MONTREAL CONFERENCE
ON PAIN CIRCUITS

Thursday, July 20, 2023

18h30 Networking event (Salon Concerto, Delta Hotel)

Friday, July 21, 2023

7h30 Breakfast available (IRCM lobby)
8h15 Welcome remarks
8h25 Remembering Bud Craig

NEUROPATHIC PAIN

Session chair: Bo Duan

8h30 **Cedric Peirs:** *Plasticity of Sensory Processing by Spinal Dorsal Horn Neurons after Neuropathy*
9h00 **Wendy Imlach:** *LanCL1: a Novel Therapeutic Target for the Treatment of Neuropathic Pain*
9h30 **Haoyi Qiu:** *Parvalbumin gates chronic pain through the modulation of tonic firing in inhibitory neurons* (Sharif-Naeini Lab)
9h45 **Nitsan Goldstein:** *A parabrachial hub for the prioritization of survival needs* (Betley Lab)

10h00 Coffee break

10h30 **Greg Corder:** *Mimicking Opioid Analgesia in Cortical Nociceptive Circuits*
11h00 **Mark Gradwell:** *Context is Key: Oxytocin to Treat Pain*
11h30 **Arnab Barik:** *Central Circuits for Pain-induced Coping Behaviors*

12h00 Buffet lunch, networking and poster viewing

CENTRAL PAIN CIRCUITS I

Session chair: Loren Orefice

13h30 **Ulrich Zeilhofer:** *Kcnip2 Neurons in Dorsal Horn Cold Processing Circuits*
14h00 **Bo Duan:** *The Dual Nature of Cold: Unraveling the Neural Circuits for Cool Sensing and Cold Allodynia*
14h30 **Feng Wang:** *Visualizing thermal sensation by in vivo functional imaging*
15h00 **Maham Zain:** *Chronic pain mediated changes in the hedonic value of gentle touch in mice* (Bonin Lab)
15h15 **Heather Allen:** *Parabrachial NPY Y1 receptor neurons as a modulator of neuropathic pain in mice* (Khanna Lab)

15h30 Coffee break

16h00 **Maria Fitzgerald:** *Key Steps in Building Healthy Pain Circuits in the Brain*
16h30 **Steven Prescott:** *The Role of Synchrony in Sensation Revealed by Spinal Cord Stimulation*
17h00 **Martyn Goulding:** *Spinal and Brainstem Circuits for Itch and Pain*
17h30 **Sung Han:** *Peptidergic transmission plays an important role in the parabrachial-to-amygdalar central pain circuit*
18h00 Plenary discussion
Discussion leaders: Mike Salter + Maria Fitzgerald

18h30 Wine and cheese networking and poster viewing (IRCM lobby)

MONTREAL CONFERENCE
ON PAIN CIRCUITS

Saturday, July 22, 2023

7h30 Continental breakfast (IRCM lobby)

ASCENDING AND DESCENDING PATHWAYS

Session chair: Cedric Peirs

8h30 **Sebastian Choi:** *Ascending Somatosensory Pathways that Shape the Sense of Touch and Pain*
9h00 **Mark Hoon:** *A Noradrenergic Descending Pain Circuit*
9h30 **Mike Salter:** *Sex and Pain: It's Not Always About the Differences*
10h00 **Xinying Zhang:** *The transcription factor Phox2a defines a thermosensing subpopulation of anterolateral system neurons in spinal Lamina I* (Kania Lab)
10h15 **Christopher Dedek:** *Reproducible and fully automated testing of nocifensive behavior in mice* (Prescott Lab)

10h30 Coffee break

11h00 **Allan Basbaum:** *Long-term Imaging of Pain Processing in the Brain and Spinal Cord of the Awake, Behaving Mouse*
11h30 **Jordan McCall:** *Endogenous Opioid Regulation of Locus Coeruleus-mediated Analgesia*

12h00 Buffet lunch, discussion and poster viewing

PERIPHERAL PAIN CIRCUITS II

Session chair: Jordan McCall

13h00 **Lauren Orefice:** *The Role of Dorsal Root Ganglia Neurons in Autism-associated Gastrointestinal Dysfunction*
13h30 **Huasheng Yu:** *Single-soma Deep RNAseq of Human DRG Neurons Reveals Novel Molecular and Cellular Mechanisms Underlying Somatosensation*
14h00 **Tomoko Ohyama:** *Comparative Connectomics and Nocifensive Behavior in Larvae of Closely Related Drosophila Species*

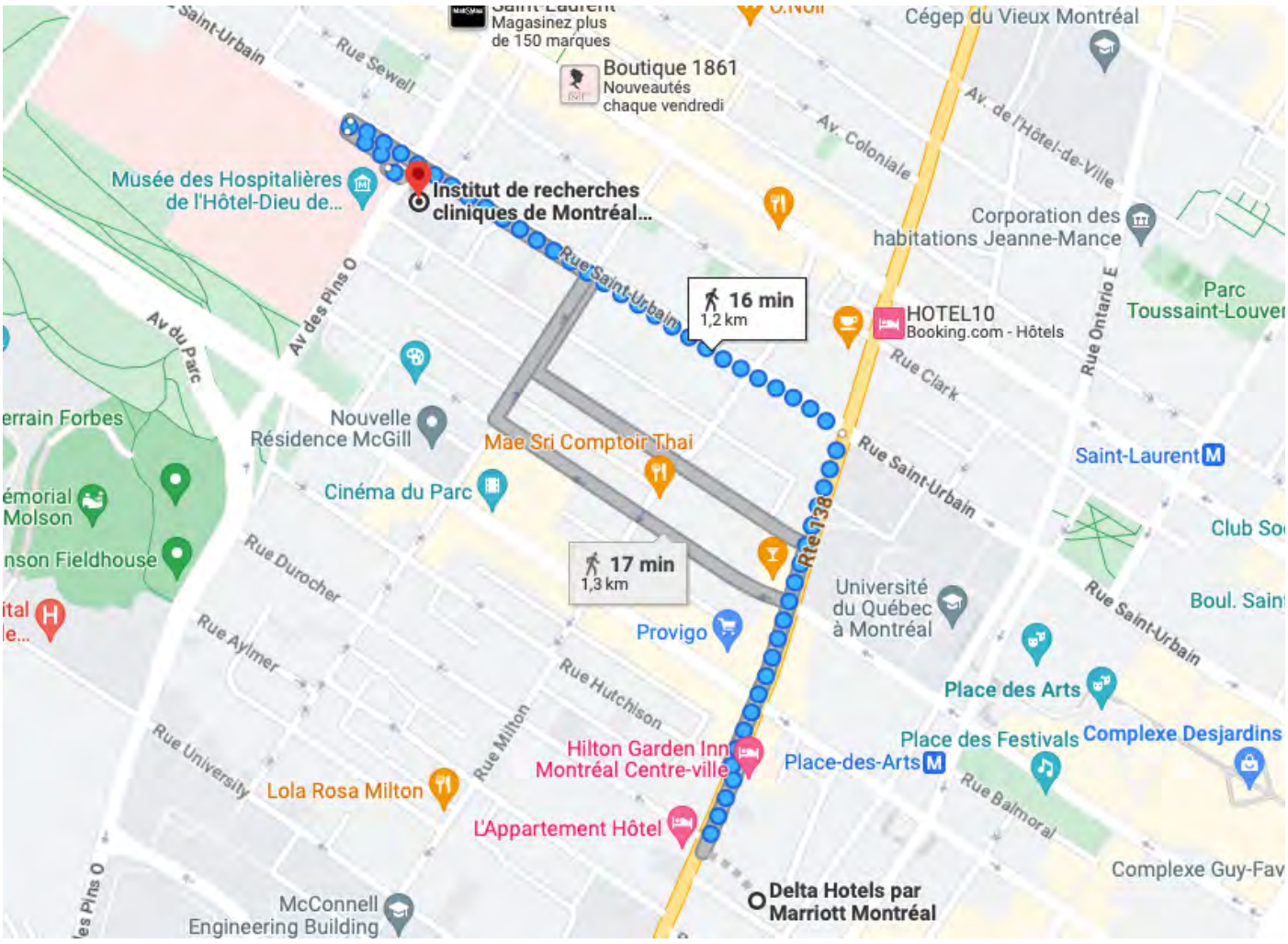
15h00 Coffee break

15h30 **Yves De Koninck:** *How chloride shapes spinal circuit processing*
16h00 **Rebecca Seal:** *Cross species organization of the dorsal horn: in search of non-opioid based therapeutics for neuropathic pain*
16h30 **R. Brian Roome:** *An embryonic atlas of spinal cord neurons post-neurogenesis* (Levine Lab)
16h45 **Robert Ganley:** *Descending Inhibitory Neurons of the RVM that Mediate Widespread Antinociception* (Zeilhofer Lab)
17h00 **Kevin Lancon:** *In Vivo Monitoring of Dopamine Release and Interneuron Activity in the Anterior Cingulate Cortex in Chronic Pain* (Séguéla Lab)
17h15 **Shamsuddin Bhuiyan:** *Harmonized cross-species cell atlases of trigeminal and dorsal root ganglia*
17h30 **Tyler Nelson:** *Uncovering therapeutic spinal cord targets for the treatment of neuropathic pain*
17h45 **Amandine Virlogeux:** *A new role for the Dorsal Column Nuclei in peripheral neuropathic pain* (Goulding Lab)
18h00 Plenary discussion
Discussion leaders: Allan Basbaum + Yves De Koninck

18h30 Conference dinner, IRCM

MONTREAL CONFERENCE
ON PAIN CIRCUITS

DIRECTIONS FROM THE DELTA HOTEL TO THE IRCM



IRCM address:
110, Avenue Des Pins Ouest, Montreal, QC, H2W 1R7

Delta Hotel par Marriott Montréal address:
475 Av. du Président-Kennedy, Montréal, QC H3A 1J7

MONTREAL CONFERENCE
ON PAIN CIRCUITS

LIST OF ABSTRACTS

1 - mTORC2 regulates phenotypic switch in spinal cord interneurons following inflammatory and neuropathic pain

Calvin Wong[1], David Rodriguez-Hernandez[2], Kevin Lister[1], Weihua Cai[1], Alfredo Ribeiro-da-Silva[1], Robert Bonin[2], Arkady Khoutorsky[1]
[1]McGill University, [2]University of Toronto

Structural reorganization of nociceptive circuits is required for the development and maintenance of chronic pain. mTORC2 is a central cellular signaling hub that modulates actin-dependent structural changes. In the central nervous system, mTORC2 has been shown to play central roles in persistent synaptic plasticity and memory formation, however, its role in spinal plasticity and chronic pain is unknown. Here we show that pharmacological activation of spinal cord mTORC2 induces acute hypersensitivity, whereas its inhibition, using downregulation of mTORC2-defining component Rictor with antisense oligonucleotide alleviates both inflammatory and neuropathic pain. We then developed transgenic conditional knockout mice with cell type-specific ablation of Rictor in excitatory and inhibitory neurons using Tac1 Cre and PV Cre driver mice. We show using this approach that selective inhibition of mTORC2 in excitatory interneurons alleviates inflammation-induced mechanical and thermal hypersensitivity, whereas inhibition of mTORC2 in inhibitory interneurons strongly alleviates nerve injury-induced mechanical hypersensitivity. Impaired spinal-LTP was observed in animals with Rictor conditionally deleted in excitatory interneurons. Our results suggest that mTORC2 plays contrasting roles in promoting pain hypersensitivity in inflammatory and neuropathic pain models. Future directions include investigation of how mTORC2 in inhibitory neurons promotes the development of neuropathic pain.

2 - Parvalbumin gates chronic pain through the modulation of tonic firing in inhibitory neurons

Haoyi Qiu[1, 2], Lois Miraucourt[1, 2], Hugues Petitjean[1, 2], Albena Davidova[1, 2], Catherine Theriault[1, 2], Reza Sharif-Naeini[1, 2]
[1]Department of Physiology, McGill University, Montreal, QC, [2]Alan Edwards Centre for Research on Pain, Montreal, QC

Spinal cord dorsal horn inhibition is critical to the processing of sensory inputs, and its impairment leads to mechanical allodynia. How this decreased inhibition occurs and whether its restoration alleviates allodynic pain is poorly understood. Here, we show that the calcium (Ca^{2+})-binding protein, parvalbumin (PV), controls the activity of inhibitory PV-expressing neurons (PVNs) by enabling them to sustain high-frequency tonic firing patterns. Upon nerve injury, PVNs transition to adaptive firing and decrease their PV expression. Interestingly, decreased PV is necessary and sufficient to the development of mechanical allodynia and the transition of PVNs to adaptive firing. This transition of firing pattern is due to the recruitment of calcium-activated potassium (SK) channels and blocking them during chronic pain restores normal tonic firing. Our findings indicate that PV is essential to the firing activity of PVNs and in preventing allodynia, these observations may lead to novel strategies for chronic pain relief.

3 - Reproducible and fully automated testing of nocifensive behavior in mice

Christopher Dedek[1, 2], Mehdi Azadgoleh[2], Steve Prescott[1, 2]
[1]University of Toronto, [2]The Hospital for Sick Children

Pain in rodents is often inferred from their withdrawal to noxious stimulation, using the threshold stimulus intensity or response latency to quantify pain sensitivity. This usually involves applying stimuli by hand and measuring responses by eye, which limits reproducibility and throughput to the detriment of preclinical pain research. Here, we describe a device that standardizes and automates pain testing by providing computer-controlled aiming, stimulation, and response measurement. Optogenetic and thermal stimuli are applied to the hind paw using blue and infrared light, respectively. Red light delivered through the same light path assists with aiming, and changes in its reflectance off the paw are used to measure paw withdrawal latency with millisecond precision at a fraction of the cost and data processing associated with high-speed video. Using standard video, aiming was automated by training a neural network to recognize the paws and move the stimulator using motorized linear actuators. Real-time data processing allows for closed-loop control of stimulus initiation and termination. We show that stimuli delivered with this device are significantly less variable than hand-delivered stimuli, and that reducing stimulus variability is crucial for resolving stimulus-dependent variations in withdrawal. Slower stimulus waveforms whose stable delivery is made possible with this device reveal details not evident with typical photostimulus pulses. Moreover, the substage video reveals a wealth of “spontaneous” behaviors occurring before and after stimulation that can considered alongside withdrawal metrics to better assess the pain experience. Automation allows comprehensive testing to be standardized and carried out efficiently.

4 - Heightened response to noxious cues following development in a noxious environment

Jean-Christophe Boivin[1, 2], Sophia Zhao[1], Jiayi Zhu[1, 2], Tomoko Ohyama[1, 3]
[1]Department of Biology, McGill University, Montreal, QC, Canada, [2]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada, [3]Alan Edwards Center for Research on Pain, McGill University, Montreal, QC, Canada

All nervous systems need to reliably transform sensory information into appropriate motor outputs that generate coordinated actions. Such actions do not arise de novo, but change over the course of development. The neural mechanisms underlying this adaptation, however, remain unclear. Here, we use nocifensive behaviour in Drosophila larvae as a model to study the neural mechanisms underlying developmental adaptation to a noxious environment. We see that chronic exposure to noxious cues leads to a hypersensitivity to noxious input, behavioural changes comprising a lower nociceptive threshold, an early detection of noxious cues and a delayed termination of nocifensive behaviour. This behavioural phenotype is affected by factors such as the frequency and timing of the stimulations and may be observed across all nociceptive modalities. This change in behaviour is sustained by octopamine signalling at the sensory level, with knock-down of octopamine receptors preventing sensitization but sparing optogenetically-induced nocifensive behaviour. Briefly, this research provides a better understanding of the mechanisms underlying experience-dependent changes in the nociceptive system of one of the most powerful animal models for studying genetics. This, in turn, may open new avenues of research on adaptive and maladaptive changes in nociceptive systems in general, shedding new light into the mechanisms underlying acute and chronic pain.

5 - Harmonized cross-species cell atlases of trigeminal and dorsal root ganglia

Shamsuddin Bhuiyan[1], Mengyi Xu[1, 2], Lite Yang[1, 3], Evangelia Semizoglou[1], Parth Bhatia[1], Katerina Pantaleo[1], Ivan Tochitsky[4], Aakanksha Jain[4], Burcu Erdogan[5], Steven Blair[5], Victor Cat[5], Juliet Mwirigi[6], Ishwarya Sankaranarayan[6], Diana Tavares-Ferreira[6], Ursula Green[7], Lisa McIlvried[3], Bryan Copits[3], Zachariah Bertels[3], Jon Del Rosario[3], Allie Widman[3], Richard Slivicki[3], Jiwon Yi[3], Clifford Woolf[4], Jochen Lennerz[7], Jessica Whited[5], Theodore Price[6], Robert Gereau IV[3], William Renthal[1]
[1]Department of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA, [2]Alan Edwards Center for Research on Pain and Department of Physiology, McGill University, Montreal, QC, H3G 1Y6, Canada, [3]Program in Neurosciences, Division of Biology and Biomedical Sciences, Washington University Pain Center and Department of Anesthesiology, Washington University School of Medicine, St Louis, Missouri 63110, USA, [4]F.M. Kirby Neurobiology Center and Department of Neurobiology, Boston Children's Hospital and Harvard Medical School, 3 Blackfan Cir. Boston, MA 02115, [5]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts, 02138, [6]Department of Neuroscience and Center for Advanced Pain Studies, University of Texas at Dallas, 800 W Campbell Rd, Richardson, TX, 75080, [7]Department of Pathology, Center for Integrated Diagnostics, Massachussetts General Hospital and Havard Medical School, Boston, MA 02114

Peripheral sensory neurons in the dorsal root ganglion (DRG) and trigeminal ganglion (TG) are specialized to detect and transduce diverse environmental stimuli including touch, temperature, and pain to the central nervous system. Recent advances in single-cell RNA-sequencing (scRNA-seq) have provided new insights into the diversity of sensory ganglia cell types in rodents, non-human primates, and humans, but it remains difficult to compare transcriptionally defined cell types across studies and species. Here, we built cross-species harmonized atlases of DRG and TG cell types that describe 18 neuronal and 11 non-neuronal cell types across 6 species and 19 studies. We then demonstrate the utility of this harmonized reference atlas by using it to annotate newly profiled DRG nuclei/cells from both human and the highly regenerative axolotl. We observe that the transcriptomic profiles of sensory neuron subtypes are broadly similar across vertebrates, but the expression of functionally important neuropeptides and channels can vary notably. The new resources and data presented here can guide future studies in comparative transcriptomics, simplify cell type nomenclature differences across studies, and help prioritize targets for future pain therapy development.

6 - Examining the function of Teneurin-3 and Latrophilin-2 in the development of spinal sensory afferent somatotopy

Kevin Sangster1, 2, Daniel del Toro3, Ashley Moses4, Shreya Mahasenan1, 2, Daniel T Pederick4, R. Brian Roome5, Elena Seiradake6, Liqun Luo4, Artur Kania1, 2
1IRCM, 2McGill, 3University of Barcelona, 4Stanford University, 5National Institute of Neurological Disorders and Stroke, 6Oxford University

Somatotopy is a recurring organisational principle within the central nervous system where neurons and their connections are arranged according to the layout of the body. Somatotopic maps have been most famously shown to exist within the primary somatosensory cortex but are also present throughout all levels of the somatosensory system — including the dorsal horn of the spinal cord. Primary sensory afferents that correspond to the distal limb project medially within the dorsal horn while those that correspond to proximal regions of the body project more laterally. This organisation is also reflected in the response properties of many dorsal horn neurons. How this somatotopic organisation develops remains poorly understood. Our lab has recently identified complementary mediolateral gradients of Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2) within the dorsal horn that correspond to its somatotopic layout. We also observe corresponding expression patterns in the primary sensory neurons of the dorsal root ganglia. Heterophilic repulsion mediated by these proteins has recently been shown to guide the wiring of hippocampal circuits during development. Our current work examines the requirement of these two proteins for the organisation of primary sensory afferents in the dorsal horn. Our preliminary results demonstrate that Ten3 is required for the ability to localise a nociceptive stimulus along the proximodistal axis. Preliminary data also suggests this mechanism might be conserved at higher level targets within ascending somatosensory pathways.

7- Genetic restriction of mGluR5 to intracellular membranes delays recovery from persistent inflammatory pain in mice

Roseanna Rought[1], Heidi Erb[1], Karen O'Malley[2], Terence Coderre[1]
[1]McGill University, [2]Washington University School of Medicine

Background: Previous pharmacological studies have implicated intracellular metabotropic glutamate receptor 5 (mGluR5) in persistent pain. Cell permeable but not impermeable antagonists effectively reduce neuropathic and inflammatory injury-induced pain hypersensitivity, as well as pain-induced enhancements of spinal cord dorsal horn signalling. To overcome limitations of pharmacological manipulation, we used mice in which mGluR5 was genetically targeted to intracellular membranes (mGluR5^{IM} mice) to determine changes in several measures of pain behaviour and downstream nociceptive signalling.

Methods: mGluR5^{IM} mice were generated by inserting an endoplasmic reticulum/ nuclear membrane targeting motif from the Lamin-B receptor immediately upstream of the mGluR5 C-terminal stop codon using CRISPR. Loss of cell surface mGluR5 was validated anatomically and functionally. Here, mGluR5^{IM} and mGluR5$^{wild\text{-}type\ (WT)}$ mice of both sexes were injected with 20 µl of 5% formalin, intraplantar to the left hind paw and the time spent licking, biting or flinching the injected hind paw was recorded for 60 minutes in 5-minute intervals. Separate groups of mice of both genotypes and sexes were injected with 20 µl of Complete Freund's Adjuvant (CFA), intraplantar to the left hind paw and were then measured for mechanical allodynia (von Frey), thermal hyperalgesia (Hargreaves) and motor performance (rotarod). Behavioural tests were conducted at several timepoints for 3-months post-injection.

Results: mGluR5^{IM} mice spent significantly longer exhibiting pain behaviour during the late phase of the formalin response compared to mGluR5^{WT} mice. Additionally, in the mGluR5^{IM} group only, females demonstrated signi-

ficantly more late phase pain behaviour than males. Following CFA injections, mGluR5^{IM} mice had significantly lower paw withdrawal thresholds (PWTs) in the injected paw at 2 weeks (males only) and 1 - 3 months (males and females) post-injection compared to mGluR5WT mice. Additionally, mGluR5^{IM} mice had significantly lower PWTs in the non-injured paw from 24 hours to 2-months post-injection, compared to baseline values. Whereas no significant differences were found in the non-injured paw of mGluR5^{WT} mice when compared to baseline values. Conversely, there were no significant differences in thermal hypersensitivity between genotypes. No signs of motor deficits were found in any group following CFA injection using the rotarod test.

Conclusions: These findings support the importance of the intracellular localisation of mGluR5 in persistent pain modulation. This work also highlights a yet unreported sex difference in mGluR5 nociceptive signalling and implicates intracellular mGluR5 as a potential contributor to mirror image pain, both of which will be further investigated. Future work will assess mGluR5^{IM} mice with a battery of behavioural tests and molecular techniques to establish differences in baseline and post neuropathic/ inflammatory injury behavioural responses and neuronal nociceptive signalling profiles.

8 - An embryonic atlas of spinal cord neurons post-neurogenesis

R. Brian Roome[1], Kaya Matson[1], Ariel Levine[1]
[1]National Institute of Neurological Disorders and Stroke

Recent single-cell transcriptomic atlases of the spinal cord integrated together have provided a broad perspective on the functional components of the spinal cord and their relationship to one another. However, the relationships between adult spinal cord neuron types and the cardinal neuron types of the embryonic spinal cord are not clear; particularly as embryonic cardinal neuron types such as v1 have been shown to show substantial diversity not verified postnatally. To resolve this disparity, we have produced an atlas of the embryonic spinal cord after neurogenesis has completed (to capture the entire set of spinal neurons), between embryonic days 14.5 and 16.5. In contrast to earlier embryonic data, a more prolonged series of temporally specified cardinal neuron subtypes is evident here. Those neurons which are born earlier generally do not have cognate neuron types in postnatal atlases; rather, their developmental gene signatures are lost, and their transcriptomes converge over time. Those neurons which are born later have cognate neuron types in postnatal atlases, but their embryonic transcriptome is less distinct from neighboring neuron types and becomes distinct over time. Using these insights, we are able to precisely correlate embryonic spinal neuron types with adult spinal neuron types. We have also identified a wealth of newly described neuron types with highly distinct marker genes, providing many new substrates for research into spinal pain mechanisms.

9 - In Vivo Monitoring of Dopamine Release and Interneuron Activity in the Anterior Cingulate Cortex in Chronic Pain

Kevin Lancon[1], Philippe Seguela[1]
[1]McGill University, Montreal Neurological Institute

Background and Aims: Dopamine (DA) is a robust neuromodulator of the anterior cingulate cortex (ACC), a prefrontal area that has been identified as a key pain structure due to its role in top-down somatosensory modulation and its dysfunction in chronic pain states. However, the impact of neuropathic pain on DA release and DA-sensitive circuits in the ACC has not been investigated.

Methods: To test whether the onset of neuropathic pain significantly affects the release of DA and DA-sensitive circuits in the ACC, we used in vivo dual-wavelength single fiber photometry in freely-moving mice expressing the DA biosensor dLight1.1 and the red-shifted calcium indicator jRCaMP1b, selectively expressed in PV+, SOM+ interneurons or pyramidal cells. DA-mediated inhibitory postsynaptic currents were measured using patch clamp electrophysiology in acute brain slices.

Results: We demonstrate that pain and reward bidirectionally control the release of DA in the ACC. Rewarding stimuli, such as sucrose, increases cortical DA release while noxious stimulation decreases it. Furthermore, we show that the onset of neuropathic pain significantly alters pain-induced inhibition of DA release in the ACC. Additionally, we observed that GABA-mediated inhibition of ACC pyramidal neurons is decreased following D1 receptor agonist application in neuropathic conditions. We will present ex vivo electrophysiological and in vivo calcium recordings of SOM+ and PV+ interneuron activity in sham and neuropathic ACC.

Conclusions: We conclude that neuropathic pain conditions promote a dysregulation of mesocortical projections and this hypodopaminergic state mediates both direct and indirect mechanisms of potentiation of pyramidal excitability in the ACC, driving typical sensory, cognitive and affective symptoms of chronic pain.

10 - Chronic pain mediated changes in the hedonic value of gentle touch in mice

Maham Zain[1], Laura Bennett[1], Hantao Zhang[1], Quinn Pauli[1], Jenny Cheung[1], Robert Bonin[1]
[1]University of Toronto

Sensory neurons expressing MrgprB4 detect gentle stroking in mice and their activation is known to be positively reinforcing. This project uses optogenetics and behavioral techniques to assess whether activation of channelrhodopsin (ChR2) expressing MrgprB4 afferents signal positively valenced tactile information, whether this is altered in chronic pain and whether this is reflected in the downstream circuits recruited.

A ceramic ferrule was surgically implanted in the lumbar vertebrae of mice expressing ChR2 in MrgprB4 lineage afferents (MrgprB4-ChR2) to deliver blue light to the central projections of the primary afferents. We used a real-time place preference (RTPP) paradigm with optogenetics to assess the motivational properties of blue light stimulation in implanted male and female MrgprB4-ChR2 mice that had either undergone a spared nerve injury (SNI) or a sham surgery and assessed whether gabapentin administration affected the response. All mice underwent a final stimulation protocol after which the brains, spinal cords and dorsal root ganglions of the mice were dissected out for immunohistochemical analysis. Light stimulation in one arm of the assay increased preference for that arm in the male and female sham surgery mice but not the SNI animals. Preference for blue light could be partially restored in the male SNI mice through treatment with gabapentin. Preliminary results from the immunohistochemistry experiments also show that stimulation successfully induced c-fos expression in the spinal dorsal horn of the stimulated animals and this expression was predominantly found in the superficial dorsal horn of the spinal cord, consistent with the innervation pattern of the MrgprB4+ afferents. Whole brain c-fos results also showed differential recruitment of brain regions following optogenetic activation of MrgprB4 lineage afferents in sham and nerve injured mice.

In conclusion, the motivational value associated with gentle touch is plastic and can be abated in models of chronic pain. Future work will continue to elucidate the subtypes of spinal dorsal horn neurons recruited and the specific brain regions activated in the processing and perception of gentle touch both in chronic pain and in control conditions.

11 - An improved conflict avoidance assay reveals modality-specific differences in pain hypersensitivity across sexes.

Samuel Ferland[1], Feng Wang[1], Yves De Koninck[1, 2], Francesco Ferrini[1, 2, 3]
[1]CERVO Brain Research Centre, [2]Université Laval, [3]University of Turin

Introduction: Reflex-based approaches in animal pain models have been largely used to study the contribution of modality-specific changes to pain pathology. However, it remains unclear whether differences in reflexes lead to significant changes in the decision-making of animals. This has sparked efforts to develop operant assays to study evoked pain in animals. To date, few of these methods can evaluate and compare sensitivity across nociceptive modalities. Understanding mechanisms underlying modality-specific hypersensitivity would lead to a better understanding of diverse pain pathologies and their treatment. To achieve this, we aim to improve an existing operant assay (conflict avoidance) to evaluate modality-specific sensitivity in mice of both sexes and to test it in two nerve-injury models.

Method: The apparatus is composed of a lit and a dark chamber linked by a corridor where thermal (15°C to 50°C) and mechanical stimuli (height-adjustable pins,0 to 4 mm) are presented. Mice naturally avoid light, but they must cross the nociceptive stimulus to escape it and move to the dark chamber. The latency to escape from the lit chamber and return from the dark chamber in the presence of various stimuli were measured from videos. A polyethylene cuff (PE-20) was inserted around the main branch of the sciatic nerve to induce neuropathic pain in a subset of mice. In another, the tibial and common peroneal branches of the sciatic nerve were cut (spared nerve injury model).

Results: We tested the impact of the lit chamber on training performance and found that mice spent less time in it with a white backdrop instead of a neutral one. The time it took for them to escape or return increased with the intensity of the stimulus for all modalities, and there were significant differences in sensitivity between male and female mice for mechanical and cold stimuli. Following cuff implantation, both sexes developed mechanical hypersensitivity, but only females developed cold hypersensitivity. SNI mice developed intense mechanical hypersensitivity in preliminary results.

Conclusion: Our research has revealed that the modified assay presents a promising substitute for evaluating modality-specific sensitivity through the utilization of voluntary behaviour instead of reflexes. This method is solid, objective, and highly reproducible, making it ideal for dissecting the contribution of specific sensory modalities in the onset of neuropathic pain symptoms.

12 - GABA B receptor dependent inhibitory synaptic pruning as a substrate of neuropathic pain

Alfredo Ribeiro-da-Silva[1, 2, 3], Simran Dhir[1], Rose Rodrigues[1]
[1]McGill University, [2]Alan Edwards Centre for Research on Pain, [3]Quebec Pain Research Network (QPRN).

Background and rationale: Neuropathic pain (NP) is a debilitating, inefficiently treated, chronic condition resulting from damage to the somatosensory nervous system. Our research focuses on investigating how spinal dorsal horn (DH) neuronal pathways involved in the coding of sensory information, in normal and chronic pain states, can be targeted to induce pain relief. While evidence suggests that deficits in DH inhibitory transmission (disinhibition) result in symptoms of NP, directly targeting this disinhibition remains challenging. In our recent studies, we discovered that following nerve injury, microglia mediate the elimination (pruning) of DH synapses resulting in a net loss of inhibitory synapses. While the mechanism that dictates the selectivity of inhibitory synapse removal in NP remains unknown, a recent study by Favuzzi et al. showed that in the cerebral cortex, during postnatal development, microglia expressing GABA B receptors ($GABA_BRs$) are responsible for sculpting inhibitory synapses. In this project, we propose to investigate the role of microglia, expressing $GABA_BRs$, in inhibitory synaptic pruning in the spinal dorsal horn.

Methods: TMEM119-tdTomato mice having either spared nerve injury, modelling peripheral neuropathy, or sham surgery are used to facilitate the visualization of microglia in DH laminae I and II, at several time points. Engulfment of excitatory (as defined by presence of VGLUT2) and inhibitory (VGAT+) synapses by microglia expressing $GABA_BRs$ are assessed using high resolution confocal microscopy. The volume of synaptic markers within the microglial lysosomes (labelled with CD68) are quantified using the IMARISs software. Antibodies against $GABA_BR1$and $GABA_BR2$ are used to identify microglia colocalizing these receptors.

Results and Discussion: Our initial data shows that $GABA_BR1$ is upregulated on microglial membranes in Lamina II a week post-SNI, suggesting that $GABA_BRs$-dependent microglial pruning of inhibitory synapses occurs in adult NP animals. Future steps include showing that both $GABA_BRs$ are co-localized in microglia selectively pruning inhibitory synapses.

13 - Superficial Tac1-lineage spinal cord neurons are heat nociceptors with low excitability

Louison Brochoire[1, 2], Pauline Larqué[1], Pascal Fossat[2], Yves De Koninck[1, 3], Feng Wang[1]
[1]CERVO Brain Research Centre, Université Laval, Québec, Canada, [2]Institute of Neurodegenerative Diseases, Université de Bordeaux, Bordeaux, France, [3]Faculty of Medicine, Université Laval, Québec, Canada

The spinal cord dorsal horn is one of the first structures relaying somatosensory information from the primary afferent axons to the brain to form various sensations. Despite its importance, the neuronal circuits and mechanisms involved in this process remain to be fully investigated. Recent studies identified a population of neurons expressing Tachykinin 1 gene (Tac1) which is essential for coping behavior induced by intense noxious stimuli. However, the nature and functional properties of these neurons are still unclear. Our study aimed to thoroughly characterize Tac1 neurons using slice electrophysiology and in vivo imaging techniques.

First, using Tac1-tdTomato mice, our immunohistochemical results show that Tac1-lineage neurons are scattered and distributed across multiple laminae in the dorsal horn.
Whole-cell patch recordings from parasagittal spinal cord slices demonstrated that superficial Tac1-lineage neurons have heterogeneous properties. Indeed, half of them are phasic neurons, while the other are single-spike, reluctant, or tonic neurons. The high rheobase and activation threshold of action potential indicated that Tac1-lineage neurons have low excitability.
In vivo calcium imaging of superficial Tac1-lineage neurons with two-photon microscopy showed that only a small subset are mechanosensitive, but most of them are thermosensitive. They respond to both heat and cold stimuli applied to the hind paw and they are activated preferably by noxious heat stimulation. Interestingly, the thermal sensory profile of these Tac1-lineage neurons is very similar to the global population of thermosensitive primary afferents, suggesting a direct connection between thermoreceptors and Tac1-lineage neurons.

Collectively, our data demonstrated that superficial Tac1-lineage spinal cord neurons are predominantly heat-nociceptors, which is consistent with their role in the nocifensive behavior

14 - The transcription factor Phox2a defines a thermosensing subpopulation of anterolateral system neurons in spinal Lamina I

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The spinal projection neurons of the anterolateral system (AS) transmit somatosensory information from the spinal cord to the brain. Previous studies in rodents, cats, and monkeys defined three major classes of AS neurons: nociceptive-specific cells, cold cells, and polymodal nociceptive cells. However, the molecular logic underlying this functional diversification remains unclear. We recently identified a population of lamina I and lamina IV/LSN AS neurons that express the developmental transcription factor Phox2a and innervate the parabrachial nucleus and other pain related brain areas. Previously proposed AS neuron markers are expressed in both interneurons and AS neurons; in contrast, Phox2a expression is exclusively confined to AS neurons encompassing over half of them in lamina I in the lumbar spinal cord. Previous work suggests their role in thermosensation; to address this idea anatomically, we used rabies tracing to identify their presynaptic inputs among dorsal root ganglia sensory neurons. To examine their physiological responses to various sensory stimuli, we used patch-clamp recordings in the semi-intact somatosensory preparation, as well as in vivio calcium imaging. Furthermore, to assess their function, we used genetic intersectional tools to ablate or chemogenetically activate them. Together, our results suggest that Phox2a AS neurons are principally involved in thermosensation.

15 - Descending Inhibitory Neurons of the RVM that Mediate Widespread Antinociception

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The rostral ventromedial medulla (RVM) is responsible for descending pain modulation, and can both facilitate and inhibit nociception via descending projections to the spinal cord. Given the importance of these descending projections in pain modulation, it is important to identify the different groups that can influence the sensory system. Here we focused on the inhibitory subset of the RVM descending neurons.

Using vGAT^{cre} transgenic mice and AAV vectors to selectively target these neurons, we show that inhibitory descending neurons can reduce sensitivity to several types of sensory stimulus and are required for normal mechanical sensitivity. Although retrogradely labelled from a single spinal cord segment, viral tracing indicates that these neurons widely innervate the spinal cord at all levels and also have axon collaterals that extend rostrally to higher brain areas. Consequently, these neurons form functional synapses in distinct spinal cord segments and can reduce cutaneous thermal sensitivity in multiple regions of the body.

Together this indicates that the descending inhibitory neurons can produce widespread inhibition of nociception, and are also required for the physiological control of mechanical sensitivity.

16 - Losing control: are Tac1 radial cells the disinhibited neurons in neuropathic pain?

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A decrease of the K^+-Cl co-transporter KCC2 in the spinal dorsal horn (DH) is one of the main mechanisms causing disinhibition and underlying hypersensitivity in neuropathic pain.
Recently, we found that KCC2 is heterogenous across DH neurons. Thus, we asked whether specific populations may constitutively express higher KCC2 and/or undergo to more dramatic changes in KCC2 in pathological conditions.

Hence, we measured EGABA from lamina II neurons of the rat DH under Cl- load. Neuronal types were characterized based on morphology and firing pattern. Interestingly, islet cells (typically, inhibitory neurons) exhibited a more depolarized EGABA (-43±2 mV, n=16) than radial, central or vertical cells (-58±2 mV, n=33; -58±2 mV, n=18; -59±2 mV, n=21, respectively; $P<0.001$). Of note, radial cells can be further split into branchy (> 3 dendrites) or simple (≤3 dendrites). Branchy radial cells (likely excitatory interneurons) exhibited an even more hyperpolarized EGABA (-71±2 mV, n=8). A similar scenario was also observed in macaque, suggesting that these features are conserved in species phylogenetically close to humans.

We replicated the EGABA measurements following nerve injury and we found that branchy radial cells undergo to the largest depolarizing shift in EGABA (about 24 mV).
Functional data were confirmed by quantitation of KCC2 expression at putative inhibitory and excitatory neurons in mice. Excitatory cells displayed the higher level of KCC2, but also the larger drop following nerve injury.
A common signature of radial cells is substance P (SP). Therefore, we targeted Tac1 (SP+) neurons and found that they correspond to branchy radial cells and their EGABA undergoes to a large depolarizing shift after nerve injury (about 20 mV).

Altogether, our data indicate that disinhibition at Tac1+ radial neurons plays a central role in the etiology of neuropathic pain. Targeting KCC2 in these cells may represent a viable strategy to treat pathological pain.

17 - A new role for the Dorsal Column Nuclei in peripheral neuropathic pain

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Chronic pain is a condition characterized by persistent and long-lasting pain that can endure for months or even years. While much of the research in this field has focused on brain regions involved in pain processing, a notable characteristic of chronic pain is the development of allodynia. To gain insight into the transformation of typically innocuous sensory stimuli into painful experiences, our study aims to investigate the gracile nucleus, the first key brain area responsible for processing touch-related sensory information.

Initially, we conducted experiments demonstrating that the inhibition of gracile neurons at a systemic level resulted in the alleviation of allodynia in mouse models of CFA (Complete Freund's Adjuvant) and SNI (Spared Nerve Injury). Building upon this finding, our subsequent objective was to unravel the neural mechanisms underlying the encoding of mechanical allodynia, which arises from nerve injury and inflammation. To achieve this, we conducted multi-unit in vivo recordings to investigate the patterns of neural activity within the gracile nucleus while applying brush or Von Frey stimuli.

Subsequently, we investigate the role in mechanical allodynia of a substantial excitatory population within the gracile nucleus projecting to the ventral posterolateral nucleus (VPL) and two inhibitory population. The results provide compelling evidence for the pivotal role played by these neuronal populations in modulating allodynia.

18 - Genetic Identification of a Spinothalamic Pathway for Somatosensory Integration During Locomotion

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Somatosensory inputs are relayed from the spinal cord to various supraspinal targets, including the thalamus, but the functional organization of such pathways remains obscure. While the lateral spinothalamic tract (STT) has been primarily associated with the relay of noxious signals to the thalamus, the ventral STT is proposed to relay crude touch and joint movements during locomotion in mammals. The cardinal transcriptional programs in the embryonic spinal cord provide insights into the molecular identity of these two STT components. Our previous work shows that lateral STT neurons derived from the lineage expressing Phox2a and Lmx1b transcription factors, contribute to the anterolateral tract involved in the relay of noxious and thermal inputs. Here, we present evidence that the neurons of the lumbar ventral STT arise from Sim1-expressing V3 neurons. This developmental divergence is mirrored by differential thalamic innervation: dI5-STT neurons primarily innervate the medial and the posterior limiting nuclei, while the V3-STT neurons predominantly innervate the ventral posterolateral (VPL) nucleus and the posterior complex. To study the role of V3-STT neurons, we used mouse intersectional genetics to ablate them and their post-synaptic VPL thalamic neurons. V3-STT neuron or VPL ablation did not alter reflexive or conscious responses to noxious stimuli. In contrast, such manipulations lead to deficient behaviors associated with the detection of innocuous touch and coordinated locomotion. Recent functional manipulation of dorsal column nuclei inputs to the VPL implicate it as a somatosensory relay site critical for forelimb movement. As past studies suggest that V3-STT neurons are activated by joint movement and muscle stretch, they may comprise a parallel ascending pathway for hindlimb kinesthesia. Thus, the somatotopically organized VPL is emerging as a major hub for the relay of non-noxious somatosensory inputs to the primary somatosensory cortex during locomotion.

19 - Uncovering therapeutic spinal cord targets for the treatment of neuropathic pain

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Objective: The development of alternative, safe, and efficacious non-opioid therapeutics for the treatment of neuropathic pain.

Background: Nociceptor activation normally provides a protective function that can reduce tissue damage in the face of potentially hazardous stimuli. However, after tissue damage occurs, pathological changes within peripheral or central neurons can lead to chronic neuropathic pain, which persists after the injury has healed and serves no protective function. Neuropathic pain affects 7-8% of the general population yet is poorly responsive to analgesic drugs, including opioids. Therefore, the need for the development of novel therapeutics for the treatment of neuropathic pain is significant. Neuropeptide Y (NPY) in the spinal cord dorsal horn exhibits long-lasting inhibitory control of nociceptive transmission after peripheral nerve injury. Dorsal horn neurons that express the neuropeptide Y1 receptor (Y1-INs), an inhibitory G protein-coupled receptor, are well positioned to mediate the anti-nociceptive actions of NPY in the setting of neuropathic pain. We hypothesize that spinal Y1-INs represent a promising pharmacotherapeutic target for the treatment of high-impact chronic neuropathic pain.

Specific Aim(s): The aims of this study were to: (i) determine if Y1-INs are necessary and sufficient for the manifestation of peripheral nerve injury-induced mechanical and cold hypersensitivities, (ii) test for conservation of Npy1r expression across the dorsal horn of mammalian species, (iii) trial intranasal administration of an NPY Y1 agonist as a non-invasive approach to treat chronic pain, and (iv) perform an in silico screen for potential novel small molecule NPY Y1 agonists for the future treatment of pain.

Results and Significance: We pharmacologically targeted Y1-INs with intrathecal administration (i.t) of the Y1 receptor selective agonist [Leu^{31}, Pro^{34}]-NPY. We found that [Leu^{31}, Pro^{34}]-NPY (0.1-10 µg / 5 µL, i.t.) dose-dependently reduced spared nerve injury (SNI)-induced mechanical and cold hypersensitivity ($p<0.05$, n=8-11 mice/group). Similarly, chemogenetic inhibition of Y1-INs with clozapine N' oxide (3.0 mg/kg, i.p.) completely abolished nerve injury-induced mechanical and cold hypersensitivity ($p<0.05$, n=7 mice/group). Conversely, chemogenetic activation of Y1-INs in un-injured mice induced multiple signs of pain including spontaneous nocifensive behaviors (lifting of hindpaw, licking, guarding, biting) ($p<0.05$, n=3-5 mice/group) and heat, cold, and mechanical hypersensitivities ($p<0.05$, n=7-8 mice/group). Chemogenetic activation of Y1-INs also induced robust conditioned place aversion ($p<0.05$, n=7-8 mice/group). Furthermore, activation of Y1-INs with wireless spinal optogenetics produced a frequency-dependent decrease in mechanical and cold withdrawal thresholds ($p<0.05$, n=8 mice/group). Next, using fluorescence in situ hybridization, we determined that spinal Npy1r expression is conserved across the dorsal horn of mouse, porcine, macaque, and human spinal cords; promising results for future pre-clinical to clinical translation. Lastly, we tested intranasal administration of [Leu^{31}, Pro^{34}]-NPY for the non-invasive treatment of chronic pain and discovered a robust inhibition of nerve injury-induced mechanical hypersensitivity in both mice and pigs. Together, these results demonstrate Y1-INs are a pharmacotherapeutic target for the treatment of neuropathic pain. Capitalizing on the enormous potential of inhibition of Y1-INs for the treatment of pain, we are currently testing novel NPY Y1 small molecule agonists from an in-silico screen for efficacy as future therapeutics for chronic pain management.

20 - Parabrachial NPY Y1 receptor neurons as a modulator of neuropathic pain in mice

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Chronic pain is associated with psychiatric co-morbidities that suggest pathology in thebrain. The parabrachial nucleus (PBN) is a hindbrain hub that relays ascending nociceptiveinformation from the spinal cord to higher brain regions. Activation of glutamatergic PBN neuronsproduces pain-like behaviors in un-injured mice, and inhibition of glutamatergic PBN neuronsreverses neuropathic pain-like behaviors. However, recent transcriptomic studies indicate thatglutamatergic PBN neurons are highly heterogenous. Pharmacological activation of glutamatergic PBN neurons expressing the neuropeptide Y Y1 receptor (NPY Y1R) reduces behavioral signs of inflammatory pain in mice. We hypothesize that the Y1R-expressing subpopulation of excitatory PBN neurons contributes to the manifestation of neuropathic pain.

Spared nerve injury (SNI), a model of neuropathic pain, was induced in miceby ligating the tibial and common peroneal branches of the sciatic nerve and leaving the sural nerve intact. Functional fluorescence in situ hybridization found that stimulus-evoked Fos expression was increased in PBN neurons expressing Y1R in mice with nerve injury compared to controls. Next, to test the functional role of this excitatory PBN subpopulation to the

manifestation of neuropathic pain, we implanted cannula in the PBN of wild type mice for in vivo pharmacology. Intra-PBN infusion of the NPY Y1 receptor-specific agonist, [Leu^{31},Pro^{34}]-NPY, reversed mechanical (up/down von Frey) and cold (acetone withdrawal duration) hypersensitivity in SNI but not sham animals. Next, we transfected Npy1r-expressing PBN neurons with an inhibitory designer receptor exclusively activated by designer drugs (DREADD, AAV8-hsyn-DIO-hM4Di-mCherry) or control (AAV8-mCherry). Chemogenetic inhibition of Npy1r-expressing PBN neurons alleviated mechanical and cold hypersensitivity in animals with nerve injury but not control animals. Saline administration had no effect on pain-like behaviors.

Conclusion: SNI sensitizes a subpopulation of PBN neurons expressing the neuropeptide Y Y1receptor (Npy1r). Inhibition of the Npy1r-expressing PBN neurons can reverse neuropathic pain-like behavior in mice.

21 - Whole human DRG transcriptomic and epigenomic landscape analyzed by single-nucleus RNA and ATAC sequencing

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Primary sensory afferents are the guardians of the nervous system and transmit internal and external sensory information to the CNS. Cell bodies of these afferents are located in the primary sensory ganglia where several non-neuronal cell-types support and nurture these neurons. In this project we aim to better understand the diversity of the primary afferents and their surrounding non-neuronal cells in order to find specific transcriptomic and epigenomic signatures of all cell types in the human dorsal root ganglia (DRGs). Taking advantage of microfluidic based barcoding technologies (10x Genomics), we have optimized single nuclei isolation from flash frozen postmortem human DRGs harvested from organ donors shortly after cross-clamp. To further enrich the data, we assess open chromatin regions of the nuclear DNA using ATAC-seq at the single-cell level as well. Currently we have analyzed a total of >150K cells. The most frequent cell type (~30%) can be associated with mesenchymal cells including fibroblasts. Satellite glial cells and macrophages constituted close to 16% each, while neurons amounted to 3.5% of total cells. Finally, ATAC-seq results enhance the detection of active genes even those that are not abundantly transcribed or those with lower capture efficiency through transcriptomic assays. Our results confirm that a thorough approach to the DRG's single-cell analysis can improve our understanding of somatosensation. By increasing our sample size, we hope to provide the pain and somatosensory research community with a valuable resource that can inform scientific observations, treatment strategies, drug development, as well as preclinical and clinical study designs.