showed delayed firing, and GRPR cells, which showed delayed or single spike firing. EPSC frequency was higher in SP and GRPR cells, suggesting they have a greater excitatory drive than GRP cells. Almost all GRP cells responded to the MOR agonist, DAMGO, but were largely unresponsive to NA or 5-HT. In contrast most SP cells were sensitive to NA and 5-HT, but not DAMGO.

Morphology was assessed in GRP cells filled with neurobiotin during patch-clamp recordings, and SP and GRPR cells in perfusion fixed tissue from mice injected with Brainbow AAVs. Analysis demonstrated that these eINs were morphologically distinct. Although GRP cells were heterogeneous, some could be classified as central cells. In contrast, many SP cells resembled radial cells. GRPR cells exhibited vertical cell morphology.

Our findings demonstrate that SP, GRP and GRPR eINs show major differences in their morphological, electrophysiological and pharmacological properties. Based on somatodendritic morphology and firing patterns, we propose that SP cells correspond to a population known as radial cells, while GRP cells are likely to overlap extensively with a population previously classified as transient central cells, and GRPR cells are a type of vertical cell. Our finding indicate that SP, GRP and GRPR cells are functionally distinct, and presumably have different roles in somatosensory processing.

39 - PVN oxytocinergic projections to rostral agranular insular cortex: a possible role in nociception modulation

Mohammed Gamal-Eltrabily[1], Antonio Espinosa de los Monteros-Zuñig[1], Guadalupe Martínez-Lorenzana[1], Miguel Condés-Lara[1], Abimael González Hernández[1]

[1]Institute of Neurobiology, UNAM

At the supraspinal level, the paraventricular nucleus of the hypothalamus (PVN) seems to play a key role modulating nociception. Indeed, electrical PVN stimulation induces antinociception at spinal level by direct oxytocinergic projections. PVN has other connections with pain-related areas, such as raphe magnus (RMg) and locus coeruleus (LC) nuclei. Certainly, the RAIC is thought to be an integration site of physical and emotional aspects of pain, where opioids, dopamine and, GABA has a modulatory effect over nociception by recruitment of descending modulatory mechanisms. In this study, we further explore the presence of PVN oxytocinergic innervations towards RAIC, their possible role in nociception modulation and the possible mechanisms that stand for it.

In male Wistar rats (280-310g), FluoroGold microinjection into RAIC stained cells bilaterally in PVN, some of them are of oxytocinergic accordingly to the immunofluorescence studies performed. Further immunofluorescence studies demonstrated: (i) the presence of oxytocin receptors (OTR) inside the RAIC; (ii) that OTR is expressed by GABAergic neurons that also show a close contact with fibers from PVN neurons (anterograde tracer FluoroRubi was injected into PVN). Later, we performed a behavioral study (5 % formalin test) to observe the effect of oxytocin microinjection (40 or 400 pmol/»40 nl) into the RAIC over nociception. Results show a decreased number of hindpaw shakes in rats which have received oxytocin into RAIC compared to control groups. This behavioral antinociceptive effect was reversed by the RAIC microinjection of the OTR antagonist (L-368,899, 400 pmol/»40nl) or bicuculline ($GABA_A$ receptor antagonist, 200 pmol/»40nl). Furthermore, intrathecal administration of BRL44408 (a selective α_{2A}-adrenoceptor antagonist) partially reversed the oxytocin-induced antinociception. Taken together, our results demonstrate that PVN and RAIC are anatomical connected; additionally, we show that the RAIC seems to be a site of oxytocin-induced antinociception mediated by local GABA levels partially promoting a descending noradrenergic inhibitory mechanism.

Acknowledgments: This study was supported by grants from PAPIIT-UNAM (Grant No. IN200415 to MCL and IA203117, IA203119 to AGH).

45 - Role of CCK-8 and its receptors (CCK1 and CCK2) on persistent pain and morphine analgesia following chronic inflammation and peripheral nerve injury in mice.

David W. Ferreira[1,2], Lucien Ruelle-le Glaunec[1,3], Cynthia M. Arokiaraj[1,2], Rebecca P. Seal[1,2]

[1]Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15260, USA., [2]Pittsburgh Center for Pain Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15260, USA, [3]Life Science Department, University of Strasbourg, Strasbourg, 67000, France.

Cholecystokinin (CCK)-8 is the most abundant neuropeptide in the nervous system and has demonstrated roles in feeding, anxiety, memory and pain. Although past efforts provided clear pharmacological evidence supporting the importance of the CCKergic system in persistent pain and morphine analgesia at the spinal level, many fundamental circuit-based questions about CCK and its receptors, CCK1 and CCK2, remain, most critically, what are the cellular sources of CCK and what neurons are involved in mediating the actions of the receptors? Here we report that intrathecal (i.t.) administration of CCK2R antagonist CI-988, but not CCK1R antagonist SR27897 reverses mechanical allodynia induced by CFA on day 5 (D5) and SNI on day 7 (D7) in mice. In naive conditions, CCK and CCK2R gene expression are significantly higher in SC than DRG, while CCK1R is higher in DRG than in spinal cord. In DRG, CCK2R gene expression is dramatically increased (~60-fold) following SNI D7, whereas a small increase in CCK2R gene expression (~2-fold) and a decrease in CCK1R gene expression (~25%) are found after CFA D5. Nevertheless, at a later chronic stage of SNI (8 weeks), the DRG levels of CCK2R gene expression are still elevated (~42-fold) and i.t. injection of CCK2R antagonist significantly reverses mechanical allodynia. We also tested the effect of spinally delivered CCK receptor antagonists on morphine analgesia after CFA or SNI. CCK2R antagonist (i.t.) enhances the effects of subcutaneous (s.c.) morphine following CFA D5 and SNI D7, whereas CCK1R antagonist (i.t.) enhances the effect of s.c. morphine on CFA, but not on SNI. Interestingly, a decrease in DRG levels of mu opioid receptor (Oprm1) (~40%) and delta opioid receptor (Oprd1) (~30%) gene expression are observed after SNI, but not after CFA. In DRG from both naïve and SNI mice, CCK1R is expressed by the TH^+ population and by myelinated neurons including a $TrkC^+$ population that expresses PV and $N52^+$, but not by $IB4^+$, $TRPV1^+$, $CGRP^+$ and SP^+ neurons. In DRG from SNI, but not in naïve mice, CCK2R is expressed by $N52^+$ and $TrkC^+/PV^-$ populations as well as a large number of unmyelinated neurons that surprisingly lack all of the common markers, potential due to SNI induced down-regulation. Finally, DRG from SNI mice shows that CCK1R and CCK2R are expressed by $Oprd1^+$, but not $Oprm1^+$ neurons. Taken together, our results show that CCK2R blockade prevents mechanical allodynia and CCK-mediated antagonism of morphine analgesia, possibly via the delta opioid receptor. Currently, we are generating floxed mouse lines (cKO) for CCK, CCK1R and CCK2R using CRISPR/Cas9, which will be crossed to cell-type specific Cre lines to identify which neuronal populations convey mechanical allodynia at the spinal level. Furthermore, based on our findings in mouse, we are also using monkey and human DRG and SC for species comparisons of the neural circuitry. Identifying and understanding the spinal level circuits that require CCK-signaling in mechanical allodynia and morphine analgesia will provide new opportunities to develop more effective, non-addictive therapies.

47 - Uneven chloride homeostasis across the spinal dorsal horn shapes synaptic plasticity and nociceptive behavior

Francesco Ferrini[1,2], Jimena Perez-Sanchez[2,5], Louis-Etienne Lorenz[2], Samuel Ferland[2], Martin Cottet[2], Chiara Salio[1], Antoine G. Godin[2,4], Nicolas Doyon[2,3], Adalberto Merighi[1], Yves De Koninck[2]

[1]Department of Veterinary Sciences, University of Turin, Turin, Italy, [2]Centre de recherche CERVO, Québec, Canada, [3]Department of Mathematics and Statistics, Université Laval, Québec, Canada, [4]Department of Psychiatry and Neuroscience, Université Laval, Québec, Canada, [5]Institut des Neurosciences de la Timone, Aix-Marseille Université, Marseille, France

Little is known about the mechanisms underlying plasticity in nociceptive pathways within specific pain modalities. Yet, thermal and mechanical pain differ in their temporal and spatial qualities, which suggests that they may undergo different central sensitization processes.

Synaptic inhibition is key in shaping nociceptive information. We thus asked whether inhibitory transmission in the spinal dorsal horn similarly regulates mechanical and thermal sensitivity. By optogenetic stimulation of spinal inhibitory interneurons, we found that enhancing inhibition actually produces a strong analgesic effect on mechanical sensitivity, while has a relatively smaller impact on thermal pain.

We speculated that the strength of synaptic inhibition may vary along a dorso-ventral axis, as thermal afferents end more superficially in the spinal dorsal horn (lamina I and outer II) than mechanical fibers (lamina II inner). To address this point, we focused on the expression and function of the K^+-Cl^- co-transporter 2 (KCC2), the main regulator of $[Cl^-]$ in central neurons. Interestingly, we uncovered a gradient in the expression of KCC2 in the superficial dorsal horn, lower in lamina I and progressively higher in lamina II. The gradient in KCC2 expression is functionally paralleled by a gradient in Cl^- extrusion capacity, which is lower in lamina I. Weak Cl^- extrusion capacity critically hampers the robustness of inhibitory transmission and increases plasticity at inhibitory synapses (ionic plasticity), resulting in an activity dependent collapse of inhibition. Importantly, higher ionic plasticity deeply affects key central sensitization processes. Indeed, lamina I neurons exhibit a form of activity-dependent synaptic plasticity expressed as a runaway, continuously-growing LTP. Finally, ionic plasticity and LTP metaplasticity both depend on TrkB receptor signaling. Blocking TrkB attenuated the KCC2 gradient in superficial dorsal horn, increased the robustness of inhibition in lamina I and leveled LTP behavior.

Our results suggest that local ionic plasticity critically shapes how different components of nociceptive pathways process sensory information. Weaker inhibition in lamina I may account for the low impact of GABAergic/glycinergic transmission in controlling nociceptive thermal input and the high propensity of this modality to cause sensitization. On the other hand, robust inhibition in lamina II neurons makes them more susceptible to decreased inhibition, which may account for the frequent occurrence of mechanical allodynia in certain pathological conditions affecting KCC2 in the dorsal horn.

15 - Characterization of direct descending projections from the somatosensory cortex to the spinal dorsal horn.

Noémie Frezel[1,2], Hauselmann Salome[1], Wildner Hendrik[1], Specht Christian[2], Triller Antoine[2], Hanns Ulrich Zeilhofer[1,3]

[1]1Institute of Pharmacology and Toxicology, University of Zurich, [2]Institute of Biology, École Normale Supérieure (ENS), [3]3Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich

Noxious stimuli are sensed by specialized sensory neurons of the peripheral nervous system called nociceptors. The nociceptive information is then processed in the spinal cord dorsal horn, which contains local interneurons and projection neurons which send axons to the brain. Supraspinal areas in turn send axons down to the spinal cord where they contribute to the gating of nociceptive signals. Exaggerated and abnormal pain sensitivity is accompanied by alterations in such descending pain control systems. The connection between somatosensory (S1) cortex in particular and the spinal cord is conserved in mammals, but very little is known about its role in modulating spinal sensory processing. Using intraspinal injections of AAV vectors, we have identified a population of pyramidal neurons in the somatosensory cortex that projet directly to the spinal dorsal horn (S1-CST neurons). In order to characterize the connectivity and function of these neurons, we used viral approaches and genetically modified mice expressing CRE under the control of a neuron type-specific promoter. We show that we can specifically target expression of various transgenes to S1-CST neurons to trace or manipulate these neurons. We found that S1-CST neurons receive monosynaptic input from layer 2/3 pyramidal neurons, PV-and NPY-positive interneurons in the cortex, as well as from thalamic relay sensory neurons that are part of the somatosensory system. We show that most S1-CST neuron axons terminate in the laminae III and IV of the dorsal horn, where they form direct synaptic contacts onto spinal interneurons. Anterograde tracing with Wheat germ agglutinin (WGA) revealed that about 60% of the spinal target neurons are inhibitory neurons.

All together these results show that we can specifically target subsets of CST neurons based on their connectivity and gene expression. CST neurons from S1 in particular receive input from sensory pathways and directly contacts spinal interneurons which are important for gating of sensory and painful stimuli.

14 - A functional topographic map for cutaneous somatosensory reflexes

Graziana Gatto[1], Steeve Bourane[1,2], Xiangyu Ren[1], Stefania Di Costanzo[1], Peter K Fenton[1], Martyn Goulding[1]

[1]Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Rd, La Jolla, CA 92037, USA., [2]Université de la Réunion, DéTROI, UMR 1188 INSERM, 2 Rue Maxime Rivière, 97490 Sainte Clotilde, France

Cutaneous somatosensory modalities play pivotal roles in generating protective reflexes, adapting ongoing movement and posture to environmental perturbations (corrective reflexes), and shaping complex motor behaviors. How these modalities are integrated by interneurons (INs) in the dorsal horn to elicit stimulus-appropriate responses is not known. We used an intersectional genetic approach to label and functionally probe select classes of excitatory INs within the spinal dorsal horn sharing different molecular signatures and/or laminar distribution patterns. We find that excitatory neurons in laminae I/II drive the itch-induced scratch reflex, while excitatory neurons in laminae IIi/III drive both noxious and non-noxious withdrawal reflexes, with the noxious pathway also recruiting lamina IIo neurons. Notably, excitatory neurons in laminae IIi-IV that broadly encode tactile information also contribute to generate corrective reflexes. We propose a functional topographic map of the dorsal horn in which the differential recruitment of excitatory neurons in specific laminae dictates the nature of the sensorimotor reflex response, largely independent of the excitatory cell types being activated.

26 - Role of m6A RNA in CFA-induced inflammatory pain

Mayank Gautam[1], Yijing Su[1], Vanessa Fleites[2], Guo-Li Ming[1], Hongjun Song[1], Mariella De Biasi[2], Wenqin Luo[1]

[1]Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania,
[2]Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania

The functional role of mRNA and DNA methylation in the development and maintenance of chronic pain and susceptibility for addiction is completely unknown. We hypothesize that m6A mRNA methylation in DRG and spinal cord plays a critical role in changing the neuronal state during the acute phase for induction/development of pain phenotypes.

To test it, we conducted a preliminary study that included two groups of adult C57BL/6J mice (both male and females). Hind paw injection of complete Freund's Adjuvant (CFA, 10 µL) was used to cause inflammation and pain and saline was used as the control. 24 h after paw injection of either CFA or saline, mice were sacrificed and L4/L5 DRGs were dissected, followed by total RNA extraction and mRNA isolation with Dynabeads Oligo (dT)25 beads. 150 ng of mRNA was then incubated with m6A antibody followed by m6A-SMART-seq. Both input and m6A immune- precipitated (IP) mRNA were used to prepare RNA-seq libraries and then sequenced on Illumina NextSeq 550. To measure the relative m6A level per gene, the ratio of m6A-IP/Input was first calculated. The Z scores were then obtained by comparing the ratios (m6A IP/Input) to the mean of the group to reflect the relative m6A level per gene on a transcriptome-wide scale. For the comparison of transcriptome and m6A epitranscriptome across different conditions, transcript levels were presented as TPM (transcripts per kilobase million). Principal Component Analysis (PCA) was performed using DESeq2 normalized RNA-seq data with a variance stabilizing transformation. Epitranscriptomic changes under saline and CFA were investigated.

Our data show that the number of m6A tagged mRNAs is almost doubled in the CFA group when compared to the saline control group. These preliminary results provide supportive evidence for our hypothesis that acute inflammation induces an elevation of m6A mRNA methylation in DRGs and spinal cord, which could play an essential role in the induction and/or prevention of chronic pain.

12 - Parvalbumin+ interneurons contribute to dorsal horn oscillations in vitro

MA Gradwell[1], KA Boyle[2], RJ Callister[1], DI Hughes[2], BA Graham[1]

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, NSW 2308, Australia, [2]Spinal Cord Research Group, Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, UK. G12 8QQ

The dorsal horn of the spinal cord is an essential region for the appropriate encoding of sensory signals. A number of studies have identified 4-aminopyridine (4AP)-induced rhythmic activity within the dorsal horn, and likened this activity to neuropathic pain states, where tactile inputs excite nociceptive circuits (Ruscheweyh and Sandkuhler, 2003; Kay et al., 2016). One population that may be relevant to these observations are Parvalbumin-positive interneurons (PVINs), as they have been shown to play an important role in the segregation of tactile and nociceptive signals within spinal circuits (Petitjean et al., 2015). Given PVINs in other brain regions exhibit synaptic coupling and generate oscillatory activity, our study examines the relevance of these properties within the dorsal horn. This work was undertaken using an optogenetic approach in transgenic mice that express Channelrhodopsin-2 (ChR2) in PVINs. In addition, pairs of PVINs and other unidentified populations were recorded to directly assess individual connections. Adult mice (30 ± 3 wks, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated before parasagittal spinal cord slices were prepared from the lumbar cord. Whole cell patch clamp recordings were made from PVINs (identified by ChR2-YFP) and slices photostimulated using brief whole-field illumination (488nm, 1ms). We demonstrate PVINs within the dorsal horn are synaptically coupled (67/110) with glycine as their primary neurotransmitter, receive convergent excitatory input, and are a source of presynaptic inhibition capable of eliciting primary afferent depolarisation (PAD)-evoked neurotransmitter release. Administration of 200μM 4AP induced rhythmic activity within the dorsal horn, as previously described. This rhythmic activity was CNQX/bicuculline-sensitive, but strychnine-insensitive mimicking the pharmacology of presynaptic inhibition. Further, 4AP induced activity was disrupted by PVIN photostimulation. Finally, we utilized a GCaMP6:PV mouse line to examine 4AP-evoked rhythmic activity at a population level and confirm its dependence on both GABA and glutamatergic signalling. Together, these findings demonstrate PVINs are coupled through inhibitory synaptic connections (feed-forward inhibition) and also provide presynaptic inhibition to the same afferent fibers that excite them (feed-back inhibition). These properties are consistent with the connectivity of PVINs in other CNS regions, suggesting they are ideally configured to drive oscillations in the sensory circuits of the dorsal horn and are capable of contributing to the dorsal horn hyperexcitability observed in neuropathic pain states.

36 - Neuropeptide Y attenuates the development of tolerance to morphine following intrathecal Co-administration in rats

Shivani Gupta[1], Rahul Kumar[1], Mayank Gautam[1], Subrata Basu Ray[1]

[1]All India Institute of Medical Sciences, New Delhi, India

Aim: Opioids like morphine are routinely used for the treatment of moderate to severe pain. However, their prolonged administration leads to tolerance and opioid-induced hyperalgesia which limits their use. The mechanism underlying the development of tolerance is not fully understood. Pain signals are transmitted to the spinal cord by A-delta and C- nerve fibres, where there is a release of different neurotransmitters and neuropeptides. Neuropeptide Y (NPY) is abundantly distributed in the mammalian nervous system. Several reports have shown it could affect the transmission of pain following tissue injury and nerve damage. Therefore, the aim of the present study was to determine the role of NPY in the development of morphine-induced antinociception and development of tolerance on a long term basis.

Methods: Male Sprague Dawley rats (275-325g), which were previously implanted with intrathecal catheters (ReCath Co, USA), were divided into groups and administered the following drugs: Saline, Morphine, NPY, and NPY+ morphine. Behavioral assessment of nociception was done by Hot-plate test daily for 9 days. Expression of NPY in the spinal cord was observed by Immunohistochemistry in morphine-tolerant rats.

Result: Repeated intrathecal administration of morphine produced an antinociceptive effect which decreased with time. Administration of NPY alone produced an antinociceptive effect which was less in comparison to morphine. However, the combined administration of NPY and morphine led to a significantly higher antinociceptive effect. Expression of NPY was down-regulated following chronic morphine treatment.

Conclusion: NPY appears to have an enhanced antinociceptive effect than morphine alone, indicating the potentiation of the effect of morphine. Decreased expression of NPY in tolerant animals revealed its role in morphine tolerance.

43 - Elucidating Neural Circuits That Transmit Affective-Motivational Pain Signals to the Amygdala

Sukjae Kang[1], Mao Ye[1], Shijia Liu[1,2], Sung Han[1,2]

[1]Salk Institute for Biological Studies, [2]Division of Biological Sciences at UCSD

Pain is a sensory and emotional experience associated with noxious stimuli that cause damages in our body. Perception of pain protects us from tissue-damaging situations by locating the source of damage, and promoting motivation to avoid the harmful situations. It has been believed that the sensory-discriminative, and affective-motivational domains of pain perception are governed by different brain structures that receive spinal nociceptive inputs. Recent discoveries pinpointed that the amygdala is a critical brain region that mediates affective-motivational component of pain. However, the circuit-based mechanism by which pain information is conveyed and processed to the amygdala is not well-understood. I will present our unpublished works about affective-motivational pain circuits that relay pain signals from the spinal cord to the amygdala.

1 - Separate pathways driving sustained pain versus reflexive-defensive reactions to external threats

Tianwen Huang[1,2], Shing-Hong Lin[1,2], Nathalie M. Malewicz[3], Martyn Goulding[4], Robert H. LaMotte[3], Qiufu Ma[1,2]

[1]Dana-Farber Cancer Institute, [2]Department of Neurobiology, Harvard Medical School, [3]Department of Anesthesiology, Yale University School of Medicine, [4]Molecular Neurobiology Laboratory, The Salk Institute of Biological Studies

Animals and humans display two types of response to noxious stimuli. The first includes reflexive defensive responses that prevent or limit injury; a well-known example of these responses is the quick withdrawal of one's hand upon touching a hot object. When the first-line response fails to prevent tissue damage (for example, a finger is burnt), the resulting pain invokes a second-line coping response—such as licking the injured area to soothe suffering. However, the underlying neural circuits that drive these two strings of behaviour remain poorly understood. Here we show in mice that spinal neurons marked by coexpression of $TAC1^{Cre}$ and $LBX1^{Flpo}$ drive coping responses associated with pain. Ablation of these spinal neurons led to the loss of both persistent licking and conditioned aversion evoked by stimuli (including skin pinching and burn injury) that—in humans—produce sustained pain, without affecting any of the reflexive defensive reactions that we tested. This selective indifference to sustained pain resembles the phenotype seen in humans with lesions of medial thalamic nuclei. Consistently, spinal TAC1-lineage neurons are connected to medial thalamic nuclei by direct projections and via indirect routes through the superior lateral parabrachial nuclei. Furthermore, the anatomical and functional segregation observed at the spinal level also applies to primary sensory neurons. For example, in response to noxious mechanical stimuli, MRGPRD- and TRPV1-positive nociceptors are required to elicit reflexive and coping responses, respectively.Our study therefore reveals a fundamental subdivision within the cutaneous somatosensory system, and challenges the validity of using reflexive defensive responses to measure sustained pain.

10 - Pruriceptive afferents and their interneuron partners in the mouse spinal cord

Noboru Iwagaki[1], Mark A Hoon[1]

[1]NIDCR, National Institutes of Health

Itch sensation causes a desire to scratch. However, the biological mechanisms of pruriception are yet incompletely understood. Here, we investigated the functional connectivity of a class of pruriceptive afferents, from Nppb-neurons, to their interneuron partners in the spinal cord. Previously, GRP-interneurons were proposed to be downstream of Nppb-neurons in the superficial dorsal horn of the mouse spinal cord, but this has not been formally established. We test whether pruriceptive afferent information converges at GRP-interneurons from Nppb afferents as well as another characterized class of pruriceptive afferents, MrgprA3 afferents. This work, combines of molecular genetics, electrophysiology and optogenetics to uncover a picture of the spinal cord and neural architecture for itch.

35 - Somatostatin modulates pain at both central and peripheral level in rats: A behavioural study

Rahul Kumar[1], Pranav Prasoon[1], Shivani Gupta[1], Subrata Basu Ray[1]

[1]All India Institute of Medical Sciences

Background: Somatostatin (SST) is widely expressed in mammalian central and peripheral nervous system. It has significant modulatory effect on the release of neurotransmitters. Several studies have observed the anti-nociceptive effect of its analogue octreotide. However, its expression at the spinal level following an acute nociceptive stimulus is not well known. Moreover, its involvement in mediating nociception at the periphery is also not well established. In the present study, the spatio-temporal expression of SST and its receptor (type-2) was observed at the spinal level. Thereafter, antinociceptive effect of somatostatin was assessed by behavioural assays.

Methods: Male Sprague-Dawley rats (N=88) were subjected to hind paw incision. The expression study of SST and its receptor type-2 was performed by Immunohistochemistry and Western blot at different post-incisional time points (2h, 8h, day 1, day 3). Comparison of anti-nociceptive effect of intra-wound (10, 30, 100mcg) and systemic (400µg/Kg i.p.) SST administration was evaluated by 3 different behavioural assays. Blood glucose level was examined. c-Fos expression in the spinal cord was also studied.

Results: Expression of SST showed an upregulation at 2 h, which decreased at 8 h and on day1. SSTr2 was also upregulated at 2h and 8h but decreased by day1. Repeated systemic administration relieved mechanical allodynia from 2h to day 3. Intra-wound SST relieved guarding pain between 2 h to day 3 and mechanical allodynia from day 4 onwards. Blood glucose level remained unaltered. c-Fos positive nuclei were significantly less after SST administration.

Conclusion: Somatostatin is involved in nociceptive modulation at both central as well as peripheral levels. This information could have clinical significance.

Keywords: Somatostatin, paw incision, anti-nociception.

46 - Conditional knockout of Prdm12 from sensory neurons in mice recapitulates aspects of human congenital insensitivity to pain

Mark Landy[1], Megan Goyal[1], Chen Liu[1], Helen Lai[1]

[1]University of Texas Southwestern Medical Center

Painful conditions are a huge medical burden with an estimated prevalence of 32% of the population, and an economic cost of $635 billion, or 4% of the GDP. Currently, the best available medical analgesics are opioids, but due to their vast side effect profile and risk of overdose, these are far from ideal drugs. Recently, the study of genetic mutations leading to congenital insensitivity to pain (CIP) has resulted in submission of novel drugs to the FDA for clinical trial. Here, we show our work on one such mutation of the gene Prdm12. We first characterized the expression pattern of Prdm12 within different sensory neurons present in the dorsal root ganglia (DRG), and found it to be expressed in both myelinated and unmyelinated small-diameter axons of the thoracic and lumbar DRGs. Further, we showed that Prdm12 is necessary during development for the maintenance of $TRKA^+$ nociceptor precursors. We then generated a new strain of mice in which Prdm12 has been conditionally knocked out of neurons in DRG and cranial ganglia, and subjected these mice to a variety of behavioral assays alongside littermate controls. We have shown that the $Prdm12^{cKO}$ mice have reduced sensitivity to nociceptive thermal and mechanical stimuli, and correlated this with a decrease in a variety of nociceptor markers, including IB4, CGRP, TRKA, and TRPV1. In ongoing studies, we are testing the analgesic and mechanistic effects of Prdm12 knockout in adult mice. We believe that further study of this mouse model, along with investigation of the downstream effectors and genomic targets of PRDM12, will provide a new avenue for the pursuit of analgesic drug discovery.

41 - Inhibitory synapse loss through a complement-microglia pathway in models of joint pain

Samantha Locke[1,3], Noosha Yousefpour[1,3], Valerie Bourassa[1,3], Alfredo Ribrieo-Da-Silva [1,2,3]

[1]Pharmacology and Therapeutics, Mcgill University, [2]Anatomy and Cell Biology, McGill University, [3]Alan Edwards Center for Research on Pain, McGill University

Joint pain affects 1 in 5 adults in developed countries and, as there are currently no optimal treatments, the identification of novel therapeutic targets is needed. Although it is known that there is a sensitization in the pain pathways, the specific mechanisms remain poorly understood. A change that could alter pain pathway excitability is an anatomical loss of inhibitory synapses in dorsal horn (DH) modulatory circuitry. In this study, we investigated the hypothesis that after prolonged joint pain there would be selective loss of inhibitory synapses in the DH mediated by microglia and complement factors. We used high resolution microscopy and two rat models of joint pain and detected, in male rats, a selective loss of inhibitory synapses, microgliosis and upregulation of complement factors in the superficial DH after sustained pain-related behavior. Complement factor tagging of inhibitory terminals and increased engulfment of inhibitory terminal material by microglia were observed. Together these data suggest that inhibitory synapse loss in the DH occurs through a microglial and complement pathway in models of joint pain which may reveal multiple novel therapeutic targets for many chronic pain conditions.

This work was supported by CIHR operating grant MOP-136903 to ARS. SL, NY and VB acknowledge studentships from the Louise and Alan Edwards Foundation.

34 - COOLING REVERSES PATHOLOGICAL BIFURCATIONS TO SPONTANEOUS FIRING CAUSED BY MILD TRAUMATIC INJURY.

B.M. Barlow[1], B. Joos[1], AK Trinh[1], A Longtin [1]

[1]Physics and Centre for Neural Dynamics, University of Ottawa

Mild traumatic injury can modify the key sodium (Na^+) current underlying the excitability of neurons. It causes the activation and inactivation properties of this current to become shifted to more negative trans-membrane voltages. This leads to a chronic influx of Na^+into the cell that eventually causes spontaneous or “ectopic” firing along the axon, even in the absence of stimuli. The bifurcations underlying this enhanced excitability have been worked out in full ionic models of this effect. Here we present computational evidence that increased temperature Tcan exacerbate this pathological state. Conversely, and perhaps of clinical relevance, mild cooling is shown to move the naturally quiescent cell further away from the threshold of ectopic behavior. The origin of this stabilization-by-cooling effect is analyzed by knocking in and knocking out, one at a time, various processes thought to be T-dependent. The T-dependence of the Na^+current, quantified by its $Q_{10\text{-}Na}$factor, has the biggest impact on the threshold, followed by $Q_{10\text{-}pump}$of the sodium-potassium exchanger. Below the ectopic boundary the steady state for the gating variables and the resting potential are not modified by temperature, since our model separately tallies the $Na^+$and K^+ions including their separate leaks through the pump. When only the gating kinetics are considered, cooling is detrimental, but in the full T-dependent model it is beneficial because the other processes dominate. Cooling decreases the pump’s activity, and since the pump hyperpolarizes, less hyperpolarization should lead to more excitability and ectopic behavior. But actually the opposite happens in the full model because decreased pump activity leads to smaller gradients of $Na^+$and K^+, which in turn decreases the driving force of the Na^+current.

9 - Preventing the collapse of augmented α2,3-GABAA-dependent inhibition rescues benzodiazepine-mediated analgesia in neuropathic pain

Louis-Etienne Lorenzo[1], Antoine G. Godin[1], Francesco Ferrini[3], Karine Bachand[1], Isabel Plasencia Fernandez[1], Simon Labrecque[1], Geneviève Brindle[1], Dominic Boudreau[1], Nicolas Doyon[1], Alfredo Ribeiro-da-Silva[2], Yves De Koninck[1]

[1]Laval University, [2]McGill University, [3]University of Turin

Spinal disinhibition has been hypothesized to underlie pain hypersensitivity in neuropathic pain. Apparently contradictory mechanisms have been reported, however; raising questions on the best target to produce analgesia in neuropathic pain. Here, we show that peripheral nerve injury (PNI) is associated with a reduction in the number of inhibitory synapses in the rat spinal dorsal horn. Paradoxically, this is accompanied by an upregulation of synaptic $GABA_A$ receptors ($GABA_ARs$) but not in glycine receptors and by an α1-to-α2 $GABA_AR$ subunit switch with ensuing enhanced strength of miniature synaptic $GABA_AR$ events. BDNF-TrkB signaling was both necessary and sufficient to explain the subunit switch. The switch provides a mechanistic rationale for the analgesic action of the α2,3 $GABA_AR$ benzodiazepine site ligand L838,417 after PNI. Yet, the BNDF-TrkB signaling concurrently mediates KCC2 hypofunction. Consistently, we demonstrate that impaired Cl^- extrusion underlies the failure of L838,417 to induce analgesia at high doses due to a resulting collapse in Cl^- gradient, dramatically limiting the therapeutic window of benzodiazepines. In turn, enhancing KCC2 expression not only potentiated L838,417-induced analgesia but also rescued its full analgesic potential at high doses. These findings reveal a novel strategy for analgesia in pathological pain, by combined targeting of the appropriate $GABA_AR$ subtypes and restoring Cl^- homeostasis.

49 - CCR2-induced neuroimmune communication drives bone cancer pain and breast tumor growth

Élora Midavaine[1], Alexandra Trépanier[1], German De Armas Guitart[1], Vera Zeugin[1], Christine Mona[1], Jean-Michel Longpré[1], Éric Marsault[1], Philippe Sarret[1]

[1]Université de Sherbrooke

The CCL2 chemokine and its cognate receptor CCR2 are well known for their pro-inflammatory role both in the nociceptive and immune systems. Acting as an excitatory neurotransmitter and signaling molecule between neurons and glia, CCL2 promotes nociceptive signaling and neuroinflammation. As a potent chemoattractant, CCL2 also drives myeloid and lymphoid cell recruitment to sites of tissue damage. CCL2 is further an important contributor to the development and progression of cancer metastasis by promoting extensive tumor cell proliferation in the bone microenvironment. Given the detrimental effects of the CCL2/CCR2 axis in diseases involving inflammatory responses, we investigated the potential of targeting CCR2 as a therapeutic strategy to reduce the tumor burden and improve the management of pain. Here, we demonstrate in a bone cancer pain model in female rats that CCR2 inhibition through either allosteric modulation using lipopeptides (pepducins) or CCR2 silencing using DsiRNA encapsulated in lipid nanoparticles resulted in robust analgesia after intrathecal injection. Bone cancer pain induces increased neurotransmitter expression, innate and adaptive immune cell recruitment and resident glial cell activation in the dorsal root ganglia, which are reduced following CCR2 allosteric antagonism. We also found that the anti-CCR2 therapeutic effects on decreased neuroimmune signaling are restricted to the dorsal root ganglia since no glial activation and immune cell infiltration are detected in the spinal cord of tumor-bearing female rats. Our results further revealed that disrupting CCR2 signaling results in decreased tumor-induced bone remodeling and tumor size, in part through the decrease in substance P and CGRP release by the crural nerve in the tumor microenvironment. In conclusion, CCR2 allosteric modulation or silencing leads to alleviation of bone cancer pain resulting from neuroglia communication disruption, limited immune cell infiltration and decreased tumor burden.

38 - Lionfish venom elicits pain predominantly through the activation of non-peptidergic nociceptors

Stephanie Mouchbahani-Constance[1], L. Stephen Lesperance[2,3], Hugues Petitjean[1], Albena Davidova[1], Aurore Bernardin[1], Jean A. Ouellet[4], Robert J. Zamboni[5], Steven A. Prescott[2,3], Reza Sharif-Naeini[1]

[1]Department of Physiology and Cell Information Systems, McGill University, Montreal, Canada, [2]Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Canada, [3]Department of Physiology and Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada, [4]Department of Surgery, McGill University, Montreal, Canada, [5]Department of Chemistry, McGill University, Montreal, Canada

The lionfish (Pterois volitans) is a venomous invasive species found in the Caribbean and Northwestern Atlantic. It poses a growing health problem because of the increase in frequency of painful stings, for which no treatment or antidote exists. In this study, we provide the first characterization of the pain and inflammation caused by lionfish venom and physiological, calcium imaging and electrophysiological testing. Intraplantar injections of the venom produce a significant increase in pain behaviour, as well as a marked increase in mechanical sensitivity for up to 24 hours after injection. The algogenic substance(s) are heat-labile peptides that cause neurogenic inflammation at the site of injection and induction of Fos and microglia activation in the superficial layers of the dorsal horn. Finally, calcium imaging and electrophysiology experiments show that the venom acts predominantly on nonpeptidergic, TRPV1-negative, nociceptors, a subset of neurons implicated in sensing mechanical pain. These data provide the first characterization of the pain and inflammation caused by lionfish venom, as well as the first insight into its possible cellular mechanism of action. Future work will be focused on isolating the algogenic toxin in the venom and identifying its receptor target in order to understand the molecular mechanism for the pain caused by lionfish venom. Furthermore, the toxin's affinity for its receptor will be characterized using cell lines expressing the receptor in calcium imaging and electrophysiology experiments. These results will 1) identify novel modulators of the pain pathway, 2) identify novel therapeutic targets in the treatment of lionfish envenomations and 3) potentially produce novel analgesics by using low-dose toxin peptides in therapeutic agents to produce analgesic effects.

21 - Characterization of the role of dorsal horn calretinin-expressing interneurons to the processing of pain inputs

Hugues Petitjean[1], Mr. Farin B. Bourojeni[2], Mrs. Susana G. Sotocina[1], Mrs. Deborah[1], Ms. Davidova Albena[1], Dr. Jeffrey S[1], Dr. Artur Kania[2], Dr. Reza Sharif-Naeini[1]

[1]McGill University, [2]IRCM

The dorsal horn of the spinal cord (DH) is the first relay centre of somatosensory information originating from the periphery. In the superficial DH laminae I-II, nociceptive information is processed by a complex network of excitatory and inhibitory interneurons whose function and connectivity remain poorly understood. In this study, we examine the role of calretinin-expressing interneurons (CR neurons) in the processing of innocuous and noxious sensory inputs. These neurons are mainly located in lamina II, where they receive direct inputs from the central endings of nociceptive fibers and polysynaptic inputs from touch-sensitive A-beta fibers. Their activation by either chemogenetic or optogenetic stimulation produces mechanical allodynia and nocifensive behaviors. Furthermore, we examined the position of CR neurons in the DH circuitry, where they would be ideally positioned to modulate the activity of pain projection neurons in lamina I. In conclusion, we propose a new neuronal pathway in which CR neurons are positioned at the junction between incoming nociceptive and innocuous circuits and ascending pain pathways of the dorsal horn.

17 - Characterization of neuronal circuits processing somatosensory information in the spinal cord

Sofia Pimpinella[1], Niccolo Zampieri[1]

[1]Max-Delbrück-Centrum für Molekulare Medizin

Somatosensory information is first detected by specialized sensory neurons in the periphery and then transmitted to the central nervous system (CNS), where sensory perception is formed in order to generate appropriate behavioral response. The projections of afferents in defined layers of spinal cord provide access to distinct neuronal pathways in the CNS, where spinal interneurons represent the first relay station controlling the coding of sensory stimuli. Despite its importance, little is known about the organization of spinal circuits involved in the integration of distinct sensory information. In order to start addressing this problem, we take advantage of the observation that rabies virus can be used to trace sensory neurons in the anterograde direction, thus allowing to link directly a sensory modality with its output connectivity. First, to make sensory neurons competent for rabies transynaptic spread and to restrict primary infection to a subset of sensory neurons of choice, we used mouse genetics to conditionally express histone-tagged GFP, TVA receptor, and rabies glycoprotein G (Rosa-HTB: Yan Li et al., 2013) under the control of Cre- recombinase activity. As a proof of principle, we first focused on proprioception, as sensory neurons in the DRGs can be efficiently target using Pv::cre mouse line (Hippenmeyer S. et al., 2005) and proprioceptive circuits in the spinal cord has been previously characterized. Therefore, we performed stereotactic rabies injections (RVDG-mCherry/EnvA) in the spinal cord of Pv::cre+/- Rosa HTB mice. First, we checked the specificity and efficiency of rabies primary infection in the DRGs and we observed that we could selectively target proprioceptors with high efficiency. Second, we mapped the distribution of post-sensory neurons labeled by rabies tracing. As expected, we obtained highly reproducible distribution patterns consistent with the known trajectory and termination of proprioceptive afferents in the spinal cord. Third, we tested whether different populations of spinal neurons, known to receive proprioceptive input, were labeled in our tracing experiments. Indeed, we observed labeling of motor neurons, V1 and V2a interneurons. Finally, we tested whether we could use this method to trace from other primary sensory neuron subsets. We focused in TRPV1::cre mouse line (Mishra SK. et al., 2011) known to be involved in noxious heat. We confirmed the specificity of the primary rabies infection in the DRGs and we observed a distinct neuronal circuit in the spinal cord by labeling mostly post-sensory neurons in the superficial laminae of the spinal cord. Altogether, these data indicate that this method allows tracing from different subset of primary somatosensory neurons paving the way for anatomical, molecular and functional characterization of spinal circuits controlling somatosensation.

44 - TrkB receptors engage different signaling cascades regulating respectively KCC2 function, trafficking and degradation.

Isabel Plasencia Fernandez[1,2], Marc J. Bergeron[1], Yves De Koninck[1,2]

[1]Laval University, [2]CERVO Brain Research Centre

The K^+-Cl^- cotransporter 2 (KCC2) is essential to maintain low intracellular chloride levels in neurons, controlling the strength of inhibition. Thus, KCC2 downregulation triggers a loss of neuronal inhibition that plays an important role in several neurological disorders. Both BDNF-TrkB and NMDAR signaling have been reported to play a role in the regulation of KCC2 expression, but how these mechanisms interact has not been studied yet. Our results show that TrkB signaling downregulates KCC2 function independently of the protein levels but NMDAR signaling has an effect on both internalization and degradation of KCC2. These distinct regulator mechanisms are dependent on different Ca^{2+} levels and sources. TrkB-mediated modulation of KCC2 is prevented by blockade of endoplasmic reticulum Ca^{2+} channels with dantrolene, indicating that it relies on intracellular Ca^{2+} pools, while NMDAR-induced KCC2 downregulation was only prevented by extracellular Ca^{2+} depletion. Interestingly, NMDAR-dependent KCC2 degradation, but not internalization, depends on voltage-gated Ca^{2+} channels (VGCC) and calpain activation as it was prevented by cadmium and MDL28170, respectively. We also found that TrkB signaling potentiated the NMDAR-mediated KCC2 downregulation, but high concentrations of NMDA are sufficient to induce downregulation of KCC2. Overall, our results show that TrkB-signaling regulates KCC2 through different signaling cascades and across different time scales. First by affecting KCC2 function and, in turn, by triggering NMDAR-mediated KCC2 internalization, followed eventually by VGCC mediated degradation.

48 - Selective melatonin MT2 receptor ligands induce an opioid-mediated antiallodynic effect by activating the opioid system in the descending brainstem antinociceptive pathway

Luca Posa[1,2], Lopez-Canul M.[1], De Gregorio D.[1], Rullo L.[3], Maione S.[4], Romualdi P.[3], Kieffer B.L.[1,5], Gobbi G.[1,2]

[1]Department of Psychiatry, McGill University, Montreal Qc, Canada, , [2]Alan Edwards Centre for Research on Pain, McGill University, Montreal QC, Canada;, [3]Department of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, [4]Department of Experimental Medicine, University of Campania, Naples, Italy;, [5]Douglas Hospital Research Center, McGill University, Montreal, QC, Canada.

Introduction: Neuropathic pain is an important health problem for which only a few treatments are available. Preclinical studies show that melatonin (MLT) has analgesic properties mediated by the melatonin MT2 receptors. Here we determined the effects of the selective MT2 receptor partial agonist (UCM924) in a rodent neuropathic pain model by examining its supraspinal mechanism of action.

Methods: Spared nerve injury (SNI) model was used to evaluate the mechanical allodynia, and the in-vivo electrophysiological modulation of the ON and OFF cells in the periaqueductal grey-rostral ventral medulla (PAG-RVM) projections was performed in SNI rats. Moreover, we investigated the morphological localization of MT2 and μ-OPr and the gene expression of the endogenous opioid receptor (OPr) ligand proenkephalin (PENK) following the treatment with UCM924 in the PAG.

Results: UCM924 (20 mg/kg) produced a prolonged antinociceptive effect. Using in-vivo electrophysiology combined with tail-flick, the microinjection of UCM924 (10 ug) into the ventrolateral PAG decreased tail-flick response, depressed the firing activity of ON cells, and activated the firing of OFF cells. Importantly, the non-selective (naloxone) and the selective (CTOP) m-OPr antagonist, blocked the antinociceptive effects of UCM924 in the SNI model and its effect on ON and OFF cells; while the selective d-OPr antagonist (naltrindole) (1 mg/kg) had no effects. Immunohistochemical results revealed that MT2 receptors are located in the rostral third part of the PAG where μ-OPr receptors are also abundant. Moreover, UCM924 increased the PENK gene expression into the PAG.

Discussion: Altogether, these data demonstrate that selective MT2 receptor agonists have analgesic properties through modulation of brainstem descending antinociceptive pathways and this effect is mediated by μ-OPr via enkephalins involvment. MT2 receptors may represent a novel target in the treatment of neuropathic pain.

40 - Cold-Response is Reduced in the African Naked Mole-Rat

Sandra J. Poulson[1], Melissa M. Holmes[1,2], Loren J. Martin[1,2]

[1]Department of Psychology, University of Toronto, [2]Cell & Systems Biology, University of Toronto

Adults develop hypersensitivity to mechanical and thermal stimuli after nerve injury. Children recover from nerve injury better than adults and are less likely to develop chronic pain. To investigate the development of chonic pain during pre-pubertal stages, we examined hypersensitivity following nerve injury using subordinate African naked mole-rats; a species where most adults do not experience puberty. Specifically, we compared responses of naked mole-rats and mice to mild mechanical, strong mechanical, and mild cold stimuli. Mechanical sensitivities were similar between the two species. However, we observed an absence of response to mild cold in adult subordinate naked mole-rats. We then tested acute responses in non-injury animals by measuring nocifensive behavior upon application of chemical activators of ion channels implicated in cold sensation. Mustard oil, an activator of TRPA1 evoked a similar amount of nocifensive responses between the two species. In contrast, icilin - an activator of TRPM8 - induced a strong pain phenotype in mice but a minimal response in naked mole-rats. We compared levels of mRNA expression of TRP ion channels (TRPA1, TRPM8 and TRPV1) using RNAscope and did not see a lack of mRNA expression in naked mole-rat DRG tissue compared to mice. We then measured whether intrathecal CGRP rescued nocifensive behavior to icilin in naked mole-rats. Our results reflect differing evolutionary adaptations in chonic and accute pain systems between these rodent species in response to environmental demands.

37 - Distribution of delta and mu receptors in rat dorsal root ganglia neurons

Béatrice Quirion[1,2,4,5], Claudie Beaulieu[1,2,4,5], Véronique Blais[1,2,4], Louis Gendron[1,2,3,4,5]

[1]Université de Sherbrooke, [2]Département de Pharmacologie-physiologie, [3]Département d'anesthésiologie, [4]Institut de Pharmacologie, [5]Centre de recherche du CHUS

Opioids, like morphine, remain the most commonly used drug to treat moderate to severe pain. Despite the existence of three opioid receptors subtypes (mu (MOP), delta (DOP) and kappa (KOP)), most opioids used clinically preferentially target MOP. However, the activation of this receptor is also responsible for the main undesired effects of opioids such as addiction, nausea, constipation and respiratory depression. One of the main interests of our laboratory is to investigate the therapeutic potential of DOP agonists. To this end, we have undertaken to identify which dorsal root ganglia (DRG) neurons express DOP in order to have a better understanding of the potential analgesic effects of these molecules.

The main objectives of this project are to 1) study the distribution and co-expression of MOP and DOP in DRG neurons in rodents as well as in higher species and 2) to verify the effect of a morphine treatment on their distribution.

In the DRG, neurons/fibers are typically divided into three different categories, which are the large diameter fibers (Aα et ß fibers; primarily responsible for touch and proprioception), the medium diameter fibers (Aδ fibers; responsible mostly for nociception), and the small diameter peptidergic and non-peptidergic fibers (C fibers; also responsible for nociception). The distribution of DOP and MOP receptors in these neurons was studied by in situ hybridization using the RNAscope™ technology. The different types of neurons were identified by immunohistochemistry using NF200, IB4 and substance P as markers. In rats, our results show that DOP is mainly present in neurons that are NF200-positive while MOP is present mostly in small neurons. Notably, DOP and MOP are co-expressed in a number of DRG neurons. Most interestingly, our preliminary results suggest that co-expression of DOP and MOP is increased following a 48-hour treatment with morphine. These results further suggest that the role of these receptors could change according to different treatments or physiological conditions.

This work is supported by the NSERC.

20 - Studying the Role of Dcc in the Development of Nociceptive Topognosis

Shima Rastegar-Pouyani[1,2], Artur Kania[1,2]

[1]Neural Circuit Development Laboratory, Institut de Recherches Cliniques de Montreal (IRCM), Montreal, QC, Canada, [2]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada

Nociceptive topognosis or the ability to localise noxious stimuli, is critical for avoidance of environmental dangers. Nociceptive information is carried from the periphery to different brain regions through spinal projection neurons (PNs). Mice with a spinal cord-specific knockout of Dcc, encoding a Netrin receptor, are unable to accurately localise noxious stimuli, similar to DCC mutant humans. However, the identity of PNs that transmit the location of a noxious stimulus to the brain remains unknown. To uncover it, I created $Dcc^{Phox2a:CreKO}$ mice, in which the embryonic Phox2a:Cre driver deletes Dcc in a specific population of PNs. To assess nociceptive topognosis of $Dcc^{Phox2a:CreKO}$ mice, thermal pain was induced by capsaicin injection into one of their hindpaws, and the accuracy with which they direct licking to the injection site was assessed. My results show that $Dcc^{Phox2a:CreKO}$ mutants erroneously lick untreated limbs more frequently than control mice. Further behavioural analyses suggest that the function of local spinal nociceptive circuits is normal in $Dcc^{Phox2a:CreKO}$ mice arguing that their topognosis deficits uncover a function of Phox2a PNs in topognosis. More generally, my results provide a genetic inroad into discriminatory versus emotive organization of ascending nociceptive pathways.

25 - Phox2a defines a developmental origin of the anterolateral system and is required for supraspinal nociception.

R. Brian Roome[1,2], Susana Sotocinal[2], Annie Dumouchel[1,2], Shima Rastegar-Pouyani[1,2], Charleen Salesse[1,2], Bishakha Mona[4], William Scott Thompson[1,2], Martyn Goulding[3], Jane Johnson[4], Lino Tessarollo[5], Jeffrey S Mogil[2], Marie Kmita[1], Artur Kania[1,2]

[1]Institut de Recherches Cliniques de Montreal (IRCM), Montreal, QC, [2]McGill University, Montreal, QC, [3]The Salk Institute, La Jolla, CA, [4]University of Texas Southwestern, Dallas, TX, [5]National Cancer Institute, Frederick, MD

The mammalian anterolateral system (ALT) is the main pathway relaying pain, itch and temperature information from the spinal cord to the brain. Classical anatomical and physiological experiments suggest a functional diversity of the spinal projection neuron (PN) subpopulations that give rise to this pathway. However, without molecular handles, their relative contribution to somatosensation remains unknown.

The Paired-like homeobox 2A (Phox2a) transcription factor is expressed in the dI5 spinal neuron lineage hypothesised to give rise to PNs. Using genetic anterograde and retrograde labelling, we show that virtually all spinal neurons of the Phox2a lineage give rise to PNs that innervate ALT nociceptive targets, such as the parabrachial nucleus, peri-aqueductal grey, nucleus of the solitary tract and the thalamus. Nearly half of spinal PNs innervating the parabrachial nucleus and the thalamus express Phox2a, revealing it as the first exclusive molecular handle of a major spinofugal PN population. Phoxa2 PNs are born concurrently with motor neurons, constituting the earliest spinal postmitotic population. The spinal dI lineage determinants Ascl1 and Ptf1a control the early aspects of Phox2a PNs specification, directing them to develop into all anterolateral system spinal PN types, first populating lamina I, then lamina V. Lamina I neurons are initially born in the intermediate spinal cord and migrate tangentially into Lamina I in a primary afferent-dependent manner. A developmental loss of spinal Phox2a function does not affect nociceptive spinal reflexive behaviours. In contrast, behaviours that involve supraspinal relay of nociceptive information are impaired in such mutants, arguing that normal ALT function requires Phox2a. Our experiments provide a molecular handle on a major constituent PN population of the ALT, allowing us to probe its specific adult function.

22 - Phox2a defines a developmental origin of the anterolateral system and is required for supraspinal nociception.

R. Brian Roome[1,2], Susana Sotocinal[2], Annie Dumouchel[1], Shima Rastegar-Pouyani[1,2], Charleen Salesse[1,2], William Scott Thompson[1], Martyn Goulding[3], Jane Johnson[4], Lino Tessarollo[5], Jeff Mogil[2], Marie Kmita[1,2], Artur Kania[1,2]

[1]Institut de Recherches Cliniques de Montréal (IRCM), Montreal, QC, Canada, [2]McGill University, Montreal, QC, Canada, [3]The Salk Institute, La Jolla, CA, USA, [4]UT Southwestern, Dallas, TX, USA, [5]National Cancer Institute, Frederick, MD, USA

The mammalian anterolateral system (ALT) is the main pathway relaying pain, itch and temperature information from the spinal cord to the brain. Classical anatomical and physiological experiments suggest a functional diversity of the spinal projection neuron (PN) subpopulations that give rise to this pathway. However, without molecular handles, their relative contribution to somatosensation remains unknown. The Paired-like homeobox 2A (Phox2a) transcription factor is expressed in the dI5 spinal neuron lineage hypothesised to give rise to PNs. Using genetic anterograde and retrograde labelling, we show that virtually all spinal neurons of the Phox2a lineage give rise to PNs that innervate ALT nociceptive targets, such as the parabrachial nucleus, peri-aqueductal grey, nucleus of the solitary tract and the thalamus. Nearly half of spinal PNs innervating the parabrachial nucleus and the thalamus express Phox2a, revealing it as the first exclusive molecular handle of a major spinofugal PN population. Phoxa2 PNs are born concurrently with motor neurons, constituting the earliest spinal postmitotic population. The spinal dI lineage determinants Ascl1 and Ptf1a control the early aspects of Phox2a PNs specification, directing them to develop into all anterolateral system spinal PN types, first populating lamina I, then lamina V. Lamina I neurons are initially born in the intermediate spinal cord and migrate tangentially into Lamina I in a primary afferent-dependent manner. A developmental loss of spinal Phox2a function does not affect nociceptive spinal reflexive behaviours. In contrast, behaviours that involve supraspinal relay of nociceptive information are impaired in such mutants, arguing that normal ALT function requires Phox2a. Our experiments provide a molecular handle on a major constituent PN population of the ALT, allowing us to probe its specific adult function. The mammalian anterolateral system (ALT) is the main pathway relaying pain, itch and temperature information from the spinal cord to the brain. Classical anatomical and physiological experiments suggest a functional diversity of the spinal projection neuron (PN) subpopulations that give rise to this pathway. However, without molecular handles, their relative contribution to somatosensation remains unknown. The Paired-like homeobox 2A (Phox2a) transcription factor is expressed in the dI5 spinal neuron lineage hypothesised to give rise to PNs. Using genetic anterograde and retrograde labelling, we show that virtually all spinal neurons of the Phox2a lineage give rise to PNs that innervate ALT nociceptive targets, such as the parabrachial nucleus, peri-aqueductal grey, nucleus of the solitary tract and the thalamus. Nearly half of spinal PNs innervating the parabrachial nucleus and the thalamus express Phox2a, revealing it as the first exclusive molecular

handle of a major spinofugal PN population. Phoxa2 PNs are born concurrently with motor neurons, constituting the earliest spinal postmitotic population. The spinal dI lineage determinants Ascl1 and Ptf1a control the early aspects of Phox2a PNs specification, directing them to develop into all anterolateral system spinal PN types, first populating lamina I, then lamina V. Lamina I neurons are initially born in the intermediate spinal cord and migrate tangentially into Lamina I in a primary afferent-dependent manner. A developmental loss of spinal Phox2a function does not affect nociceptive spinal reflexive behaviours. In contrast, behaviours that involve supraspinal relay of nociceptive information are impaired in such mutants, arguing that normal ALT function requires Phox2a. Our experiments provide a molecular handle on a major constituent PN population of the ALT, allowing us to probe its specific adult function.

30 - Cellular and circuit mechanisms underlying sensorimotor processing in the spinal cord

Anupama Sathyamurthy[1], Kory Johnson[1], Kaya J.E. Matson[1], Arnab Barik[1], Courtney I Dobrott[1], Li Li[1], Alexander Chesler[1], Ariel J Levine[1]

[1]National Institutes of Health

Our survival and well-being are predicated on the ability of the nervous system to transform complementary sensory cues into stimulus-appropriate motor responses. The spinal cord represents a crucial node in this process of sensorimotor transformation. A diverse array of spinal neurons is tasked with converting somatosensory stimuli into actionable cues and transforming descending motor instructions into specific muscle activation patterns. In order to dissect the neural mechanisms by which the spinal cord brings about sensorimotor transformations, it is necessary to identify the cell types that constitute the cord, reveal the molecular repertoire that endows the cell types with their functional specializations, and illuminate the network architecture in which the cell types are embedded. First, to define the cell types of the adult mammalian spinal cord, we adapted new techniques in single cell biology. Using gene expression profiles of thousands of individual spinal cord cells, we identified and molecularly characterized 43 distinct neuronal cell types and established the first major atlas of adult spinal cord cell types. Majority of neuronal cell types were defined by neurotransmitter status and dorso-ventral address of constituent neurons, suggesting that transcriptional variation correlates with neurotransmitter status and spatial identity. While dorsal neurons formed discrete clusters, ventral neurons displayed overlapping gene expression patterns, suggesting the latter could have a continuum of phenotypes. Furthermore, by performing this technique immediately following formalin-injection and locomotion, and mapping transient molecular signatures of neuronal activity onto single neurons, we identified both previously known and novel populations of spinal neurons that were active during these tasks. With this atlas in hand, we next sought to systematically probe how spinal cell types interact with different brain regions. Virus-based mapping of descending inputs revealed both previously known and uncharacterized pathways from the brain to the spinal cord. Current studies are geared towards using intersectional genetic strategies to target specific descending pathways to the spinal cord and unravel how spinal interneuron function is modulated by descending inputs to shape motor outcomes. Together, this work greatly expands our knowledge of adult spinal cord neurons and will facilitate our understanding of the neural networks underlying sensorimotor processing.

8 - Multi-modality of a Single Afferent Subset Transmitting Itch or Pain is Achieved by Differential Recruitment of TRP Channels

Behrang Sharif[1], Ariel Ase[1], Alfredo Ribeiro-da-Silva[1], Philippe Seguela[1]

[1]McGill University

Despite the known anatomical and behavioral overlap of pruriception and nociception, the underlying neurophysiological basis of itch and its relation to pain is still unclear. To examine the multimodal capacity of first-order somatosensory afferents, we focused on a genetically-defined subpopulation of primary C-fiber pruriceptors that express the chloroquine receptor MrgprA3. To be able to assess the effects of a wide variety of activation conditions, we took advantage of Cre-dependent optogenetic and chemogenetic actuators for selective activation of MrgprA3^{+} neurons. Quantitative behavioral analysis was performed after anatomical and functional validation of heterologous excitatory opsins and DREADDs in these primary sensory neurons. Our behavioral analysis shows that chemogenetic activation of MrgprA3^{+} afferents evokes stereotypical itch behaviors rather than pain responses. Unexpectedly, light-gated ionotropic activation of these same neurons, through ChR2, predominantly induces pain responses and avoidance behaviors rather than itch. Pharmacological interventions confirm that the itch modality transmitted by this afferent subset is sensitive to gastrin releasing peptide receptor antagonist, while the pain modality is μ opioid-sensitive. Furthermore, we show that several types of calcium-permeable TRP channels expressed in MrgprA3^{+} neurons are exclusively engaged in the induction of itch behavior yet optically-induced nocifensive behavior is not affected by blockade of these channels. Our findings support the existence of intrinsic peripheral mechanisms for sensory discrimination at the level of primary sensory neurons to mediate either itch or pain depending on their mode of activation.

32 - Microglia-mediated removal of perineuronal nets contributes to the development of hypersensitivity in neuropathic pain models

Shannon Tansley[1], Noosha Yousefpour[2], Annie Castonguay[3], Godin Antoine[4], Jordyn Heal[2], Ji Zhang[2], Luda Diatchenko[2], Yves de Koninck[4], Jeffrey Mogil[2], Arkady Khoutorsky[2]

[1]McGill, [2]McGill University, [3]INRS-IAF, [4]Université Laval

Introduction/Aim:

In the nervous system, neurons and glial cells are embedded within an extracellular matrix (ECM), whose components not only provide structural support, but also regulate synapse formation and function, and modulate neuronal excitability. The ECM restricts synaptic and structural plasticity, and enzymatic digestion of ECM affects acquisition of memories, and promotes cognitive flexibility and extinction. In this study, we investigated how remodeling of ECM components, specifically perineuronal nets (PNNs) contributes to the sensitization of spinal nociceptive circuits after injury.

Methods:

Immunohistochemical analysis was used to assess varying components of PNNs in the spinal cord, as well as microglial engulfment. To label projection neurons, AAV2/9-CMV-CRE-eGFP virus was injected into parabrachial nuclei of TdTomato reporter mice. Behavioural studies were performed after intraspinal delivery of AAV2/9-CMV-chABC on 6-8-week-old C57BL/6 mice. Both sexes were used in all studies.

Results:

In the dorsal horn of the spinal cord, perineuronal nets are preferentially found around projection neurons in the lamina I, and the lateral spinal nucleus. After spared nerve injury (SNI), microglia mediate removal of components of PNNs via engulfment processes. Viral delivery of an enzyme that degrades PNNs (AAV 2/9-CMV-chABC) promotes hypersensitivity in mice in both SNI and CCI (chronic constriction injury) models.

Discussion/Conclusions:

Nerve injury causes robust microglia-mediated remodeling of the ECM in the dorsal horn of the spinal cord. Removal of perineuronal nets promotes hypersensitivity in mice, suggesting that PNNs are involved in regulation of spinal nociceptive circuits. Ongoing studies are aiming to assess the role of PNNs in chloride regulation after nerve injury.

7 - Methylglyoxal, a glycolytic metabolite associated with painful diabetic neuropathy, alters C-fibre activity-dependent slowing and induces thermal hyperalgesia in a sex-dependent manner.

Atanaska Velichkova[1], Menekse Mutlu-Smith[1], Amy L. Hall[1], Carole [1]

[1]Centre for Discovery Brain Sciences, University of Edinburgh, United Kingdom

Background and aims: Diabetic neuropathic pain is associated with raised plasma concentrations of the glycolytic metabolite methylglyoxal (MG)[1]. One proposed mechanism of MG-induced pain is post-translational modification of the voltage-gated sodium channels Nav1.7 and Nav1.8[1]. Both of these voltage-gated sodium channels are involved in activity-dependent slowing (ADS) of C-fibre nociceptors[2,3], whereby repetitive stimulation (≥1Hz) results in a progressive slowing of action potential conduction velocity, which manifests as a progressive increase in response latency[4] that may influence spinal pain processing and thermal sensitivity[5]. We have recently shown that C-fibre ADS is altered in a chemotherapy-induced neuropathic pain model[6] and also the CFA inflammation model in a sex-dependent manner[5]. We therefore aimed to explore whether the glycolytic metabolite, methylglyoxal, alters C-fibre ADS and induces thermal hyperalgesia in both sexes.

Methods: All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and IASP ethical guidelines for animal research. Lumbar (L4 & L5) dorsal roots (DR) were isolated from juvenile (~P21) naïve Sprague-Dawley rats of both sexes. The isolated DRs (without dorsal root ganglia) were incubated in 100μM MG or vehicle control for 3 hours prior to electrophysiological recordings. Population compound action potentials (CAPs) were recorded in the continued presence of 100μM MG or vehicle. C-fibre ADS was assessed in response to x40 stimuli at 1Hz, 2Hz and 10Hz. ADS was quantified by assessing the change in latency (negative peak) and the change in width (positive peak to positive peak) of the triphasic C-fibre response. To negate any influence of varying dorsal root length, the latency/width change was normalised to the length of the stimulated root. Area under the curve (AUC) analysis was used to compare treatment groups. Thermal sensitivity was assessed in a separate group of animals before and after i.p. administration of 5mg MG or vehicle control.

Results: Chronic MG application had no effect on the threshold, amplitude or conduction velocity of the C-fibre response in both sexes. C-fibre ADS was observed in isolated dorsal roots as a progressive increase in the latency and width of the C-fibre component of the CAPs. In females, using both the latency and width measures, chronic MG application increased the frequency-dependent C-fibre ADS compared to vehicle treatment ($p<0.001$). In contrast, in males MG decreased the frequency-dependent ADS as assessed by the latency change in the C-fibre response ($p=0.0006$). However, chronic MG application did not significantly alter male ADS as reflected by the width change of the C-fibre response. Behaviourally, MG induced thermal hyperalgesia in males but not females.

Conclusion: Chronic MG application alters C-fibre ADS in a sex-dependent manner and induces thermal hyperalgesia in males only. This may contribute to our understanding of the mechanisms underlying sex differences in the manifestation of diabetic neuropathic pain.

Source of Financial Support: The Carnegie Trust for The Universities of Scotland, University of Edinburgh, Edinburgh Medical School: Biomedical Sciences International Career development PhD Studentship

13 - Different coding strategies for heat and cold by primary sensory neurons

Feng Wang[1], Erik Bélanger[1,2], Sylvain L. Côté[1], Patrick Desrosiers[1,3], Steven A. Prescott[4,5], Daniel C. Côté[1,2,3], Yves De Koninck[1,2,6]

[1]CERVO Brain Research Centre, Québec Mental Health Institute, Quebec City, QC, Canada, [2]Center for Optics, Photonics and Lasers (COPL), University Laval, Quebec City, QC, Canada, [3]Department of Physics, Physical Engineering, and Optics, University Laval, Quebec City, QC, Canada, [4]Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada, [5]Department of Physiology and the Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [6]Department of Psychiatry and Neuroscience, University Laval, Quebec City, QC, Canada

Primary somatosensory afferent neurons transduce environmental stimuli into neuronal activity that is transmitted into central nervous system to be decoded into corresponding sensations. How different stimuli are encoded by an ensemble of afferents remains unclear. To address this question, we performed in vivo video-rate two-photon functional imaging from thousands of dorsal root ganglion (DRG) neurons in anesthetized mice, and applied natural mechanical and thermal stimuli to the hind paws. We found that approximately half of the DRG neurons were polymodal (including >30% being both mechano- and thermosensitive). Further parametric analysis revealed that thermoceptive neurons used distinct encoding strategies in the heat vs. cold ranges. As temperature increased, more heating-sensitive neurons were activated and most individual neurons responded with stronger activity. It is consistent with graded coding at population and single-cell levels, respectively. In contrast, most cooling-sensitive neurons responded in an ungraded fashion, which is inconsistent with graded coding and instead suggests combinatorial coding based on the co-activation pattern of a population of DRG neurons. Thus, our study found that polymodality is a common phenomenon of primary sensory afferent neurons, and thermoceptive afferents use different strategies to encode heat and cold.

6 - Chemogenetic control of primary afferents

Greg Weir[1,2], Steven Middleton[1], Andrew Todd[2], David Bennett[1]

[1]Nuffield Department of Clinical Neurosciences, University of Oxford, [2]Institute of Neuroscience and Psychology, University of Glasgow

Recent advances have been made in stratifying primary afferent sub-populations by molecular profiling, however our understanding on the functional contributions that these distinct groups make to normal and pathological pain processing is lacking. This information will be critical for the rational design of therapeutics capable of targeting neurons that underlie pathological pain, while sparing other afferents and their contributions to normal somatosensation. We have developed a targeted chemogenetic approach capable of non-invasive, long-term and reversible silencing of primary afferents. A modified form of the glutamate-gated chloride channel (GluCl) is insensitive to glutamate, but highly sensitive to the anti-parasitic drug Ivermectin (IVM). Viral delivery of GluCl selectively to primary afferents allows for afferent silencing and renders animals hyposensitive to sensory stimuli for up to 3 days following a single dose of IVM. The GluCl system therefore represents an ideal approach to study the primary afferent drivers of chronic pain and the consequence of long-term afferent activity to neural plasticity. We have generated a toolkit of cre-dependent GluCl vectors and combined these with transgenic mouse lines in which cre expression is restricted to discreet afferent populations. Using behavioural assays of evoked and spontaneous pain, we have begun to profile the role of molecularly discreet afferent populations in the naïve state and following traumatic nerve injury. These studies will reveal important information on primary afferent circuitry and the neural drivers that underlie the different components of pathological pain.

19 - The role of mTORC2 in the spinal cord in the development of chronic pain

Calvin Wong[1,2], Kevin lister[1,2], Neil Wu[1], Shannon Tansley[1,2,3], Sonali Uttam[1,2], Jieyi Yang[1], Alfredo Ribeiro-da-Silva[2,4,5], Arkady Khoutorsky[1,2,6]

[1]Department of Anesthesia, McGill University, Montreal, Canada, [2]Alan Edwards Centre for Research on Pain, McGill University, Montreal, Canada, [3]Department of Psychology, McGill University, Montreal, Canada, [4]Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada, [5]Department of Anatomy and Cell Biology, McGill University, Montreal, Canada, [6]Faculty of Dentistry, McGill University, Montreal, Canada

The development of pain is associated with the reorganization of spinal nociceptive circuits. mTOR is a highly evolutionarily conserved serine/threonine kinase that regulates cell homeostasis through key cellular processes, including cell growth and proliferation, translation, autophagy, and cytoskeleton organization. mTOR is present in two structurally and functionally distinct multiprotein complexes: mTOR Complex 1 (mTORC1) and mTORC2. mTORC1 regulates the rate of mRNA translation. Much less is known about mTORC2, which has recently emerged as a key signalling molecule in a variety of cellular processes including synaptic plasticity.

Our experiments revealed an increase in p-AKT (S473), which is a proxy for increased mTORC2 activity, in the spinal cord in the model of inflammation-induced pain (Complete Freund's Adjuvant) and neuropathic pain (spared nerve injury). To study the role of mTORC2 in pain, we selectively ablated Rictor, a key protein within the mTORC2, in the dorsal horn of the lumbar spinal cord via intraspinal injection of AAV9-CMV-Cre into rictor$^{fl/fl}$ mice. Our behavioural experiments demonstrate that mice with reduced levels of Rictor in the spinal cord exhibit decreased hypersensitivity in a model of inflammatory pain. Conversely, intrathecal administration of the mTORC2 activator A-443654 induced prolonged mechanical hypersensitivity.

Our study shows for the first time that spinal mTORC2 is activated following peripheral tissue injury and demonstrates the central role of mTORC2 in spinal dorsal horn neurons in the development of pain hypersensitivity in response to inflammation.

42 - A complement-microglia pathway drives spinal inhibitory synapse loss in neuropathic pain

Noosha Yousefpour[1], Shannon Tansley[1], Samantha Locke[1], Chengyang Wang[1], Valerie Cabana[1], Arkady Khoutorsky[1], Yves de Ko[2], Alfredo Ribeiro-da-Silva[1]

[1]McGill University, [2]Université Laval

Aim of Investigation: Inhibitory synapse loss in the dorsal horn of the spinal cord strongly correlates with pain hypersensitivity in animal models of neuropathic pain. This selective synapse loss potentially contributes to spinal cord disinhibition and maintenance of neuropathic pain. The present study aims to find the mechanisms underlying this inhibitory synapse loss. Specifically, we investigate the role of microglia and the complement system, a well characterized synaptic pruning pathway, in selective inhibitory synapse loss in neuropathic pain. Methods: Using super resolution and electron microscopy, in a mouse model of neuropathic pain, we analysed the integrity of inhibitory and excitatory synapses and their colocalization with complement factors using antibodies targeting pre- and post-synaptic elements and complement proteins. We further investigated microglial involvement in phagocytosing inhibitory presynaptic inputs by performing an engulfment assay. Lastly, we depleted spinal microglia and complement factors in neuropathic and control mice and assessed the effect of microglial depletion on dorsal horn synapse loss and pain-related behaviour. Results: In a mouse model of neuropathic pain, there was a reduction in the number of intact inhibitory synaptic structures. A great proportion of the remaining synapses colocalized with initiating protein of complement system C1q and complement factor 3. Microglial engulfment assay studies showed colocalization of inhibitory pre-synaptic markers with a microglia-specific lysosomal protein. Furthermore, microglia and complement depletion prevented inhibitory synapse loss and pain hypersensitivity. Conclusions: Together, these findings suggest that microglia contribute to disinhibition in neuropathic pain through engulfing inhibitory synapses in the spinal dorsal horn. The selectivity of microglia mediated synapse pruning in neuropathic pain is likely dependent on complement factors.