The Challenge of Chronic Pain

Wellcome Genome Campus Conference Centre, Hinxton, Cambridge, UK
4-6 March 2019

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Dear colleague,

I would like to offer you a warm welcome to the Wellcome Genome Campus Advanced Courses and Scientific Conferences: The Challenge of Chronic Pain. I hope you will find the talks interesting and stimulating, and find opportunities for networking throughout the schedule.

The Wellcome Genome Campus Advanced Courses and Scientific Conferences programme is run on a not-for-profit basis, heavily subsidised by the Wellcome Trust.

We organise around 50 events a year on the latest biomedical science for research, diagnostics and therapeutic applications for human and animal health, with world-renowned scientists and clinicians involved as scientific programme committees, speakers and instructors.

We offer a range of conferences and laboratory-, IT- and discussion-based courses, which enable the dissemination of knowledge and discussion in an intimate setting. We also organise invitation-only retreats for high-level discussion on emerging science, technologies and strategic direction for select groups and policy makers. If you have any suggestions for events, please contact me at the email address below.

The Wellcome Genome Campus Scientific Conferences team are here to help this meeting run smoothly, and at least one member will be at the registration desk between sessions, so please do come and ask us if you have any queries. We also appreciate your feedback and look forward to your comments to continually improve the programme.

Best wishes,

Dr Rebecca Twells
Head of Advanced Courses and Scientific Conferences
rebecca.twells@wellcomegenomecampus.org
General Information

Conference Badges
Please wear your name badge at all times to promote networking and to assist staff in identifying you.

Scientific Session Protocol
Photography, audio or video recording of the scientific sessions, including poster session is not permitted.

Social Media Policy
To encourage the open communication of science, we would like to support the use of social media at this year’s conference. Please use the conference hashtag #CCP2019. You will be notified at the start of a talk if a speaker does not wish their talk to be open. For posters, please check with the presenter to obtain permission.

Internet Access
Wifi access instructions:
- Join the ‘ConferenceGuest’ network
- Enter your name and email address to register
- Click ‘continue’ to send an email to the registered email address
- Open the registration email, follow the link ‘click here’ and confirm the address is valid
- Enjoy seven days’ free internet access!
- Repeat these steps on up to 5 devices to link them to your registered email address

Presentations
Please provide an electronic copy of your talk to a member of the AV team who will be based in the meeting room.

Poster Sessions
Posters will be displayed throughout the conference. Please display your poster in the Conference Centre on arrival. There will be two poster sessions during the conference.

Odd number poster assignments will be presenting in poster session 1, which takes place on Monday, 4 March, at 18:10 – 19:10.

Even number poster assignments will be presenting in poster session 2, which takes place on Tuesday, 5 March, at 19:15 – 20:15.

The abstract page number indicates your assigned poster board number. An index of poster numbers appears in the back of this book.

Conference Meals and Social Events
Lunch and dinner will be served in the Hall, apart from on Monday 4 March when lunch will be served in the Conference Centre. Please refer to the conference programme in this book as times will vary based on the daily scientific presentations. Please note there are no lunch or dinner facilities available outside of the conference times.

All conference meals and social events are for registered delegates. Please inform the conference organiser if you are unable to attend the conference dinner.

The Hall Bar (cash bar) will be open from 19:00 – 23:00 each day.
**Dietary Requirements**
If you have advised us of any dietary requirements, you will find a coloured dot on your badge. Please make yourself known to the catering team and they will assist you with your meal request.

If you have a gluten allergy, we are unable to guarantee the non-presence of gluten in dishes even if they are not used as a direct ingredient. This is due to gluten ingredients being used in the kitchen.

**For Wellcome Genome Campus Conference Centre Guests**

**Check in**
If you are staying on site at the Wellcome Genome Campus Conference Centre, you may check into your room from 14:00. The Conference Centre reception is open 24 hours.

**Breakfast**
Your breakfast will be served in the Hall restaurant from 07:30 – 09:00

**Telephone**
If you are staying on-site and would like to use the telephone in your room, you will need to contact the Reception desk (Ext. 5000) to have your phone line activated - they will require your credit card number and expiry date to do so.

**Departures**
You must vacate your room by 10:00 on the day of your departure. Please ask at reception for assistance with luggage storage in the Conference Centre.

**Taxis**
Please find a list of local taxi numbers on our website. The conference centre reception will also be happy to book a taxi on your behalf.

**Return Ground Transport**
Complimentary return transport has been arranged for 11:45 on Wednesday, 6 March to Cambridge station and city centre (Downing Street), and Stansted and Heathrow airports.

A sign-up sheet will be available at the conference registration desk from 15:10 on Monday, 4 March. Places are limited so you are advised to book early.

Please allow a 30 minute journey time to both Cambridge and Stansted Airport, and two and a half hours to Heathrow.

**Messages and Miscellaneous**
Lockers are located outside the Conference Centre toilets and are free of charge.

All messages will be posted on the registration desk in the Conference Centre.

A number of toiletry and stationery items are available for purchase at the Conference Centre reception. Cards for our self-service laundry are also available.

**Certificate of Attendance**
A certificate of attendance can be provided. Please request one from the conference organiser based at the registration desk.

**Contact numbers**
Wellcome Genome Campus Conference Centre – 01223 495000 (or Ext. 5000)
Wellcome Genome Campus Conference Organiser (Lucy) – 07780 333332
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Conference Sponsors

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The Challenge of Chronic Pain
4-6 March 2019

Wellcome Genome Campus,
Hinxton, Cambridge, UK

Lectures to be held in the Francis Crick Auditorium
Lunch and dinner to be held in the Hall Restaurant
Poster sessions to be held in the Conference Centre

Spoken presentations - If you are an invited speaker, or your abstract has been selected for a spoken presentation, please give an electronic version of your talk to the AV technician.

Poster presentations – If your abstract has been selected for a poster, please display this in the Conference Centre on arrival.

Monday 4 March 2019

11:30-12:30 Registration with buffet lunch
Conference Centre Event Space

12:30-12:40 Welcome and Introductions
John Wood, University College London, UK

12:40-13:40 Keynote Lecture
Introduced by John Wood, University College London, UK

Cortical circuits and oscillatory rhythms in pain
Rohini Kuner
University of Heidelberg, Germany

13:40-15:10 Session 1: Pain in the brain
Chair: Rohini Kuner, University of Heidelberg, Germany

13:40 Imaging Mechanisms relate to Chronic Pain and its Relief
Irene Tracey
University of Oxford, UK

14:10 Brain circuits involved in phantom limb pain
Herta Flor
Institute of Cognitive and Clinical Neuroscience, Germany

14:40 Role of Piezo2 in trigeminal corneal mechanotransduction
Jorge Fernandez Trillo
Instituto de Neurociencias de Alicante UMH-CSIC, Spain

14:55 Human labor pain is influenced by the voltage-gated potassium channel
Kv6.4 subunit
Michael Lee
University of Cambridge, UK
Afternoon Tea

Session 2: Development and transition to chronic pain
Chair: Jon Levine, University of California, USA

15:40 Pain in early life - setting the scene
Maria Fitzgerald
University College London, UK

16:10 Mechanisms of transition to chronic pain
Theodore Price
University of Texas at Dallas, USA

16:40 Pathophysiological basis of pain in fibromyalgia
David Andersson
Kings College London, UK

16:55 A novel molecular mechanism underlying tumor-associated pain
Larry Sherman
Oregon Health & Science University, USA

Lightning talks
Chair: John Wood, University College London, UK

18:10-19:10 Poster Session 1 (odd numbers) with drinks reception

19:10 prompt Dinner

Tuesday 5 March 2019

07:30-09:00 Breakfast

Session 3: Genetics of pain
Chair: John Wood, University College London, UK

09:00 Sensory neuron populations – genetics and function
Paul Heppenstall
EMBL- Rome, Italy

09:30 Gene therapy for neuropathic pain: Can we dial down excitability?
David Bennett
University of Oxford, UK

10:00 NIHR Bioresource - Neuropathic Pain Disorders: Analysis of 193 whole genome sequencing data to understand neuropathic pain disorders.
Andreas Themistocleous
University of Oxford, UK

10:15 Attributing function to classes of colonic sensory neuron revealed by single-cell RNAseq
James Hockley
University of Cambridge, UK
10:30-11:00  **Morning Coffee**

11:00-12:30  **Session 4: Neural circuits in pain and effects of opioids**  
Chair: Rohini Kuner, University of Heidelberg, Germany

11:00  Spinal cord circuits in touch and pain  
Rebecca Seal  
University of Pittsburgh, USA

11:30  Neural circuits of pain unpleasantness and its control by opioids  
Gregory Scherrer  
Stanford University, USA

12:00-13:30  **Lunch**  
Hinxton Hall Restaurant

13:30-14:30  **Session 4 continued: Neural circuits in pain and effects of opioids**  
Chair: Rohini Kuner, University of Heidelberg, Germany

13:30  Opioids and brain function: Pain relief, reward, addiction  
Catherine Cahill  
University of California, Los Angeles, USA

14:00  The role of neuropeptide Y-expressing dorsal horn inhibitory interneurons in nociceptive and pruriceptive circuits  
Kieran Boyle  
University of Glasgow, UK

14:15  Spinofugal nociceptive projection neurons defined by Phox2a expression  
Artur Kania  
IRCM / McGill University, Canada

14:30-15:15  **Afternoon Tea and Group Photograph**

15:15-17:00  **Session 5: Molecules and cells of nociception**  
Chair: Cheryl Stucky, Medical College of Wisconsin, USA

15:00  Molecules and cell biology of nociception  
Aziz Moqrich  
Marseile University, France

15:30  Ion channels in somatodetection and nociception  
Thomas Voets  
Leuven, Belgium

16:00  Skin cells detect touch and pain  
Cheryl Stucky  
Medical College of Wisconsin, USA

16:30  Peripheral mechanisms of cold allodynia in neuropathic pain  
Donald Ian McDonald  
University College London, UK
16:45  Profiling the proteome landscape of chronic pain – opportunities for mechanism-based research
Manuela Schmidt
MPI of Experimental Medicine and University of Goettingen, Germany

17:00-18:00  Lightning talks
Chair: Rohini Kuner, University of Heidelberg, Germany

18:00-18:15  Comfort break

18:15-19:15  Keynote Lecture
Introduced by Jon Levine, University of California, USA

Mechanically activated ion channels in touch and pain
Ardem Patapoutian
Scripps, USA

19:15-20:15  Poster Session 2 (even numbers) with drinks reception

Wednesday 6 March 2018

07:30-09:00  Breakfast

09:00-10:30  Session 6: Translation and New Therapeutics in pain
Chair: John Wood, University College London, UK

09:00  Bench to Bedside in Pain Treatment: Following, Leading, or Misleading?
James Eisenach
Wake Forest, USA

09:30  Cancer-induced bone pain: understanding the role of glial cells and P2X7
Anne-Marie Heegaard
University of Copenhagen, Denmark

10:00  Escape the rodent trap: iPSC derived sensory neurons for pain research
Angelika Lampert
Uniklinik RWTH Aachen, Germany

10:15  Genome-wide Association Study of Multisite Chronic Pain in UK Biobank
Keira Johnston
University of Glasgow, UK

10:30-11:00  Closing remarks
Rohini Kuner, University of Heidelberg, Germany
11:00-11:30  Morning Coffee

11:45 prompt  Coaches depart to Cambridge City Centre and Train Station, Stansted Airport via Heathrow Airport

These abstracts should not be cited in bibliographies. Materials contained herein should be treated as personal communication and should be cited as such only with consent of the author.
Spoken Presentations

Cortical circuits and oscillatory rhythms in pain

Rohini Kuner
University of Heidelberg, Germany

Not available at the time of printing
Notes
Imaging Mechanisms related to Chronic Pain and its Relief

Irene Tracey

Nuffield Chair Anaesthetic Science & Head of Nuffield Department Clinical Neurosciences, University of Oxford, England UK.

The ability to experience pain is old and shared across species. Acute pain is the body’s alarm and warning system, and as such a good thing. Chronic pain is the system gone wrong and now one of the largest medical health problems worldwide. The brain is key to both these experiences and relating specific neurophysiologic measures from advanced brain imaging to perceptual or non-perceptual changes in pain perception induced by peripheral or central sensitisation, psychological or pharmacological mechanisms has tremendous value. Identifying non-invasively where functional and structural plasticity, sensitisation and other amplification or attenuation processes occur along the pain central neuraxis (i.e. brain, brainstem and spinal cord) for an individual and relating these neural mechanisms to specific pain experiences, measures of pain relief, persistence of pain states, degree of injury and the subject’s underlying genetics, has neuroscientific and potential diagnostic relevance. A key area of development has been pharmacological imaging where objective evidence of pharmacodynamic efficacy can be obtained providing useful information to aid analgesic drug development that oftentimes is hampered by over-reliance on subjective ratings which can mask genuine mechanistic efficacy. More recently, researchers have been investigating through brain imaging whether there is a pre-disposing vulnerability or resilience in neural networks towards developing chronic pain. As such, advanced neuroimaging studies can powerfully aid explanation of a subject’s multidimensional pain experience, analgesia and even what makes them vulnerable to developing chronic pain.

Learning Objectives:

1. The basic neuroanatomy of pain processing in the human brain – concept of a flexibly accessible network
2. How different neuroimaging techniques provide insight into chronic and acute pain (and analgesia)
3. The basics of pharmacological neuroimaging to identify pharmacodynamic efficacy

Key References and Reviews:
Brain circuits involved in phantom limb pain

Herta Flor
Department of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Heidelberg University

Phantom limb pain is a frequent sequel of an amputation occurring in up to 80% of the amputee population. Peripheral factors such as local changes at the residual limb, alterations in severed nerves and associated dorsal root ganglia, as well as central changes such as spinal and supraspinal plastic changes have been examined. Moreover, input from the amputated limb has been discussed as a crucial factor. New insights about central changes have also come from studies on somatosensory illusions and altered body perception. These hypotheses have led to new behavioral and pharmacological treatment options that target maladaptive plasticity and learning and sensory incongruence phenomena. These approaches include prosthesis use, discrimination training, mirror treatment, video feedback, imagery and virtual reality applications, brain-computer interfaces as well as combinations of these treatments with pharmacological interventions. We will provide a critical overview of these recent developments on sources and treatments of phantom limb pain.
Role of Piezo2 in trigeminal corneal mechanotransduction

Jorge Fernandez-Trillo, Danny Florez-Paz, Almudena Iñigo, Omar González-González, Alejandro González, Félix Viana, Carlos Belmonte and Ana Gomis

Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, 03550 San Juan de Alicante, Spain

Transduction and coding of mechanical forces is crucial for many of the most important physiological processes required for survival of living organisms. Nervous sensory terminals respond to mechanical stimuli evoking tactile and proprioceptive sensations when the stimulus exclusively activates low threshold mechanoreceptors, and painful sensations when nociceptors are recruited. Significant progress has been made in the understanding of the cellular and molecular transduction mechanisms in touch receptors. However, transduction of mechanical forces by nociceptors is less understood.

The Piezo2 channel is mainly responsible for touch sensation and proprioception. However, mechanosensitivity of skin nociceptors is unaffected in mice deficient for Piezo2. Very recent behavioural experiments suggest that Piezo2 is required for mechanical allodynia but has only a partial role in noxious mechanosensation.

The cornea, innervated by the trigeminal ganglion neurons contains three functionally different populations of sensory nerve terminals. Polymodal nociceptors and pure mechano-nociceptors are mechanosensitive, and their activation evokes pain. However, the transducing channels conferring them mechanosensitivity have not been yet identified. Thus, the cornea appears a simple and good model to approach the characteristics of the transducing mechanisms underlying mechanotransduction in two functionally distinct populations of peripheral sensory neurons evoking mechanical pain sensations.

In the mouse cornea, immunostaining of peripheral nerve branches showed fibers that express only Piezo2 as well as others that co-express Piezo2 and the polymodal nociceptor ion channel TRPV1. Whole cell patch clamp recordings combined with simultaneous mechanical indentation performed in mouse corneal trigeminal (TGC) neurons retrogradely marked with the dye FM1-43 applied onto the cornea, allow to identify pure mechanooceptor and polymodal nociceptor neurons, by their response to capsaicin; both classes of neurons displayed the three well-defined types of mechanically activated currents: RA, IA and SA currents. After recordings, neurons were fixed on the coverslips and treated for Piezo 2 immunocytochemistry. This showed that Piezo2 is expressed in neurons that displayed the three types of mechanically-activated currents. Furthermore, functional results using a conditional Piezo2 KO mouse, in which the channel was eliminated exclusively in sensory neurons, evidenced a significant reduction of mechanical responses in all mechanosensory corneal neurons. These findings provided direct evidence that the ion channel Piezo2 plays a role in mechanotransduction in corneal neurons signaling mechanical pain.

Supported by: Ministerio de Ciencia, Innovación y Universidades, SAF2016-77233-R co-financed by the European Regional Development Fund (ERDF), and the "Severo Ochoa" Program for Centers of Excellence in R&D SEV-2013-0317 and SEV-2017-0723.
Human labor pain is influenced by the voltage-gated potassium channel Kv6.4 subunit

Michael Lee1†, Michael S. Nahorski2†, James R.F. Hockley3†, Van B. Lu4†, Kaitlin Stouffer2, Emily Fletcher2, Gillian Ison1, Christopher Brown5, Daniel Wheeler1, Patrik Ernfors6, David Menon1# , Frank Reimann4#, Ewan St John Smith3*#, C. Geoffrey Woods2*#

1. University Division of Anaesthesia, University of Cambridge, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 0QQ, UK
2. Cambridge Institute for Medical Research, Wellcome Trust MRC Building, Addenbrooke’s Hospital, Hills Rd, Cambridge CB2 0QQ, UK.
3. Department of Pharmacology, Tennis Court Road, Cambridge, CB2 1PD, UK
4. University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, CB2 0QQ, UK
5. Department of Psychological Sciences, Institute of Psychology, Health and Society, University of Liverpool, L69 7ZA
6. Department of Medical Biochemistry and Biophysics, Karolinska Institute, SE-171 77 Stockholm, Sweden.
† These authors contributed equally to this paper.
# These authors supervised the work presented in this paper

We sought Mendelian genetic influences on labor pain by studying healthy women who neither requested nor used drug-based analgesia during their first labor; a discovery cohort of 116 were exome sequenced, and an additional 80 had targeted sequencing. Thirty-three of these 196 women underwent comprehensive sensory and psychometric tests, which revealed higher experimental pain thresholds, particularly to deep somatic pressure, when compared to matched controls. We found an excess of heterozygotes carrying the rare allele SNP rs140124801 p.Val419Met in KCNG4 (encoding the voltage-gated potassium channel subunit KV6.4); 6 versus an expected 1.57, P < 0.001. We show that the rare variant Kv6.4-Met419 fails to traffic to the plasma membrane and, unlike Kv6.4, does not modulate the voltage-dependence of Kv2.1 inactivation. In vivo, we observed Kcng4 (Kv6.4) to be present in 40% of retrolabelled mouse uterine sensory neurons, all of which expressed Kcnb1 (Kv2.1), and over 90% of which expressed the nociceptor markers Trpv1 and Scn10a. Moreover, the voltage-dependence of inactivation for Kv2.1-mediated currents is more depolarized when Kv6.4-Met419 is overexpressed in mouse sensory neurons compared to when Kv6.4 is overexpressed and hence expression of Kv6.4-Met419 produces less excitable sensory neurons. Lastly, we show that Kv6.4-Met419 has a dominant-negative effect on wild type Kv6.4, consistent with the reduction of labor pain observed in the individuals of our cohort who were heterozygotes for the KCNG4 SNP rs140124801 allele. Hence Kv6.4 can influence human labor pain by modulating the function of nociceptors that innervate the uterus.
Pain in early life - setting the scene

Maria Fitzgerald FMedSci FRS
Neuroscience, Physiology, Pharmacology,
University College London
Gower St, London WC1E 6BT

All newborn mammals display pain related protective reflex movements and physiological reactions essential for the preservation of life, but individual sensitivity to pain is established in infancy and childhood. Thus, nociceptive experience in early life shapes future pain behaviour and increases susceptibility to chronic pain states in adulthood. Since cortical networks undergo substantial postnatal maturation, alterations in cortical pain circuits are likely to underlie developmental shaping of pain sensitivity. However, little is known about the postnatal development of cortical pain circuitry and its susceptibility to repeated or excess noxious stimulation in the first weeks of life.

In this talk I will present our discoveries on the pattern and vulnerability of developing central nociceptive pathways in the young mammalian brain. Our group takes a translational approach, using both telemetric local field potential (LFP) recordings of cortical pain related activity in awake, active rat pups together with EEG recordings from hospitalised human infants exposed to clinically required noxious stimulation.

Evidence will be presented for the gradual functional development of evoked and background pain activity in postnatal somatosensory and medial prefrontal cortex, the existence of sex differences in cortical noxious evoked activity from birth and the influence of early life tissue injury upon the subsequent structural and functional development of cortical pain networks.

Funding: Biotechnology and Biological Sciences Research Council and Medical Research Council #MR/M006468/1; #MR/L019248/1

References

Mechanisms of Transition to Chronic Pain

Theodore Price PhD
University of Texas at Dallas

How does acute pain become chronic? In this talk I will argue that nervous and/or immune system plasticity mechanisms are the key drivers of the transition from acute to chronic pain and that translation regulation signaling is a key target to block or reverse this transition. The first part of the talk will focus our experiments showing that a mitogen activated protein kinase (MAPK) family kinase called MNK1/2, which phosphorylates eukaryotic initiation factor (eIF) 4E, is critical for the generation and maintenance of nociceptor plasticity in mice. Importantly, using human dorsal root ganglion neurons removed during vertebrectomy surgery from patients with neuropathic pain, we demonstrate that inhibiting MNK1/2 reverses spontaneous action potential generation in human nociceptors. The second part of the talk will focus on how MNK1/2 inhibitors might be used to reverse chronic neuropathic pain. Targeting this pathway genetically or pharmacologically has little effect on mechanical allodynia caused by peripheral nerve injury in mice; however, MNK1/2 targeting blocks the development of spontaneous pain and cognitive deficits that arise following nerve injury. Our findings indicate that translation regulation signaling via MNK1/2-mediated phosphorylation of eIF4E is a key component of the transition from an acute to chronic pain state and that this signaling pathway is a prime target for the generation of therapeutics that might be capable of reversing chronic pain.
Pathophysiological basis of pain in fibromyalgia

David Andersson, Andreas Goebel, Clive Gentry, Ulku Cuhadar, Nisha Vastani, Serena Sensi, Katalin Sandor, Alexandra Jurczak, Eva Kosek, Camilla Svensson and Stuart Bevan

Pain Research Institute, University of Liverpool, UK; Wolfson CARD, King's College London, UK; Department of Physiology and Pharmacology, Karolinska Institutet

Fibromyalgia syndrome (FMS) is a common chronic pain condition associated with a very low health-related quality of life. Widespread pain and tenderness, together with anxiety, fatigue and mood disorders are characteristic symptoms. There are no diagnostic tests or effective pharmacological treatments available, and the aetiology and pathophysiology of fibromyalgia have remained unknown. Here we show that the painful hypersensitivity in fibromyalgia is caused by sensitization of peripheral nociceptive sensory neurons. We demonstrate that hypersensitivities experienced by patients can be passively transferred to mice by administration of patient IgG. IgG from individual patients, and IgG pooled from independent groups of patients, increased the mouse hind paw pain sensitivity to stimulation with noxious cold and mechanical pressure. In contrast, transfer of IgG from healthy control subjects was without effect on pain sensitivity. Mechanical stimulation of the receptive fields of A- and C-nociceptors evoked a significantly increased number of action potentials in ex vivo skin-nerve preparations from mice treated with IgG from patients, compared to preparations from mice treated with IgG from control subjects. Our results demonstrate that fibromyalgia pain is caused by IgG autoantibodies that act by sensitizing peripheral nociceptive afferents.
Patients with a variety of benign and metastatic tumors often experience significant pain. We have been studying mechanisms underlying tumor-associated pain in patients with schwannomatosis, a disease characterized by multiple benign peripheral nerve tumors (schwannomas) and intractable pain that can occur in the absence of a detectable mass and which is not always relieved by tumor resection. Patients with schwannomatosis have a high rate of mutations in the SMARCB1 gene which encodes a subunit of the SWI/SNF chromatin remodeling complex that is involved in regulating gene transcription. We found that inducible conditional disruption of the Smarcb1 gene in Schwann cells does not lead to changes in peripheral nerve morphology, Schwann cell proliferation, or cell cycle-related gene expression. However, mice with targeted Smarcb1 disruption in Schwann cells demonstrate increased pain sensitivity. Dorsal root ganglion (DRG) and trigeminal ganglion neurons from mice with Schwann cell-targeted disruption of Smarcb1 express elevated levels of the TRPV1, a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin. We also find that TRPA1, an ion channel that acts as a sensor for environmental irritants, and the calcitonin gene related peptide (CGRP), which has been implicated in pain signaling, are elevated in sensory neurons of mice with Schwann cell-targeted Smarcb1 mutations. Wild type DRG cells grown in Smarcb1-null Schwann cell conditioned media demonstrated elevated cobalt uptake, a marker of TRPV1 activity, compared to cells grown with wild type Schwann cell conditioned media, and DRG cultures treated with Smarcb1-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients expressed elevated levels of TRPV1, TRPA1 and CGRP as indicated by immunocytochemistry. Proteomic, DNA microarray and chromatin immunoprecipitation analyses identified several proteins that are elevated in Smarcb1 mutant Schwann cells and whose transcription is directly regulated by Smarcb1. Several of these proteins directly influence the expression of TRPV1 in sensory neurons. Collectively, these data indicate that loss of Smarcb1 in Schwann cells leads to the secretion of factors that induce the expression of pain mediators in sensory neurons, and suggest a novel mechanism for schwannomatosis pain in patients bearing SMARCB1 mutations. The data also suggest a novel model for the molecular mechanisms underlying neuropathic pain.
Sensory neuron populations – genetics and function

Paul Heppenstall
EMBL Monterotondo, Italy

Recent mouse genetic studies have demonstrated that distinct subpopulations of primary afferent contribute to behavioral responses to different types of mechanical, thermal and chemical nociceptive stimuli. Focusing on work from my laboratory in which we have identified a population of sensory neurons marked by TrkB involved in mechanical hypersensitivity, I will discuss how opto- and pharmacogenetic approaches can be used to control sensory input from the periphery and thus regulate behavior in transgenic mice. Taking this approach further I will show how these genetic strategies can also be translated into pharmacological technologies by means of the ligands that bind to membrane receptors expressed on these distinct populations of neuron. Using BDNF, the ligand for TrkB, as an example, I will describe how cargoes such as photosensitizers can be attached to these molecules and delivered in vivo in mice. Application of light to the skin then results in retraction of TrkB neurons from their end organs and long-term reversal of mechanical hypersensitivity in animal models of traumatic and diabetic neuropathic pain. Finally, I will discuss how this technology can be applied to other ligands and cargoes with the ultimate aim of gaining optical and chemical control over neuronal activity, thus inhibiting pain at its source.
Notes
Gene therapy for neuropathic pain: Can we dial down excitability?

David Bennett
Oxford University, UK

The development of ectopic activity in sensory neurons is critical for the induction and maintenance of neuropathic pain. Local anaesthetics and anti-epileptic drugs can suppress hyper-excitability however these drugs are complicated by unwanted effects on motor, CNS and cardiac function and alternative more selective treatments to suppress hyper-excitability are therefore required. I will discuss pharmacogenomics as an alternative strategy. We used a glutamate-gated chloride channel (GluCl) modified to be activated by low doses of Ivermectin (but not glutamate) and found that this was highly effective in silencing sensory neurons and reversing neuropathic pain related hypersensitivity. Activation of GluCl expressed in either rodent or human iPSC-derived sensory neurons in vitro potently inhibited their response to both electrical and algogenic stimuli. Silencing is achieved both at nerve terminals and the soma. We used intrathecal adeno-associated virus serotype-9 based delivery to target GluCl to mouse sensory neurons in vivo. This resulted in high level and long lasting expression of GluCl selectively in sensory neurons and enabled reproducible and reversible modulation of pain related hypersensitivity following nerve injury with no side effects observed. We are now using this system to target specific sub-populations of sensory neurons to define which populations are the key drivers of pain related behaviour. I will discuss alternative gene therapy approaches such as the targeting specific ion channels. Finally I will draw parallels with other neurological disorders such as neuromuscular disorders and epilepsy and how they have translated promising preclinical results into clinically approved gene therapy approaches.
Extreme pain phenotypes, such as erythromelalgia and insensitivity to pain, caused by rare high impact genetic variants, offer us insight into mechanisms that may apply to more common causes of neuropathic pain. The aim of our study was to identify singleton patients and families with extreme pain phenotypes to determine whether variants/mutations were present in genes known to cause neuropathic pain. We included participants with: congenital insensitivity to pain; painless sensory neuropathy; chronic pain caused by erythromelalgia, small fibre neuropathy, and sensory neuropathy. NeuPSIG grading criteria for neuropathic pain were used to stratify the cohort. A total of 219 participants were recruited from secondary care clinics across the UK. Neuropathic pain was classified as: not present (n=9, 4.1%), definite (n=125, 57.1%), probable (n=62, 28.3%), possible (n=12, 5.5%) or unlikely (n=1, 0.5%). Ten participants were unaffected family members. Whole genome sequencing data, acquired using next generation sequencing technology, were available for 193 participants. Previously characterised pathogenic variants in SCN9A, the gene encoding the sodium channel (Nav) 1.7, were identified in 11 participants. For example, the SCN9A pathogenic variant, c.2543T>C (p.Ile848Thr), was identified in a pair of sisters diagnosed with erythromelalgia. Novel uncharacterised variants, predicted through in silico analysis to be pathogenic and confirmed in multi-disciplinary team meetings, were identified in SCN9A, SCN10A, SCN11A and SPTLC1 genes. We have demonstrated that a meticulous phenotyping approach combined with next generation sequencing provides a powerful platform to explore pathophysiological mechanisms of chronic neuropathic pain.
Attributing function to classes of colonic sensory neuron revealed by single-cell RNAseq

James RF Hockley¹, Tereza Bautzova², Teresa Perez-Berezo², Julien Pujo², Michael M Tranter³, Cleo Desormeaux², Maria Raffaella Barbaro⁵, Lilian Basso², Pauline Le Faouder⁴, Corinne Rolland², Pascale Malapert⁶, Aziz Moqrich⁶, Helene Eutamene⁷, Alexandre Denadai-Souza², Nathalie Vergnolle², David I Hughes⁸, Giovanni Barbara⁵, Gilles Dietrich², David Bulmer¹, Nicolas Cenac², Ewan St John Smith¹

¹ Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB1 2PD, UK
² INSERM, UMR1220, IRSD, Université de Toulouse, INRA, ENVT, UPS, Toulouse, France
³ National Centre for Bowel Research and Surgical Innovation, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AJ, UK
⁴ INSERM UMR1048, Lipidomic Core Facility, Metatoul Platform, Université de Toulouse, Toulouse, France
⁵ Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy
⁶ Aix-Marseille-Université, CNRS, Institut de Biologie du Développement de Marseille, UMR 7288, Marseille, France.
⁷ Neuro-Gastroenterology and Nutrition Team, UMR 1331, INRA Toxalim, INP-El-Purpan, Université de Toulouse, Toulouse, France
⁸ Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, United Kingdom

Integration of nutritional, microbial and inflammatory events along the gut-brain axis can alter bowel physiology and organism behaviour. Colonic sensory neurons activate reflex pathways and give rise to conscious sensation, but the diversity and division of function within these neurons is poorly understood. The identification of signalling pathways contributing to visceral sensation is constrained by a paucity of molecular markers. Here we address this by comprehensive transcriptomic profiling and unsupervised clustering of individual mouse colonic sensory neurons. Unbiased single-cell RNA-sequencing was performed on retrogradely traced mouse colonic sensory neurons isolated from both thoracolumbar (TL) and lumbosacral (LS) dorsal root ganglia associated with lumbar splanchnic and pelvic spinal pathways, respectively. Identified neuronal subtypes were validated by single-cell qRT-PCR and immunohistochemistry (IHC). Transcriptomic profiling and unsupervised clustering of 314 colonic sensory neurons revealed seven neuronal subtypes. Of these, five neuronal subtypes accounted for 99% of TL neurons, with LS neurons almost exclusively populating the remaining two subtypes. Using this framework, we investigate a previously undescribed visceral-projecting Mrgprd+ pathway responsible for transducing abdominal pain to a lipid mediator, 5-oxoETE, which is upregulated in constipation-predominant irritable bowel syndrome (IBS). These results provide a pathway to molecular interrogation of colonic sensory innervation in health and disease, together with identifying novel targets for drug development.
Persistent pain remains an enormous clinical problem. First-line treatments such as non-steroidal anti-inflammatory drugs and opioid-based pain killers have serious side-effects, can lose efficacy and are not effective for all types of pain. Efforts in our laboratory are focused on delineating the neural circuitry for persistent pain in the spinal dorsal horn. This area of the nervous system serves as a major site for the central integration of peripheral somatosensory information including circuits that give rise to persistent forms of pain. Here, we have identified a number of excitatory populations in mice that are required for mechanical allodynia, a common and persistent condition in which touch becomes painful after injury. Interestingly, chemogenetic analyses of these populations revealed that this central mechanical allodynia network differs by the nature of the injury. Current efforts to transfer these findings to the clinic are now focused on studies that allow us to identify conserved elements of the dorsal horn circuitry in primates, and ultimately humans, and to develop strategies to target them therapeutically.
Neural circuits of pain unpleasantness and its control by opioids

Grégory Scherrer1,2
1Department of Anesthesiology, Perioperative, and Pain Medicine, Department of Molecular and Cellular Physiology, Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305, USA.
2New York Stem Cell Foundation – Robertson Investigator, Stanford University, Stanford, CA 94305, USA.

Pain is a complex experience with sensory and affective dimensions. The aversive quality of pain (i.e. pain unpleasantness) causes the majority of chronic pain patients’ suffering, and often results in comorbid disorders (anxiety/depression). However, the neural circuits and codes by which our brain assigns a negative emotional valence to nociceptive information remain unresolved. Opioids are useful drugs because for many patients they provide substantial pain relief by dampening pain affect. Where and how opioids act in emotional circuits to alter pain codes is unknown. To begin addressing these questions, we examine the mechanisms by which the amygdala, a region of the temporal lobe essential for emotions, contributes to pain unpleasantness and opioid analgesia. We used histological techniques to characterize neurons active during pain and opioid receptor distribution in the amygdala, in vivo calcium imaging to record activity of large ensembles of amygdalar neurons in freely moving mice experiencing pain, and chemogenetics to establish the function of these neurons in pain unpleasantness. Neuroanatomical studies indicated that in basolateral amygdala (BLA), c-Fos+ neurons activated by nociceptive stimuli comprised a population of mid-anterior Camk2a+ and Rspo2+ principal aversive neurons. Additionally, we found that the mu opioid receptor (MOR) is densely expressed by neurons of the capsular part of the central amygdala and by intercalated cells, while delta opioid receptor-expressing amygdalar neurons are mostly found in the BLA, suggesting that the two receptors have distinct functions in amygdalar circuits modulating pain affect. Next, in calcium imaging experiments, we identified a distinct neural ensemble in the BLA that encodes the negative affective valence of pain. Silencing this nociceptive ensemble with chemogenetics alleviated pain affective-motivational behaviors without altering the detection of noxious stimuli, withdrawal reflexes, anxiety, or reward. Following peripheral nerve injury, innocuous stimuli activated this nociceptive ensemble to drive dysfunctional perceptual changes associated with neuropathic pain, including pain aversion to light touch (alodynia). Collectively, these results clarify the functional organization of DOR and MOR in the amygdala, and identify the amygdalar representations of noxious stimuli that are functionally required for the negative affective qualities of acute and chronic pain perception.
Kappa Opioid Receptors Drive a Tonic Aversive Component of Chronic Pain

Catherine Cahill, Shiwei (Steve) Liu, Sarah Pickens, Anna M.W. Taylor, Hongyan Yang, Lihua Xue, Piper Williams, Chris Cook, Nicole Burma, Joshua K. Hakimian, Amie Saverino, Ines Ibarra, Lindsay Lueptow, Kristina Komarek, F. Ivy Carroll, Anne M. Andrews, Mary C. Olmstead, Wendy Walwyn, Tuan Trang, Christopher J. Evans, Frances Leslie

Department of Pharmacology, University of California Irvine, School of Medicine, Irvine, California, USA
Hatso Center for Neuropharmacology, Semel Institute for Neuroscience and Human Behavior, University of California Los Angeles, Los Angeles, California, USA
Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, California, USA
Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada
Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, California, USA
Department of Biomedical and Molecular Sciences, Queen’s University, School of Health Sciences, Kingston, Ontario, Canada
Children’s Hospital Los Angeles and the Keck School of Medicine of the University of Southern California, 4650 Sunset Boulevard, Los Angeles, California, USA
Department of Comparative Biology and Experimental Medicine, University of Calgary, Calgary, Alberta, Canada, Department of Physiology and Pharmacology, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada
Research Triangle Institute, Research Triangle Park, North Carolina, USA
Department of Psychology, Queen’s University, Kingston, Ontario, Canada

Chronic pain is second only to bipolar disorder as the major cause of suicide among all medical illnesses. Co-occurring psychopathology in chronic pain patients significantly impacts pain perception (heightened pain intensity), increases pain-related disability, decreases response to treatment and increases risk of prescription opioid misuse. In humans, kappa opioid receptor (KOR) activation causes anxiety, discomfort, agitation, depression and dysphoria. Considering that the circuitry involved in pain processing and affective/motivational systems overlaps extensively, we asked whether KOR contributes to the aversive nature of chronic pain. In a rodent model of chronic neuropathic pain, we show that the endogenous tone of the KOR system within mesolimbic dopaminergic circuitry is robustly increased. Importantly, we show that KOR blockade or elimination of KOR in midbrain dopamine neurons alleviates a tonic-aversive component of chronic neuropathic and inflammatory pain in male, but not female mice. KOR blockade also alleviates depressive and anxiogenic effects associated with neuropathic pain but this effect is not sex-dependent, suggesting a diversion of mechanisms between affective dimensions of chronic pain and the on-going tonic-aversive states. Our results strongly support the use of KOR antagonists as therapeutic adjuvants to alleviate the emotional, tonic-aversive component of chronic pain, which is argued to be the most significant component of the pain experience that impacts a patient's quality of life.
The role of neuropeptide Y-expressing dorsal horn inhibitory interneurons in nociceptive and pruriceptive circuits

Kieran A Boyle, Maria Gutierrez-Mecinas, Erika Polgar, Allen C. Dickie and Andrew J. Todd

Institute of Neuroscience & Psychology, University of Glasgow, Glasgow, UK

Previous work from our laboratory has identified neuropeptide Y (NPY)-expressing neurons of the dorsal horn as a population of inhibitory interneurons (INs) that are well placed to modulate spinal nociceptive circuits through connections with nociceptive projection neurons and other dorsal horn interneurons (Iwagaki et al. (2016). The aims of the current study were 1) to use intraspinal injection of adeno-associated viruses (AAVs) in adult RH26 NPY::Cre mice to restrict chemogenetic activation to dorsal horn INs that express NPY in the adult (NPY-INs), 2) to assess the effects of this activation on dorsal horn nociceptive and pruriceptive circuit activity and the associated behavioural output, and 3) to test the hypothesis that chemogenetic activation of NPY-INs will suppress the pain hypersensitivity observed in the plantar Complete Freund's Adjuvant (CFA) model of inflammatory pain.

To achieve these aims, we unilaterally injected a Cre-dependent AAV construct that expresses the excitatory DREADD hM3Dq fused to mCherry (AAV.hM3Dq.mCherry), into the L3-5 dorsal horn of young adult RH26 mice. Systemic administration of the hM3Dq agonist CNO induced Fos expression (a marker of neuronal activation) in the vast majority of mCherry-expressing neurons in laminae I-III, and this was largely restricted to NPY-immunoreactive cells. However the proportion of mCherry-negative neurons in laminae I-II that expressed Fos following noxious heat or a pruritic stimulus was significantly lower in CNO-treated animals compared to vehicle-treated controls, suggesting that NPY-IN activation can inhibit spinal neurons normally activated by these stimuli. Consistent with this conclusion, NPY-IN activation increased the noxious heat withdrawal threshold and markedly reduced chloroquine-induced itch behaviour, as well as increasing noxious mechanical and cold withdrawal thresholds. NPY-IN activation also reversed mechanical and thermal hypersensitivity in the plantar CFA model.

Based on these findings, we draw the following conclusions: 1) Intraspinal injections of AAV.DREADDs into adult RH26 mice allows the specific manipulation of dorsal horn NPY-IN activity, 2) NPY-IN activation suppresses activity in dorsal horn circuits that process pain- and itch-related information, resulting in reductions in acute pain- and itch-related behaviours and 3) recruitment of NPY-INs reduces inflammatory pain. Dorsal horn NPY-INs therefore represent a potential target for treatment of acute and/or pathological pain and itch.
Spinofugal nociceptive projection neurons defined by Phox2a expression

Artur Kania, R. Brian Roome (1,2), Susana Sotocinal (1,2,3), Annie Dumouchel (2), Shima Rastegar-Pouyani (1,2), W. Scott Thompson (1,2), Samuel Ferland (4,5), Cyril Bories (4,5), Yves de Koninck (4,5), Jeff Mogil (1,2,3), Marie Kmita (1,2) and Artur Kania (1,2).

(1) McGill University, Montréal, QC, (2) Institut de Recherches Cliniques de Montréal, Montréal, QC, (3) Department of Psychology, McGill University, Montréal, QC, (4) CERVO Brain Research Centre, Québec, QC, (5) Laval University, Québec, QC

The relay of nociceptive signals from spinal neuronal circuits to the brain remains poorly understood. Classical experiments demonstrate that spinal dorsal horn projection neurons relay such signals to the parabrachial nucleus, thalamus, periaqueductal gray and other brain regions. To directly study the specific function of these pathways, we generated the Phox2a:Cre transgenic mouse line expressing Cre recombinase from the Paired-like Homeobox 2a (Phox2a) locus that encodes a developmentally-expressed transcription factor. Phox2a:Cre labels neurons in Lamina I and V of the spinal cord exhibiting classical projection neuron morphology. At least 90% of Phox2a:Cre neurons are spinofugal projection neurons, demonstrated by retrograde tracing from supraspinal locations. Activation of spinal Phox2a:Cre neurons using chemogenetics produces nocifensive behaviours in the absence of noxious stimuli. Furthermore, optogenetic activation of their axonal termini in the parabrachial nucleus results in escape behaviours and conditioned place aversion. Together, our data indicate that Phox2a:Cre is a genetic handle of the spinal nociceptive projection neurons that comprise the anterolateral tract, possibly permitting the dissection of the emotive, discriminative, motor and homeostatic components of pain.
Mechanisms underlying the transition from acute to chronic pain: Insights from an typical myosin protein

Moqrich Aziz, Reynders Ana, Jhumka Anissa, Gaillard Stéphane, Castests Francis
Aix-Marseille University, Institute of Developmental Biology of Marseille, France

Transition from acute to chronic pain represents a major medical problem worldwide. Its impacts on health care costs and social lives of patients are dramatic. Chronic inflammatory, neuropathic or postoperative pain occurs as a consequence of aberrantly prolonged sensitization of pain pathways both in the peripheral and central nervous systems, causing either increased facilitation or loss of inhibition in pain-transmitting circuits. We know a great deal about the molecular and cellular mechanisms underlying both peripheral and central sensitization that control the onset of injury-induced acute pain. However, our knowledge on the molecular and cellular events that trigger the transition from acute to chronic pain is still limited. As a consequence, to date, there are no efficient strategies that can either prevent or treat chronic pain. In the last years, my laboratory, identified an unconventional myosin protein whose loss-of-function (LoF) converts an acute and reversible inflammatory, neuropathic and postoperative pain into a long lasting and irreversible chronic pain. We also used this mouse model in combination with state-of-the-art RNA deep sequencing technology and behavioral pharmacology to i) decipher the molecular mechanisms that trigger the transition from acute to chronic pain and ii) to design "à la carte" pharmacological therapies to prevent the establishment of chronic pain.
TRP channels in acute and inflammatory pain

Thomas Voets

Laboratory of Ion Channel Research, VIB Center for Brain and Disease Research & KU Leuven Department of Cellular and Molecular Medicine, Leuven, Belgium

Transient receptor potential (TRP) channels form a superfamily of cation channels involved in a wide variety of physiological and pathophysiological processes. In particular, several mammalian TRP channels are expressed in sensory neurons, where they act as molecular sensors of thermal and chemical cues. Using single and combined mouse knockout models, we have investigated the role of various somatosensory TRP channels in acute pain sensation, and assessed how their expression and function changes in inflammatory conditions associated with hyperalgesia and ongoing pain. We further explore how pharmacological targeting of specific TRP channels may be developed to treat a variety of chronic pain conditions.
Skin cells detect and convey somatosensory stimuli

Cheryl L. Stucky, Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, USA.

The conventional view of somatosensation initiation is that sensory nerve terminals are the principal detectors as well as conveyors of environmental stimuli. However, the first point of our body’s contact with our physical environment is the epidermis, the outermost layer of skin that is largely composed of keratinocytes. Thus, it makes sense that keratinocytes could contribute to our ability to detect tactile, temperature and painful stimuli in the environment and convey these signals to nearby sensory nerve terminals. This talk will highlight our lab’s recent work using optogenetics to selectively inhibit keratinocyte signaling in vivo, skin-nerve assays to measure sensory nerve function ex vivo, and “cell sniff,” calcium imaging, and patch clamp approaches to measure keratinocyte function and released factors to discover the roles that keratinocytes play in the detection and transmission of tactile and temperature stimuli in multiple species. We believe that these experiments will lay an important foundation for subsequent studies to uncover the dysfunctional signaling that occurs in cutaneous pain and itch disorders, and ultimately, the development of novel topical therapeutics for peripheral disease conditions.

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Peripheral mechanisms of cold allodynia in neuropathic pain

Donald Iain MacDonald, Edward C. Emery, Ana P, Luiz and John N. Wood

Molecular Nociception Group, UCL

Chronic pain patients suffering from cold allodynia experience normally innocuous cooling as excruciating pain. While cold sensing in the healthy state is relatively well understood, the cells and molecules that drive cold allodynia remain elusive. We used in vivo calcium imaging of lumbar dorsal root ganglia to investigate how the activity of peripheral cold-sensing neurons was altered in mouse models of neuropathic pain. Cold hypersensitivity was elicited in mice by intraplantar injection of the chemotherapeutic oxaliplatin, which evokes prolonged cold allodynia in humans. In control mice, cold-sensitive neurons were rare with small diameters. Oxaliplatin caused a subset of cold-insensitive, large-diameter neurons to acquire a de novo responsiveness to cooling. These 'silent' cold-sensing neurons were also unmasked in cold allodynia induced by the marine toxin ciguatoxin-2. Silent cold-sensing neurons were identified as nociceptors based on their response to noxious mechanical stimulation and expression of the nociceptive marker NaV1.8. Consistent with this, diptheria toxin-mediated ablation of NaV1.8-positive nociceptors decreased oxaliplatin-induced cold hypersensitivity. Voltage-gated potassium channels KV1.1 and KV1.2 - hypothesized to mediate a hyperpolarizing 'brake' current against cooling-induced depolarization - were highly expressed in NaV1.8-positive neurons under basal conditions. Pharmacological inhibition of KV1 channels with 4-AP or alpha-dendrotoxin consequently induced cold responsiveness in cold-insensitive neurons within minutes, pointing to functional downregulation of KV1 channels as potentially triggering pathological cold activation. Collectively, we reveal that silent-cold sensing neurons contribute to cold allodynia in neuropathic pain and identify a molecular mechanism driving de novo cold sensitivity, in vivo
Profiling the proteome landscape of chronic pain – opportunities for mechanism-based research

Manuela Schmidt, Julia Sondermann, Allison Barry, David Gomez-Varela

Somatosensory Signaling and Systems Biology Group, Max Planck Institute of Experimental Medicine & University of Goettingen, Faculty of Biology and Psychology; Goettingen, Lower Saxony, Germany
Present Address AB: Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

Developing new therapeutic strategies to managing chronic pain will undoubtedly depend on a better understanding of the molecular mechanisms underlying chronic pain states. A prerequisite for such a mechanism-based approach is a thorough, systems level understanding of the complex and dynamic interplay of proteins and their networks contributing to chronic pain.

To date, extensive efforts have been invested to achieve this goal, in particular on the level of the genome and transcriptome. These studies have yielded important insights into genetic variants and transcriptome alterations relevant for chronic pain. In order to complement and further advance these research lines, we implemented cutting-edge proteomics technology to comprehensively profile proteome changes during chronic pain in mouse.

We revealed global differences in proteome dynamics across models of chronic pain, and in different regions of the mouse pain-axis. These data are summarized in a freely accessible online database (painproteome.em.mpg.de). In this way we provide the scientific community with a unique protein-centric systems biology framework, which facilitates mechanistic insights into somatosensory biology and chronic pain.

For example, in a proof-of-concept study we could show the functional relevance of vesicle transport through interaction with t-SNAREs homolog 1B (Vti1b) for TRPV1 pathology upon inflammation: Vti1b promotes TRPV1 sensitization specifically during inflammatory pain while capsaicin-induced nociceptive pain is unaffected. Moreover, in our current work we explore the involvement of a previously uncharacterized mitochondrial transmembrane protein in chronic pain. Strikingly, KO mice exhibit WT-like nociceptive pain, and a modality-specific phenotype in chronic pain models (CFA and spared-nerve injury, SNI), i.e. attenuation of tactile but normal thermal hypersensitivity.

Taken together, the discovery of novel candidate molecules implicated in chronic pain demonstrates the immense utility of exploring proteome dynamics in a region-resolved, pain model-dependent, and, eventually, longitudinal manner.

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Mechanically activated ion channels in touch and pain

Ardem Patapoutian
Scripps Research/HHMI, USA

Mechanotransduction is perhaps the last sensory modality not understood at the molecular level. Proteins/ion channels that sense mechanical force are postulated to play critical roles in sensing touch/pain (somatosensation), sound (hearing), shear stress (cardiovascular function), etc.; however, the identity of ion channels involved in sensing mechanical force has remained elusive. We identified PIEZO1 and PIEZO2, mechanically-activated cation channels that are expressed in many mechanosensitive cell types. We showed that PIEZO2 is the major transducer of mechanical forces for touch sensation in mice. I will discuss recent data showing that PIEZO2 is required for mechanical allodynia in mice and men. I will also describe our efforts to find novel mechanically activated ion channels.
Bench to Bedside in Pain Treatment: Following, Leading, or Misleading?

James Eisenach, MD

Basic science pain research seeks to identify psychological-social-biological mechanisms and new targets for treatment of pain in humans. One would not expect a high likelihood of translation, given the reductionist approach to much of basic science, species differences, and reliance on simplistic models of disease. Nonetheless, basic pain research has arguably usually followed clinical observations or misled understanding and only occasionally translated into clinically meaningful treatments. Below are examples of each:

Following: The original description and studies of presumed neuropathic pain after spinal nerve ligation in rodents showed effective treatment by sympathectomy, but poor efficacy of intrathecal opioids, similar to clinical experience at the time. Subsequent clinical studies flipped these results, with poor response of neuropathic pain to sympatholytic maneuvers but acute efficacy from intrathecal morphine. Remarkably, the rodent literature results also flipped following these clinical observations.

Leading: Several new chemical entities and biologics have been developed based on bench work suggesting a role for calcitonin gene related peptide, nerve growth factor, transient receptor for vanilloid-1, and nicotinic receptors, for example, in the treatment of pain. None of these are commonly used or did not reach the market, due in most cases to poor tolerability.

Misleading: Several approaches based on a wealth of preclinical studies failed to alter clinical pain in humans, most spectacularly glial inhibitors and neurokinin-1 antagonists. In our own work examining spinal analgesia, we replicated efficacy in animals of intrathecal cyclooxygenase inhibitors to reduce hypersensitivity after peripheral injury or inflammation, but failed to observe analgesia in humans with acute or chronic pain after intrathecal ketorolac, despite its efficacy to reduce prostaglandin concentrations in cerebrospinal fluid. Curiously, the role of cyclooxygenase inhibitors for pain treatment continues unabated in the laboratory.

Bench research can mislead us especially when it focuses on what is easy at the expense of what is relevant. We rely on hypersensitivity to mechanical stimuli after injury in rodents whereas this is relatively uncommon in patients with chronic pain. We focus on low threshold mechanoreceptive afferents whereas these become desensitized after surgery or chronic nerve injury. We focus on C-fibers but their role in chronic pain is unclear. We aim to study the transition from acute to chronic pain, but don’t use trajectory of recovery as an outcome measure in either animals or humans. Most importantly, poor scientific rigor and selective reporting are common in many leading laboratories, resulting in failure in replication by others and misleading drug development. Solutions to all of these problems are available or under development, but the culture of science in the field must change for them to be implemented.
CANCER-INDUCED BONE PAIN: UNDERSTANDING THE ROLE OF GLIAL CELLS AND THE P2X7 RECEPTOR

Anne-Marie Heegaard, Marta Díaz del Castillo, Camilla Appel, Sarah Falk, and Rie Bager Hansen
Department of Drug Design and Pharmacology, University of Copenhagen, Denmark

Pain remains one of the most feared and debilitating consequences of cancer. Pain from metastatic bone disease is the most common cause of cancer-related chronic pain, and it is a frequent complication in patients suffering from metastatic breast, lung, prostate or kidney cancer. Animal models of cancer-induced bone pain typically rely on inoculation of cancer cells into the marrow cavity of either the femur or tibia. Using these models, the behavioral phenotype and the nociceptive mechanisms of cancer-induced bone pain have been investigated. Data from our group and others suggest differential pain-related behaviors and nociceptive mechanisms in models of cancer-induced bone pain as compared to models of neuropathic pain and pain induced by proinflammatory adjuvants. In mouse models of neuropathic pain, ATP signaling via purinergic receptors such as the P2X3, P2X4 and P2X7 receptors seems to be involved in nociceptive signaling. Also, spinal microglia reaction has been shown to play an important role in the development of neuropathic pain. We have used a range of mouse and rat models to elucidate the involvement of the P2X7 receptor and spinal glial cells in cancer-induced bone pain. Contrary to neuropathic pain, targeted deletion of the P2X7 receptor was pro-nociceptive and pharmacological inhibition of the P2X7 receptor was found to be either pro-nociceptive or anti-nociceptive depending on the duration of inhibition. These data question the P2X7 receptor as a relevant therapeutic target in cancer-induced bone pain. As the P2X7 receptor is expressed in spinal microglia, we further investigated alterations of the spinal glial cells in mouse and rat models of cancer-induced bone pain. We found the presence of astrogliosis to depend on the specific model, and although other groups have published strong microglia reaction in rat models of cancer-induced bone pain, we found no evidence of microglia reaction in neither mouse nor rat models. The surgical technique, source of cancer cells and animal vendor are some of the factors that vary among laboratories and the results suggest a strong need for full and transparent reporting and standardization of the animal models of cancer-induced bone pain.
Escape the rodent trap: iPSC derived sensory neurons for pain research

Angelika Lampert, Barbara Namer, Jannis E. Meents, Diana Schmidt, Elisangela Bressan, Stephanie Sonntag, Alec Foerster, Esther Eberhardt, Michele Maroni, Petra Hautvast, Elena Dragicevic, Aaron Gerlach, Zhixin Lin, Jürgen Schüttler, Inge Petter Kleggetveit, Torhild Warncke, Ellen Jørum, Martin Zenke, Beate Winner

1Institute of Physiology, Uniklinik RWTH Aachen, 52074 Aachen, Germany
2Department of Cell Biology, Institute for Biomedical Engineering, Uniklinik RWTH Aachen, 52074 Aachen, Germany
3Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, 52074 Aachen, Germany
4Institute for Physiology and Pathophysiology, Friedrich-Alexander-University Erlangen-Nürnberg, 91054 Erlangen, Germany
5Division of Stem Cell Biology and Cellular Engineering, Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, 52074 Aachen, Germany
6Section of Clinical Neurophysiology, Department of Neurology, Oslo University Hospital-Rikshospitalet, 0424 Oslo, Norway
7Institute of Clinical Medicine, University of Oslo, 0318 Oslo, Norway
8Interdisciplinary Center for Clinical Research within the faculty of Medicine at the RWTH Aachen University, 52074 Aachen, Germany
9Department of Stem Cell Biology, Friedrich-Alexander-University Erlangen-Nürnberg, 91054 Erlangen, Germany
10IZKF Junior Research Group III, and BMBF Research Group Neuroscience, Friedrich-Alexander-University Erlangen-Nürnberg, 91054 Erlangen, Germany
11Department of Anesthesiology, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), 91054 Erlangen, Germany.
7Icagen, Durham, NC 27703, USA
9Nanion Technologies GmbH, 80636 Munich, Germany

A major challenge in human pain research is the translational gap - findings from mice are not easily translated to humans. Therefore, we established a human induced stem cell (iPS-cell) based approach to differentiate nociceptors of patients with genetic pain syndromes. This system allows us to study the role of voltage gated sodium channels, e.g. Nav1.7, in action potential firing. Using patch-clamp electrophysiology, we find that Nav1.7 is not active during subthreshold depolarizations, but that its activity defines the action potential threshold and contributes significantly to the action potential upstroke.

Ultimately, our goal is to identify personalized medicine for individual, treatment-resistant patients using iPSC derived human nociceptors. We report an example of in-vitro predicted individualized treatment success in a Caucasian patient suffering from severe small fiber neuropathy (SFN) with a genetic variant in Nav1.9. Using iPSC-derived patient nociceptors, we tested an FDA-approved compound, lacosamide, which abolished the disease specific phenotype in-vitro. The patient responded promptly with significant pain relief. Thus, this stem-cell based approach ended a history of 10-years of severe pain for the patient.
Genome-wide Association Study of Multisite Chronic Pain in UK Biobank

Keira J. A. Johnston, Mark J. Adams4, Barbara I. Nicholl1, Joey Ward1, Rona J. Strawbridge1,5, Amy Ferguson1, Andrew McIntosh4, Mark E.S. Bailey3, Daniel J. Smith1.

1Institute of Health and Wellbeing, University of Glasgow, Scotland, UK
2Deanery of Molecular, Genetic and Population Health Sciences, College of Medicine and Veterinary Medicine, University of Edinburgh, Scotland, UK
3School of Life Sciences, College of Medical, Veterinary & Life Sciences, University of Glasgow, Scotland, UK
4Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Scotland, UK
5Department of Medicine Solna, Karolinska Institute, Stockholm, Sweden

Chronic pain is highly prevalent worldwide, contributing a significant socioeconomic and public health burden. Several aspects of chronic pain, for example back pain and a severity-related phenotype, chronic pain grade, have been shown to be complex, heritable traits with a polygenic component. Additional pain-related phenotypes capturing aspects of an individual's overall sensitivity to experiencing and reporting chronic pain have also been suggested. We have here made use of a measure of the number of sites of chronic pain in individuals within the general UK population. This measure, termed Multisite Chronic Pain (MCP), is also a complex trait, but its genetic architecture has not previously been investigated. To address this, a large-scale genome-wide association study (GWAS) of MCP was carried out in ~380,000 UK Biobank participants to identify associated genetic variants. Findings were consistent with MCP having a significant polygenic component with a SNP heritability of 10.2%, and 76 independent lead single nucleotide polymorphisms (SNPs) at 39 risk loci were identified. Additional gene-level association analyses identified neurogenesis, synaptic plasticity, nervous system development, cell-cycle progression and apoptosis genes as being enriched for genetic association with MCP. Genetic correlations were observed between MCP and a range of psychiatric, autoimmune and anthropometric traits including major depressive disorder (MDD), asthma and BMI. Furthermore, in Mendelian randomisation (MR) analyses a bi-directional causal relationship was observed between MCP and MDD. A polygenic risk score (PRS) for MCP was found to significantly predict chronic widespread pain (pain all over the body), indicating the existence of genetic variants contributing to both of these pain phenotypes. These findings support the proposition that chronic pain involves a strong nervous system component and have implications for our understanding of the physiology of chronic pain and for the development of novel treatment strategies.
Poster Presentations

Promiscuous GPCR Modulation of TRPM3: A Novel Analgesic Pathway?

Omar Alkhatib, Robson Costa, Stuart Bevan and David Andersson

Wolfson Centre for Age-Related Diseases, King’s College London and School of Pharmacy, Universidade Federal do Rio de Janeiro

We have recently shown that transient receptor potential melastatin 3 (TRPM3), a non-selective cation channel that is involved in nociception and inflammatory pain, can be modulated by Gβγ subunits liberated from Goi/o protein-coupled receptors (Badheka et al., 2017; Dembla et al., 2017; Quallo et al., 2017). Here, we demonstrate that TRPM3 channels can also be modulated by Gβγ released from Gαs and Gαq subunits. We used both patch clamp electrophysiology and calcium imaging to observe the effect of GPCR activation on TRPM3-mediated responses in both human embryonic kidney (HEK293) cells and mouse dorsal root ganglia (DRG) neurons. Pharmacological and molecular techniques were utilised to determine the responsible mechanism for GPCR-mediated inhibition of TRPM3. To test the physiological relevance of our findings, we examined whether GPCR agonist intraplantar administration in mice was able to modulate TRPM3-mediated nociception. The activity of heterologously expressed TRPM3 in human embryonic kidney (HEK293) cells was inhibited by activation of Gαs-coupled adenosine 2B receptors (A2B) and Gαq-coupled protease-activated receptor 2 (PAR2). Inhibition by PAR2 activation did not rely on PtdIns(4,5)P2 hydrolysis. A2B- and PAR2-mediated inhibition of TRPM3 activity was reversed when the Gβγ sequestering tool (C-terminus of the β adrenergic receptor kinase 1) was co-expressed with TRPM3 in HEK293 cells. In isolated mouse dorsal root ganglia (DRG) neurons, TRPM3 activity was inhibited by activation of the Gαs-coupled prostaglandin EP2 and the Gαq-coupled bradykinin B2 (BK2) receptors. Preincubation with the Gi/o inhibitor (pertussis toxin) had no effect on EP2- and BK2-mediated inhibition of TRPM3. Additionally, this effect is not dependent on protein kinase A or C activity. Gs and Gq modulation of TRPM3 was also shown in vivo where EP2 and BK2 receptor agonists inhibited pain induced by TRPM3 agonists.

Our results demonstrate that TRPM3 is inhibited by Gβγ following activation of Gi/o-, Gq- and Gs-coupled GPCRs. This modulatory mechanism can potentially be utilised for the development of novel analgesic rationales and identifies TRPM3 as a promiscuous effector for GPCR signalling.
Relationship between NGF/TrkA axis and ADRA2b in osteoarthritic knee pain

Juan Carlos Arévalo, S. Lisa1, L. Calvo2, J. Valente-Sousa3, C. Vicente-Garcia1, I. Arisi4, M. D’Onofrio4, M. Malcangio5

1 Institute for Neuroscience Castilla and Leon (INCyL), Department of Cell Biology and Patology, University of Salamanca, Salamanca, Spain.
2 Unit of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.
3 Vascular Biology and Inflammation Section, Cardiovascular School of Medicine and Science, British Heart Foundation Centre of Excellence, King’s College London, London, United Kingdom.
4 European Brain Research Institute-Fondazione Rita Levi Montalcini, Roma.
5 Wolfson Centre for Age-Related Diseases, King’s College London, London, United Kingdom.

Acute pain is an unpleasant but necessary sensation for survival, but when it becomes chronic is a major social, health and economical burden. Osteoarthritis (OA), a degenerative joint disease, is nowadays responsible for a large percentage of chronic pain observed in humans. Nerve growth factor (NGF) and its receptor, TrkA, play an important role in the generation of acute and chronic pain and blocking NGF alleviates pain symptoms of knee OA patients. To identify genes downstream of NGF/TrkA axis responsible for the enhanced mechanical sensitivity in OA, we have used a mouse expressing a mutant TrkA that develops mechanical hypersensitivity compared to wild type (WT) mice in the monoiodoacetate (MIA) model of OA. We have carried out gene expression analysis from WT and KI ipsilateral L3-5 DRGs collected at early pain phase, 5 days post-saline or MIA injection. One of the most significant genes altered in the microarrays and qPCR analysis was ADRA2B, which belongs to the α2 adrenergic receptor family, whereas ADRA2A and ADRA2C, the other two members of the family, showed no differences in gene expression. At the protein level, ADRA2B was upregulated exclusively in the ipsilateral DRGs of mice in response to MIA. In addition, blocking ADRA2B, with the specific inhibitor Imiloxan, enhanced the mechanical sensitivity in MIA-injected mice in the ipsilateral paw. Finally, in vitro studies showed that ADRA2B expression regulates specifically TrkA expression. Altogether these results support the idea that ADRA2B participates together with NGF/TrkA axis in a functional network that may play a relevant role in pain modulation in osteoarthritis.
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 recherche cliniques de Montréal, Montréal, QC, Canada
2 Integrated Prog. in Neuroscience, McGill University, Montréal, QC, Canada
3 Salk Institute, San Diego, CA, USA

Nociception relies on the appropriate 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 sensory inputs to the cortex where an appropriate reactionary motor response is computed. This pathway may be involved in the processing of noxious stimulus intensity and location, and spinothalamic (ST) neurons are central to its organisation. However, little is known about ST developmental organisation and relative contribution to nociception.

Here, we demonstrate that ST neurons arise from multiple developmental lineages and migrate to populate distinct dorsoventral 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 cardinal group that expresses 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. The central endings of primary nociceptors form appositions on Lmx1b ST neurons. In contrast, Sim1 ST neurons coincide with proprioceptive inputs. Using a recombinase intersection approach coupled with virally-driven circuit labelling, we further show that Lmx1b and Sim1 ST neurons display regional innervation biases in the thalamus. Collectively, we propose that the developmental origin of spinothalamic neurons defines their functional role in the relay of nociceptive, tactile and proprioceptive inputs for thalamic integration.
Effects of modulating the Nav 1.8 conductance in human DRG neurons depends on baseline ion channel densities: a computational study

Oliver J. Britton¹, Blanca Rodriguez¹

¹. Department of Computer Science, University of Oxford

The sodium channel Nav 1.8 is selectively expressed in small diameter DRG neurons and has an important role in pain signalling. Gain-of-function mutations in Nav 1.8 contribute to painful peripheral neuropathy, and Nav 1.8 has been shown to support repetitive firing and the characteristic broad shoulder of DRG neuron action potentials.

The effect of an ion channel on neuronal electrophysiology is dependent on the background ensemble of other ion channels expressed in the neuron. A demonstration of this principle was provided by Rush et al. (PNAS, 2006), who showed that a gain-of-function Nav 1.7 mutation caused hyper-excitability in DRG neurons with Nav 1.8 and hypo-excitability in sympathetic ganglion neurons without Nav 1.8. However, understanding how multiple ionic currents will affect one another is challenging. This is further complicated by inter-neuronal variability in ion channel densities, which will cause different neurons to respond differently to the same experimental conditions, e.g. application of the same concentration of a drug.

In this study we constructed a population of 224 computational human DRG neuron models that integrate data from electrophysiological recordings of human DRG neurons at the cellular and ion channel levels. Each model in the population shares the same underlying kinetics for seven ionic currents, but has a different set of ion channel densities, reflecting inter-neuronal heterogeneity. Importantly, every model in the population produces action potential behaviour in range with the experimental variability reported in human DRG neurons by Davidson et al. (PAIN, 2014) when simulated with equivalent protocols and measured with eight action potential biomarkers.

We investigated the effects of modulating the Nav 1.8 conductance on neuronal excitability in the context of ion channel heterogeneity by varying the Nav 1.8 conductance in all models and analysing the effects this had on action potential firing rate, width and firing pattern. We found that the baseline Nav 1.8 conductance in each model is highly correlated with its delayed rectifier potassium conductance and that models with low values of both conductances tend to develop repetitive spiking at lower stimulus amplitudes than models with high values of both conductances, although a minimum level of Nav 1.8 conductance is required for repetitive firing to occur at all.
In silico identification and electrophysiological characterisation of novel acid-sensing ion channel 3 (ASIC3) modulators.


Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1PD, UK.

Acid-sensing ion channels (ASICs) are voltage-independent cation channels activated by extracellular protons. They are involved in pain, fear, learning, and neurodegeneration after ischemic stroke. Of all the ASIC subunits, ASIC3 has been suggested as the key sensor of acid-induced pain and has also been shown to have a pivotal in different models of inflammatory pain including models of rheumatoid arthritis and osteoarthritis. Therefore, the identification of new ASIC3 modulators and the mechanistic understanding of how these modulators modulate ASIC3 could be important for the development of new strategies to counteract the detrimental effects of dysregulated ASIC3 activity in inflammatory conditions. Here, we report the identification of novel ASIC3 modulators based on the ASIC3 specific agonist, 2-guanidine-4-methylquinazoline (GMQ) and the ASIC3 specific inhibitory toxin APETx2. Through an in silico ligand (here, GMQ)-guided screening of FDA-approved drugs, we selected 6 different compounds and tested them against ASIC3 using whole-cell recording. Of the chosen drugs, guanabenz, an α2-adrenoceptor selective agonist, produced similar effects to GMQ on ASIC3, activating the channel at neutral pH and potentiating its response to mild acidic stimuli. Sephin1 is a guanabenz derivative that lacks α2-adrenoceptor activity and has been proposed to act as a selective inhibitor of a regulatory subunit of the stress-induced protein phosphatase 1 (PPP1R15A). However, we found that like guanabenz, sephin1 activates ASIC3 at neutral pH and potentiates its response to acidic stimulation, i.e. sephin1 is a novel modulator of ASIC3. Besides this ligand-guided approach, we also modelled the interaction of the selective peptide inhibitor of ASIC3 APETx2 and predicted two regions of the APETx2 sequence that mediate interaction with ASIC3. Based on this, we designed peptides predicted to bind to the ASIC3-APETx2 binding sites and identified a peptide that inhibits the transient component of ASIC3 current whilst also dramatically increasing its inactivation time constant; a control peptide where proposed ASIC3-binding residues have been mutated to alanine had no such effect. Overall, these data demonstrate the utility of computational analysis for identifying novel ASIC3 modulators, which can be validated with electrophysiological analysis and may lead to the development of better compounds for targeting ASIC3 in the treatment of arthritic conditions.
Human spinal cord injury (SCI) is associated with chronic pain that is refractory to medical therapy. Human induced pluripotent stem cells (iPSCs) derived neural progenitor cells (hiNPCs) show promise as a regenerative therapy for SCI. While these findings are exciting, it’s unclear whether neural repair strategies for SCI might inadvertently cause or exacerbate post SCI-pain. We hypothesized that hiNPCs implanted into severely lesioned spinal cord will modify post lesion molecular and sprouting responses to attenuate chronic pain. We utilized high thoracic and severe SCI to better model and understand mechanisms associated with post SCI-pain. We grafted hiNPCs expressing GFP into the SCI lesion site one week after severe T3 compression in immunodeficient rats. Severe T3 compression caused substantial loss of motor function in both hiNPC and no cell grafted (control) groups within two weeks (BBB <2). The control group maintained a BBB score <6 for the duration of the study (4 months). In contrast, rats grafted with hiNPCs demonstrated consistent and significant motor improvement by 16 weeks (*p<0.01; BBB 15). Grafted hiNPCs survived, filled the lesion cavity and showed evidence of neuronal maturation. Cell fate mapping revealed a large number of βIII tubulin positive axons emerging from the lesion site and extending to the T7 spinal segment. Triple-label immunofluorescence revealed motor (ChAT, MNX1) and sensory (Lbx1) neuron phenotypes in the lesioned area. All rat groups developed pain related behaviors within one week that included increased spontaneous lifts, tactile allodynia and cold sensitivity. Place escape avoidance testing confirmed that the stimulus represented active pain related aversion. Gabapentin attenuated both spontaneous and evoked pain. However, rats grafted with hiNPCs showed no worsening or improvement in pain outcomes over 4 months. Notably, RNAseq of DRGs from control and hiNPC groups revealed differential expression of pain related genes. The potassium channel related gene, KCNN1 and neurotransmitter, VIP, were unchanged after 8 weeks in grafted hiNPCs rats, but KCNN1 was down regulated (*p<0.05), and VIP was upregulated (*p<0.05) by 16 weeks compared to controls. Thus, although functional pain outcomes appeared unchanged by 16 weeks, genes potentially involved in restoring sensation were regulated in DRGs. Collectively, these data are important for optimizing grafting approaches and assessing safety in translating hiNPC for patient use with SCI.
Dorsal Root Ganglion Neuromodulation as an Alternative to Opioids in the Evolving Healthcare Crisis

Adam J Carinci,
University of Rochester School of Medicine and Dentistry

Background: The opioid epidemic is the most pressing healthcare crisis of our time. There is increasing recognition that opioids have limited long-term efficacy and are associated with hyperalgesia, addiction and increased morbidity and mortality. Therefore, alternative strategies to combat chronic pain are paramount. We initiated a multicenter retrospective case series to review the efficacy of DRG stimulation in facilitating opioid tapering, opioid discontinuation and as a viable alternative to chronic opioid therapy.

Purpose: The dorsal root ganglion (DRG) plays a key role in the development and maintenance of pain. Recent innovations in neuromodulation, specifically, dorsal root ganglion stimulation, offers an effective alternative to opioids in the treatment of chronic pain. A Retrospective case series demonstrates preliminary evidence that DRG stimulation facilitates opioid tapering, opioid discontinuation and presents a viable alternative to chronic opioid therapy.

Procedure: This small multicenter retrospective case series provides preliminary evidence that DRG stimulation facilitates opioid weaning, opioid tapering and is a viable option to opioid therapy in the treatment of chronic pain. A retrospective analysis was completed. Visual analog scale pain scores and pain medication usage were collected at the baseline visit and after four weeks, 3 months and 6 months of treatment. Ten consecutive patients across two study centers were included. The pain was rated 7.38 at baseline and decreased to 1.50 at the 4-week follow-up, a reduction of 79.5%. All patients significantly decreased their opioid pain medication use with an average >30% reduction in morphine equivalents and four were able to discontinue their medications entirely.

Conclusion: This Retrospective case series demonstrates preliminary evidence that DRG stimulation facilitates opioid tapering, opioid discontinuation and presents a viable alternative to chronic opioid therapy. Goals / Learning Objectives:
To discuss and describe the role and mechanism of action of DRG neuromodulation and present preliminary outcome data supporting DRG stimulation in facilitating opioid tapering, opioid discontinuation and as a viable alternative to chronic opioid therapy.
In-vitro Inflammatory Knee Pain: Of Mice and Men

Sampurna Chakrabarti, Luke A. Pattison1, Kaajal Singhal1, David C. Bulmer1, Deepak R. Jadon2, Ewan St. John Smith1

1Department of Pharmacology, 2Department of Medicine, University of Cambridge, United Kingdom.

The ongoing pain associated with arthritis reduces the quality of human life. Upon injection of complete Freund's adjuvant (CFA) into the knee of mice, we observed knee inflammation and a concomitant decrease in their natural digging behavior within 24-hours. To understand the neural basis of this behavioral deficit, we performed retrograde tracing to label knee-innervating dorsal root ganglion neurons (knee neurons) and recorded their electrical and chemical sensitivity using whole-cell patch-clamp. After inflammation, knee neurons showed a decreased threshold of action potential generation and increased transient receptor potential vanilloid-1 (TRPV1) expression. Subsequently, administration of a TRPV1 antagonist normalized mouse digging behavior, likely due to inhibition of pain signaling. To test the translational potential of these results, we recorded from mouse sensory neurons incubated overnight with osteoarthritic (OA) synovial fluid from human patients with chronic pain. We find that OA synovial fluid directly sensitizes mouse knee neurons by decreasing the action potential threshold and increasing the resting membrane potential. Using Ca2+ imaging we also find that OA synovial fluid acutely activates mouse sensory neurons, but that overnight incubation does not increase the proportion of TRPV1-positive neurons. Taken together, our data suggests synovial fluid/neuron systems can be used as an in-vitro translational model to study inflammatory joint pain.
A role for TRPM3 channel in osteoarthritis pain

Fabiana C. Dias1,2, Robson da Costa1,2,3; Clive Gentry1; Stephan Philipp 4, David A. Andersson1, Stuart Bevan1

(1) Wolfson Centre for Age Related Diseases, King’s College London, London, UK. (2) School of Pharmacy, UFRJ, Rio de Janeiro, Brazil. (3) Newton International Fellow, The Royal Society, UK. (4) Institute for Experimental & Clinical Pharmacology and Toxicology, Universität des Saarlandes, Germany

Introduction: Osteoarthritis (OA) pain is poorly treated by existing drugs and new analgesic therapies are needed. TRPM3 is a non-selective cation channel that is expressed in peripheral sensory neurons and its activation in vivo evokes nociception in mice. Thus, inhibition of TRPM3 is a potential tool for OA pain relief. The current study used a combination of genetic and pharmacological approaches to determine the role of TRPM3 in joint pain using two different animal models. Methods: Female C57BL/6N wild-type (WT) and TRPM3 knockout (Trpm3-/-) mice (8-10 weeks old) were subjected to model of joint pain induced by intra-articular injection of monosodium iodoacetate (MIA) (0.5 mg/ site) into the knee. Pain was assessed by measuring the differential weight bearing on the affected and unaffected limbs and quantifying the number of vocalizations to joint manipulation. Referred hypersensitivities to mechanical (paw pressure) and cold (cold plate 10°C) stimuli applied to the paw were also assessed. After the end of behavioural assessments, joint knees were dissected and submitted to micro CT and histological analysis. The pharmacological effects of ononetin (10 mg/kg, i.p.), a selective TRPM3 antagonist, were also assessed in WT mice in either the MIA joint pain model or a meniscectomy surgical model of OA pain. Results: In the MIA model, pain behaviours and referred hypersensitivities developed in WT mice within the first week and were maintained for four weeks, whereas no pain behaviours or hypersensitivities were observed in the Trpm3-/- mice over this period. With micro CT analysis we found a decrease in bone mineral density in MIA-injected WT mice but not in Trpm3-/- mice. Toluidine blue and safranin-O staining of section from mouse knees revealed histological differences between MIA-injected WT and Trpm3-/- mice. Importantly, treatment with ononetin reversed established mechanical (paw pressure) and cold hypersensitivities (cold plate) in both the MIA and meniscectomy OA models in WT mice. Conclusion: We conclude that TRPM3 has a key role in OA pain in these experimental models, suggesting that inhibitors of TRPM3 could be therapeutic agents for the treatment of chronic joint pain.
INVESTIGATING THE PATHOPHYSIOLOGY OF HEREDITARY SENSORY NEUROPATHY TYPE 1 (HSN1) USING HUMAN IPSC-DERIVED SENSORY NEURONS

Alex Clark, Maiya Kugathasan, Iulia Blesneac, Mary Reilly, David Bennett

Neural Injury Group, Nuffield Department of Clinical Neurosciences, University of Oxford

HSN1 is a slowly progressive sensory neuropathy leading to profound sensory loss and lancinating pain. HSN1 is most commonly caused by mutations in the genes SPTLC1/2. These encode subunits of the Serine Palmitoyltransferase (SPT) enzyme, required for the biosynthesis of complex sphingolipids. Mutations in these genes alter the substrate specificity of SPT leading to the synthesis of atypical metabolites called deoxysphingolipids (dSLs). Plasma levels of dSLs are raised in HSN1 patients, and are toxic when exogenously applied to mammalian DRG cultures. However, the mechanism of dSL neurotoxicity is unknown. Therefore, we have investigated the pathogenicity of elevated dSLs using human sensory neurons derived from induced pluripotent stem cells (iPSCs) from HSN1 patients.

HSN1 patient sensory neurons were all found to autonomously produce elevated dSLs. Electrophysiological examination revealed patient neurons were hyperexcitable with increased repetitive firing in response to current injection. Elevated expression of the axonal injury marker - ATF3 is observed in mature neurons, as well as increased expression of the apoptotic protein - Caspase III. To study the pathophysiology of dSL accumulation in human sensory neurons we investigated the expression of phosphorylated ERM proteins, which are involved in neurotrophic signalling. We found membrane expression was almost completely absent in HSN1 neurons. Furthermore, analysis of lipid rafts has revealed a profound reduction in patient neurons, with an associated reduction of the p75 neurotrophic receptor. Neurite outgrowth is decreased in patient neurons, however this can be dramatically improved with L-Serine supplementation (the normal substrate for the SPT enzyme). Using a myelinating coculture technique developed in lab, we found that myelination is disturbed in HSN1 neurons with an overall reduction in myelin formation, abundant myelin blebbing and fragmentation of internodes. This phenotype can also be rescued with the supplementation of L-Serine to the cultures.

We have for the first time, demonstrated that human iPSC-derived sensory neurons can be used as an in vitro model for HSN1. We are beginning to unravel a novel pathophysiological mechanism for the neurotoxicity associated with dSL accumulation, which appears to relate to impaired neurotrophic signalling. Furthermore, we have shown we can reverse certain aspects of the neuropathology in vitro, demonstrating the ability to test potential therapeutic agents in our model system.
The selective TRPV4 channel antagonist HC-067047 attenuates mechanical allodynia in diabetic mice

Robson Costa1,2., Fabiana C. Dias1,2, Vinicius S. Alves1, Claudia P. Figueiredo1,2, Ana Luisa P. Miranda1,2, Giselle F. Passos1

1Faculdade de Farmácia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil. 2Programa de Pós-graduação em Ciências Farmacêuticas, UFRJ, Rio de Janeiro, Brazil.

Painful diabetic neuropathy (PDN) is a serious symptom that compromises the patient’s quality of life and remains without effective pharmacological treatment. The transient receptor vanilloid 4 (TRPV4) is a cation-permeable channel implicated in sensory transduction and pain signalling. Therefore, drugs that act on TRPV4 may have therapeutic applications to treat PDN. In the present work, we assessed the effect of the selective TRPV4 channel antagonist HC-067047 on painful neuropathy associated to streptozotocin (STZ)-induced diabetes in mice. STZ-treated animals presented both mechanical and cold allodynia at 6 weeks after diabetes induction. Notably, HC-067047 (1 mg/kg, s.c.) given daily between 2 and 6 weeks after diabetes induction significantly prevented the development of mechanical allodynia. Additionally, both single and repeated treatments with HC-067047 (10 mg/kg, s.c.) significantly reversed established mechanical allodynia induced by STZ. However, HC-067047 was not capable of affecting neither thermal cold allodynia nor increased blood glucose concentration. Similarly, HC-067047 treatments did not alter body weight, temperature, locomotor activity or motor coordination of normal mice. Western blotting and immunohistochemistry assays showed that TRPV4 expression was not different in sciatic nerve, DRG, spinal cord or hind paw plantar skin from diabetic and non-diabetic mice, suggesting that HC-067047 acts on constitutive receptors to inhibit mechanical allodynia. Taken together, the data generated in the present study show the potential relevance of using TRPV4 antagonists to treat painful neuropathy associated with diabetes.
Passive Transfer of CRPS Pain from Patient to Mouse

Ulku Cuhadar (1), Clive Gentry (1), Nisha Vastani (1), Andreas Goebel (2) and David Andersson (1)

(1) Wolfson CARD, King’s College London (2) Liverpool University

Complex Regional Pain Syndrome (CRPS) is a severe, usually post-traumatic, chronic pain condition confined to one limb, with unknown neurophysiological basis. Removal of IgG by plasma exchange produces a marked pain reduction in a subset of patients, indicating that CRPS is an autoimmune condition. The objective of our study is to determine the neurophysiological mechanisms responsible for pain and hypersensitivity in an IgG passive transfer/trauma model of CRPS.

IgG from CRPS patients or HC subjects was administered to female mice on 4 consecutive days. On the second day, mice were subjected to a plantar skin-muscle incision in one hind paw. Behavioural tests of mechanical and thermal nociception were performed daily on the injured and uninjured hind paws. On day 5, skin-saphenous nerve dissection was performed, and in vitro electrophysiological recording used to measure nerve fibre activity. Administration of IgG from CRPS patients, but not from HC, exacerbated and prolonged the mechanical and thermal hypersensitivities produced by a paw incision. Interestingly, in vitro electrophysiological recordings showed a long lasting, high frequency ectopic firing in CRPS-IgG treated mice, indicative of spontaneous pain. In vitro recordings in the same mice also revealed an increased impulse rate of Aδ- and C-mechano-nociceptors to mechanical stimulation, when compared to control groups.

Our results establish the IgG passive transfer/trauma model of CRPS as a translational model of excellent face and construct validity. Finally, electrophysiological recordings identify an enhanced ectopic and evoked impulse rate in nociceptive afferents as a cause of CRPS pain.
Natural killer cells degenerate partially injured axons to aid recovery from painful nerve injury

Alexander J. Davies1,10*, Hyoung Woo Kim2, Rafael Gonzalez-Cano3, Jahyang Choi1, Seung Keun Back4, Seung Eon Roh5, Errin Johnson6, Melanie Gabria6, Mi-Sun Kim1, Jaehee Lee4, Jeong Eun Lee4, Yun Sook Kim8, Yong Chul Bae8, Sang Jeong Kim7, Kyung-Mi Lee4, Heung Sik Na4, Priscilla Riva3, Alban Latremoliere9, Simon Rinaldi10, Sophie Ugolini7, Michael Costigan3, Seog Bae Oh1,2

1 Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul 03080, Republic of Korea.
2 Department of Brain and Cognitive Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Republic of Korea.
3 Departments of Anesthesia and Neurobiology, Children’s Hospital Boston and Harvard Medical School, Boston, MA 02115 USA.
4 Departments of Physiology, Biochemistry and Molecular Biology, College of Medicine, Korea University, Seoul 02841, Republic of Korea.
5 Department of Physiology, Seoul National University College of Medicine, 03087, Seoul, Republic of Korea.
6 Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, United Kingdom.
7 Centre d'Immunologie de Marseille-Luminy, Aix Marseille Univ, CNRS, INSERM, 13288 Marseille, France.
8 Department of Anatomy and Neurobiology, School of Dentistry, Kyungpook National University, Daegu 700-412, Korea.
9 Neurosurgery Department, Johns Hopkins School of Medicine, Baltimore, MD 21287 USA.
10 Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom.

The immune response to peripheral nerve injury is critical for the adaptive mechanisms of axonal regeneration but may also trigger maladaptive changes leading to neuropathic pain. Understanding the roles of immune cell populations in one or other of these processes is therefore likely to help in the resolution of pain after nerve injury. Sensory axons degenerate following separation from their cell body but incomplete injury to peripheral nerves may leave the integrity of damaged axons preserved leading to ongoing neuropathy. Using in vitro and in vivo approaches we show that an endogenous ligand for the Natural Killer (NK) cell receptor NKG2D, Retinoic Acid Early protein 1 (RAE1), is re-expressed in adult dorsal root ganglion neurons following nerve peripheral injury triggering selective degeneration of injured axons. Infiltration of cytotoxic NK cells into the sciatic nerve by extravasation occurs within three days following crush injury. Using a combination of genetic cell ablation and cytokine-antibody complex stimulation we show that NK cell function correlates with an acute loss of sensation due to degeneration of injured afferents within the injured sciatic nerve without effecting long-term functional regeneration. This mechanism of selective NK cell-mediated degeneration of damaged but intact sensory axons, via a genetically encoded neuro-immune ligand-receptor interaction, complements Wallerian degeneration and suggests the therapeutic potential of modulating NK cell function to resolve sensory neuropathy through the clearance of partially damaged nerves.
The Role of RorB-expressing Lamina II Interneurons in Gating Nociceptive C-fibre Input

Olivia Davis (1), Allen Dickie (1), Kieran Boyle (1), Marami Mustapa (1), Kelly Smith (2), Robert Callister (2), Brett Graham (2), Andrew Todd (1), David Hughes (1)

1: University of Glasgow, Glasgow, United Kingdom; 2: University of Newcastle, Callaghan, Australia

Inhibitory interneurons in the spinal dorsal horn play a crucial role in controlling transmission of somatosensory information from the periphery to the brain. Spinal inhibition is diminished in some chronic pain states, and inhibitory interneurons therefore represent a potential target for therapeutic intervention. The organisation of synaptic circuitry through which inhibitory interneurons modulate the transmission of sensory information is poorly understood, largely due to the difficulty in identifying distinct interneuron populations. We have found a population of lamina II inhibitory interneurons that co-express both the calcium binding protein calretinin (CR) and the RAR-related orphan receptor beta (RorB). The dendritic arbor of these cells overlaps directly with the central arbors of C-fibre mechano-nociceptors that are defined by their expression of CMrgD or capacity to bind the lectin IB4. We have used a combination of anatomical and electrophysiological approaches to show that the dendrites of the RorB interneurons receive synaptic inputs from CMrgD afferents, whilst their axon terminals form axo-axonic synapses onto the central terminals of Type I glomeruli, which are derived from these afferents. Furthermore, peripheral stimulation conducted in vivo under terminal anaesthesia shows that the RorB cells are preferentially activated by noxious mechanical stimulation of skin, rather than noxious chemical stimulation (capsaicin) or noxious heat (water at 52°C). We therefore believe these interneurons play a critical role in setting mechanical pain thresholds.
Investigating the Role of Nav1.8 in Action Potential Propagation in Nociceptors Using the Selective Blocker PF-01247324

Fahm Deen, Martin Koltzenburg

Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom

The tetrodotoxin (TTX) resistant Nav1.8 is preferentially expressed in thin myelinated and unmyelinated nociceptors and generates the majority of depolarising inward current during an action potential (AP). The expression of Nav1.8 is upregulated in rodent models of inflammatory & neuropathic pain and genetic inactivation/knockdown of Nav1.8 have shown analgesic effects. Here, we asked using PF-01247324, a structurally novel blocker of Nav1.8, whether pharmacological blockade of Nav1.8 influences AP propagation in peripheral nociceptors.

In vitro skin-saphenous nerve preparation or sciatic nerve segments from C57Bl/6 mice or Wistar rats were used. Nerves were dissected rapidly and mounted in an organ bath superfused with oxygenated synthetic interstitial fluid. The nerve was stimulated electrically and the A- and C-fibre compound action potential (CAP) was recorded. Drugs were applied to a metal reservoir placed on the nerve between the recording and stimulation site. 10µM PF-01247324 had little, non-differential effect on A- and C-fibre CAP. We hypothesised that the perineurium was the diffusion barrier and carried out experiments on mechanically desheathed nerves. In desheathed mice sciatic-tibial nerve preparations, 1µM PF-01247324 had no effect whereas 10µM decreased A- and C-fibre CAP by 36% and 48%. Although the application of 100µM PF-01247324 had little, non-significant additive effect, changes in conduction velocities were observed which is consistent with Nav blockade. Unlike non-desheathed nerves, 1µM TTX abolished the A- and C- responses completely in all desheathed nerves and was used as a positive control.

Next, we investigated the effect of PF-01247324 on desheathed rat saphenous nerves. 1µM PF-01247324 inhibited A-fibres by 17% but higher concentrations (10 and 100µM) showed no additive effect. Interestingly, C-fibres showed a similar response at 1 and 10µM, but an additional inhibition of 9% at 100µM. In the final set of experiments, we used the highest concentration of the drug (100µM) to investigate the frequency dependant blockade of AP. Compared to baseline (0.25Hz) 10, 50 or 100 Hz stimulation did not show any additional block in A-fibre CAP. Interestingly, 1 Hz stimulation revealed a frequency dependant block (10% inhibition; baseline-0.25Hz vs 1Hz) in C-fibres CAP, however, higher frequency stimulations failed to show significant inhibition.

PF-01247324 attenuates but does not abolish AP propagation in rodent A- or C-fibres. The lack of differential action on A- and C-fibre suggests that the effects are not selective for Nav1.8 but may act on other subtypes. It raises concerns that blocking Nav1.8 may have limited therapeutic advantages over non-selective Nav blockers.
DIFFERENTIAL PAIN-RELATED BEHAVIORS IN IMMUNOCOMPETENT MOUSE MODELS OF MULTIPLE MYELOMA

Marta Diaz-delCastillo, Marta Diaz-delCastillo1, Danna Kamstrup1, Rikke Brix Olsen1, Rie Bager Hansen1, Thomas Pembridge2,3, Brigita Simanskaite2,3, Juan Miguel Jiménez-Andrade4, Michelle Anne Lawson2,3, Anne-Marie Heegaard1*

1 Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Jagtvej 160, Copenhagen Ø DK-2100, Denmark
2 Department of Oncology & Metabolism, University of Sheffield, Sheffield, S10 2RX, United Kingdom.
3 Mellanby Centre for Bone Research, University of Sheffield, Sheffield, S10 2RX, United Kingdom.
4 University Autonomus of Tamaulipas, Campus Reynosa Aztlán, Reynosa Tamaulipas, México.

Bone pain is a serious and debilitating symptom of multiple myeloma (MM) that impairs the quality of life of patients. The underlying mechanisms of myeloma-induced bone pain are unknown and understudied, as there is a lack for immunocompetent preclinical models that allow further investigations of this condition. We hypothesized that a widely used syngeneic model of MM, established by systemic inoculation of 5TGM1-GFP myeloma cells in the tail vein of immunocompetent C57BL/KaLwRijHsd (BKAL) mice, would present pain-related behaviors. Disease phenotype was confirmed by splenomegaly and tumor infiltration in the bone marrow of the hind limbs; however, myeloma-bearing mice did not present pain-related behaviors or substantial bone disease. Thus, we investigated an alternative model in which 5TGM1-GFP cells were directly inoculated into the intrafemoral medullary cavity of BKAL mice. This localized myeloma model presented the hallmarks of the disease, including tumor growth and osteolytic bone lesions. Additionally, the localized model of MM displayed a pain-like phenotype, which could be reversed by morphine (p<0.05 30 and 60 min after morphine administration, p<0.01 120 min after). Furthermore, intrafemorally injected mice presented a profound denervation of the myeloma-bearing femurs, a previously unknown feature of the disease. Our study reports the intrafemoral inoculation of 5TGM1-GFP cells as an immunocompetent model of myeloma-induced bone pain, with consistent bone loss. We propose that this model can be used to study the underlying mechanisms of this condition and its pharmacological modulation. Moreover, we suggest that myeloma-induced bone pain is caused by a combinatorial mechanism including osteolysis and bone marrow denervation.
Substance P and gastrin-releasing peptide are expressed by morphologically and functionally distinct populations of excitatory interneuron in the superficial 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) and Andrew J. Todd (1)

1. Spinal Cord Group, Institute of Neuroscience and Psychology, University of Glasgow, Glasgow G12 8QQ, UK.
2. The Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK.
3. Department of Anatomy, University of California, San Francisco, San Francisco, CA 94158;
4. Department of Anatomy, Hokkaido University School of Medicine, Sapporo 060-8638, Japan

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

Tac1Cre;GRP::eGFP mice that had received a spinal injection of AAV.Flex.tdTom were used to investigate overlap between eGFP+ (GRP) and tdTom+ (SP) cells. There was minimal overlap between eGFP and tdTom, confirming that GRP and SP cells are two distinct populations. Because some cells may not be detected in the GRP::eGFP mouse, we also compared the distribution of GRP mRNA with eGFP in Tac1Cre mice that were injected with AAV.Flex.eGFP. We found that although there is limited overlap between SP and GRP cells, they are largely separate populations.

Patch-clamp electrophysiology, in spinal slices from GRP::eGFP or Tac1Cre mice injected with AAV.Flex.eGFP or AAV.Flex.tdTom, was used to study GRP and SP cells. Action potential firing patterns in GRP cells were predominantly transient or single spike, and this differed greatly from SP cells, which mostly showed delayed firing. sEPSC and mEPSC frequency was higher in SP cells, suggesting they have a greater excitatory drive than GRP cells. Almost all GRP cells responded to the MOR agonist, DAMGO, but they 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 that were filled with neurobiotin during electrophysiological recordings, and in SP cells in perfusion fixed tissue from Tac1Cre mice injected with Brainbow AAVs. Analysis of somatodendritic morphology demonstrated that GRP and SP cells were morphologically different. Although GRP cells were heterogeneous, some could be classified as central cells. In contrast, many SP cells resembled radial cells. Our findings demonstrate that GRP and SP cells 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. Our finding indicate that GRP and SP cells are functionally distinct, and presumably have different roles in somatosensory processing.
The antinociceptive effects of low dose morphine but not pregabalin are enhanced by the selective Cav2.2 blocker CNV2197944 in the rat

A. S. FISHER, N. UPTON, A. S. FISHER, C. TAYLOR, K. GIBSON, Z. ALI

Transpharmation, London, United Kingdom; Calchan, London, United Kingdom

Cav2.2 remains a compelling analgesic target but despite 2 decades of intensive research, a selective small molecule blocker efficacious at safe doses in humans remains elusive. At the spinal level, there is the opportunity to modulate presynaptic release from primary terminals of primary afferents by targeting µ-opioid, α2δ pathways as well as Cav2.2. This study aimed to determine if low doses of the Cav2.2 blocker CNV2197944 could potentiate the effects of low doses of morphine and/or pregabalin (PGB) preclinically—given the convergent nature of modulation between all three mechanisms.

Inflammatory pain; Intraplantar injection of Complete Freund's Adjuvant (CFA) induced hypersensitivity was detected by a shift in weight-bearing between injured and non-injured hind paws at 24 hrs post dose. Both Celebrex (10mg/kg p.o.) and CNV2197944 (30mg/kg p.o.) at all test times significantly reversed the hypersensitivity. Morphine alone, produced a dose-related reversal of the hypersensitivity over the dose-range of 0.3-10mg/kg i.p.

From this and previous studies, minimally effective doses of CNV2197944 (3 & 10mg/kg p.o.) and Morphine (0.3mg/kg i.p.) were selected for combined administration to evaluate potential additive/synergistic effects. Overall, the effects of CNV2197944 appeared to be largely additive to those of morphine (0.3mg/kg i.p.) at the time points evaluated.

Neuropathic pain; The chronic constriction injury (CCI) model of neuropathic pain was used to determine the effects of CNV2197944 (1 & 10mg/kg p.o.) alone and in combination with PGB (3mg/kg p.o.) following a sub chronic dosing regimen.

The control dose of PGB (30mg/kg p.o.) produced a clear reversal of the mechanical allodynia, equivalent to shams at the 1 hr time point, indicating efficacy.

CNV2197944 dose-dependently attenuated CCI-induced mechanical allodynia, with a significant increase in PWT observed at the 1 hr time-point in the 10mg/kg p.o. group.

CNV2197944 (10mg/kg p.o.) in combination with PGB (3mg/kg p.o.) increased PWT at both the 1 hr and 3 hr time-point but, the effect was not greater than that observed with CNV2197944 alone at 10mg/kg p.o. As such, there was no indication of a synergistic effect of these two compounds on PWT.

Using the current repeat-dose protocol PGB and CNV2197944 caused no overt sedative effects on the day of testing either alone or, in combination, compared to vehicle treated CCI animals CNV2197944 could enhance the effect of morphine in the CFA model but not PGB in the CCI model. Suggestive of a different modulation of opioid versus α2δ mechanism and/or inflammatory versus neuropathic pain.
Functions of sodium-activated potassium channels in pain processing

Cathrin Flauaus 1, Katharina Metzner 1, Fangyuan Zhou 1, Anne Bausch 2, Peter Ruth 2, Robert Lukowski 2, Achim Schmidtko 1 and Ruirui Lu 1

1 Pharmakologisches Institut für Naturwissenschaftler, Goethe-Universität, Fachbereich Biochemie, Chemie, und Pharmazie, 60438 Frankfurt am Main, Germany
2 Pharmakologie, Toxikologie und Klinische Pharmazie, Institut für Pharmazie, Universität Tübingen, 72076 Tübingen, Germany

Accumulating evidence indicates that different subtypes of potassium channels specifically control the processing of pain. Slack (sequence like a Ca2+-activated K+ channel; also termed KNa1.1 or KCNT1) and Slick (sequence like an intermediate conductance K+ channel; also termed KNa1.2 or KCNT2) are two members of Na+-activated K+ channels, which are mainly activated by increases in cytoplasmic levels of Na+. Previous studies suggest that Slack and Slick are expressed in the nociceptive system, regulate cellular excitability and contribute to pain processing. However, their expression pattern in pain-relevant tissues and contribution to pain processing are still not fully understood. To shed light on this issue we here characterized the expression pattern of Slack and Slick in dorsal root ganglia (DRGs) and the spinal cord, and analyzed the pain behavior of mice lacking Slack or Slick. In immunohistochemical experiments we detected Slack and Slick in distinct subpopulations of DRG neurons and in dorsal horn neurons of the spinal cord. Mice lacking Slack globally (Slack−/−) or specifically in sensory neurons (SNS-Slack−/−) demonstrated increased mechanical hypersensitivity after peripheral nerve injury. By contrast, Slick seems not to be critically involved in this context. Together, our data indicate that modulating activity of Slack, but not Slick, might represent a novel strategy for management of neuropathic pain.
Expression of cholecystokinin by neurons in mouse spinal dorsal horn.

Maria Gutierrez-Mecinas1, Andrew Bell1, Fraser Shepherd1, Erika Polgar1, Andrew Todd1


Excitatory interneurons account for the majority of dorsal horn neurons, and are required for perception of normal and pathological pain. We have identified largely non-overlapping populations in laminae I-III, based on expression of substance P, gastrin-releasing peptide, neurokinin B and neurotensin. Cholecystokinin (CCK) is expressed by many dorsal horn neurons, particularly in the deeper laminae. Here we have used immunocytochemistry and in situ hybridisation to characterise the CCK cells. We show that they account for ~7% of excitatory neurons in laminae I-II, but between a third and a quarter of those in lamina III. They are largely separate from the neurokinin B, neurotensin and gastrin-releasing peptide populations, but show limited overlap with the substance P cells. Laminae II-III neurons with protein kinase Cγ (PKCγ) have been implicated in mechanical allodynia following nerve injury, and we found that around 50% of CCK cells were PKCγ-immunoreactive. Neurotensin is also expressed by PKCγ cells, and among neurons with moderate to high levels of PKCγ, ~85% expressed CCK or neurotensin. A recent transcriptomic study (Häring et al, Nat. Neurosci., 2018) identified mRNA for thyrotropin-releasing hormone (TRH) in a specific subpopulation of CCK neurons, and we show that these account for half of the CCK/PKCγ cells. These findings indicate that CCK cells are distinct from other excitatory interneuron populations that we have defined. They also show that PKCγ cells can be assigned to different classes based on neuropeptide expression, and it will be important to determine the differential contribution of these classes to neuropathic allodynia.
MECHANISMS OF CYSTEIN-RICH PROTEIN 4 DEPENDENT PAIN PROCESSING

Lea Kennel1, Jörg Isensee2, Jonas Petersen1, Tilman Groß1, Cathrin Flauaus1, Ruirui Lu1, Julia Straubinger4, Hannes Schmidt3, Peter Ruth4, Tim Hucho2, Robert Lukoswki4, Achim Schmidtko1

1 Pharmakologisches Institut für Naturwissenschaftler, Goethe-Universität Frankfurt am Main, Germany
2 Experimentelle Anästhesiologie und Schmerzforschung, Universität Köln, Germany
3 Interfakultäres Institut für Biochemie, Universität Tübingen, Germany
4 Institut für Pharmazie, Universität Tübingen, Germany

Cystein-rich protein 4 (CRP4) is a member of the LIM domain protein family that organizes multiprotein complexes and regulates various cellular functions including the processing of persistent pain. In earlier studies we showed that CRP4 is highly expressed in subpopulations of sensory neurons and phosphorylated by cGMP-dependent protein kinase Iα (cGKIα). However, the mechanisms regulating CRP4 activity in pain processing are still poorly understood. We here investigated the distribution of cGMP-producing guanylyl cyclases, cGKIα and CRP4 in dorsal root ganglia and the spinal cord of mice by combined in situ hybridization and immunofluorescence. We demonstrate that particulate guanylyl cyclase B (GC-B), but not GC-A or NO-sensitive guanylyl cyclase, is expressed in subpopulations of sensory neurons and co-expressed with cGKIα. Behavioral experiments revealed that stimulation of GC-B triggers CRP4-dependent pain hypersensitivity. These data suggest that CRP4 phosphorylated by GC-B/cGMP/cGKIα signaling contributes to pain processing. Further investigations are necessary to elucidate the downstream mechanisms of CRP4-mediated pain processing in sensory neurons.
Rheumatoid Arthritis-associated monoclonal autoantibodies can drive pain-like behaviour and bone loss in mice

Emerson Krock, Alexandra Jurczak1, Katalin Sandor1, Gustaf Wigerblad1, Akilan Krishnamurthy1, Katarzyna Rogoz1, Arisai Martinez-Martinez3, Enriqueta Munoz-Islas3, Payam Emami Khoonsari1,2, Kim Kultima2, Anca Catrina1, Vivianne Malmström1, Miguel Jimenez Andrade1, Lars Klareskog1, Camilla I Svensson1

1Karolinska Institutet, 2Uppsala University, 3Universidad Autonoma de Tamaulipas

Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint inflammation, pain and bone erosion. Interestingly, RA-associated pain and bone erosion are not simply reflective of inflammation; they often develop prior to RA joint inflammation and diagnosis. Anti-citrullinated protein antibodies (ACPA) are often found in individuals’ sera years before diagnoses. We previously found that injecting pooled, purified ACPA IgG from RA patients into mice causes long lasting pain-like behavior but not inflammation. To better understand the mechanisms of autoantibody-driven pain, we are now investigating RA patient derived, murinized monoclonal ACPA (mACPA) that bind different citrullinated epitopes. Two different mACPA (B09 and C03) induce transient mechanical hypersensitivity that lasts approximately one week compared to control IgG when injected intravenously (i.v.) into female mice. Interestingly, combining B09 and C03 resulted in mechanical hypersensitivity that lasted at least 28 days. Either mACPA alone, or in combination, did not promote the development of paw inflammation and diclofenac or naproxen did not reverse mechanical hypersensitivity induced by B09, C03 or B09/C03. These results suggest ACPA-induced pain-like behaviour is mediated by unconventional, prostaglandin-independent mechanisms. As expected, buprenorphine transiently reversed mACPA-induced mechanical hypersensitivity. The B09/C03 combination promoted bone loss, evaluated by μCT scanning, and increased gene expression of the osteoclast (bone resorbing cells) related genes Clcn7, Tcrg1, and Acp5, as well as Cxcl2. We then tested the effects of zoledronate, a bisphosphonate that inhibits osteoclasts and bone resorption, and reparixin, a CXCR1/2 (IL-8 and CXCL2 receptor) antagonist. Both zoledronate and reparixin reversed B09/C03 driven mechanical hypersensitivity, suggesting a link between ACPA-induced pain, osteoclast activity, and IL-8 signaling. Intriguingly, B09 alone does not activate osteoclasts (Krishnamurthy et al, 2015) or cause bone erosion, but C03 does activate osteoclasts (Krishnamurthy et al, 2015). This suggests that some ACPA drive pain through osteoclast-dependent mechanisms, whereas other ACPA function through different pronociceptive pathways. Altogether, we conclude that targeting osteoclasts or IL-8 pathways could be an effective therapeutic strategy for RA-associated pain in some cases. Our findings also highlight the importance of using patient-derived monoclonal autoantibodies to better understand the relation between autoantibody specificities, RA pathophysiology and pain.
Synthetic 5β-reduced neurosteroids in the treatment of neuropathic pain

Eva Kudova1, Jiri Palecek2, Ladislav Vylicky2, Jan Jakubik2

1 Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam.2, Prague 6, 16610, Czech Republic
2 Institute of Physiology, Czech Academy of Sciences, Videnska 1083, Prague 4, 14220, Czech Republic

Neurosteroids are endogenous steroids that are synthesized from cholesterol and act in the central nervous system. Neurosteroids produce rapid effects on neuronal excitability and synaptic function that involve direct or indirect modulation of neurotransmitter-gated ion channels, or other neurotransmitter receptors and transporters, rather than classic, nuclear hormone receptors. The central effects of these compounds are mediated by interactions with ligand-gated ion channels such as glutamate, GABAA, glycine and nicotinic acetylcholine receptors. N-Methyl-D-aspartate receptors (NMDARs) are glutamate gated, calcium-permeable ion channels that are activated during excitatory synaptic transmission and are implicated in various forms of synaptic plasticity, which underlies learning and memory processes. On the contrary, excessive increase in NMDAR/GluN2B activity has been associated with various disorders such as neuropathic pain and neuronal death following hypoxia. Several allosteric modulators, including neurosteroids, can influence the activity of NMDARs.

Here, we describe the state of the art of the novel synthetic neurosteroids in the treatment of neuropathic pain in the models of: (a) paclitaxel-induced neuropathy, (b) bortezomib-induced neuropathy, (c) spinal nerve ligation, and (d) iodoacetamide model of osteoarthritis pain. Gabapentin, a medication that is used primarily to treat neuropathic pain, was used as comparator drug. Our results indicate that synthetic neurosteroid may be beneficial therapeutics and offers new avenue of investigation in the treatment of neuropathic pain. This work was supported by grant TE01020028 Center for Development of Original Drugs from the Technology Agency of the Czech Republic and Czech Academy of Sciences grant 17-02300S and research projects RVO 67985823, RVO 61388963.

This work was supported by grant TE01020028 Center for Development of Original Drugs from the Technology Agency of the Czech Republic and Czech Academy of Sciences grant 17-02300S and research projects RVO 67985823, RVO 61388963.
Chemogenetic Silencing as a Tool to Understand Primary Afferent Contributions to Chronic pain

Steven J Middleton, Greg Weir, Alex Clark, David Bennett

Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK

Recent advances have been made in stratifying primary afferent sub-populations by molecular profiling, however information of their functional role in normal and pathological nociceptive processing is lacking. Neuropathic pain patients can suffer from sensory dysfunction that includes spontaneous lancinating or burning pain, and/or abnormal processing of noxious and non-noxious stimuli (hyperalgesia and alldynia respectively). Understanding the neural pathways that underlie the different components of chronic pain will greatly aid rational design of novel therapeutics. We have recently 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). We have used AAV-based intrathecal delivery to target GluCl solely to primary afferents in order to silence and thus study their role in normal and pathological nociceptive transmission. To address specific sub-population contributions, we have recently designed FLEX-switch AAV that express GluCl only in the presence or absence of Cre. We have validated the fidelity of this approach in vitro and in vivo. Restricting and targeting GluCl expression in this manner will allow us to control which DRG sub-populations we silence and study. When GluCl is delivered to a broad range of primary afferent subtypes, IVM administration can silence their activity and render animals hyposensitive to sensory stimuli for up to 3 days. This approach was efficacious in ameliorating mechanical and cold hypersensitivity in a rodent model of neuropathic pain. Taking these findings forward, we have developed cre-dependent GluCl-AAV. Current efforts centre on delivering this AAV to transgenic mouse lines where expression is restricted to discreet afferent populations. We are using classical withdrawal assays in addition to operant tests of spontaneous pain, to investigate the role these populations play in normal sensation and pathological pain. Our initial study non-selectively delivering GluCl to a wide range of primary afferents demonstrate the importance of aberrant afferent input in the maintenance of pain following injury and the potential for targeted chemogenetic silencing as a new treatment modality in neuropathic pain. By targeting GluCl to sub-populations of primary afferents, we are able to test their role in normal sensation and their contribution to differing pain modalities following injury. More detailed information of the neural circuitry underlying different aspects of pain will be valuable for the design of novel therapeutic strategies.
Characterisation of Two Kv1.6 Channel Knock-out Mice: Inclusion of a LacZ (E.coli β-galactosidase) Cassette as a Gene Knock-out Strategy May Yield Confounding Effects Including Primary Afferent Neurotoxicity

Peck, LJ (1), Patel, R (2); Dawes, JM (1); Dickenson, AH (2); Todd, AJ (3); Bennett, DLH (1)

(1) Nuffield Department of Clinical Neurosciences, University of Oxford
(2) Department of Neuroscience, Physiology and Pharmacology, University College London
(3) Institute of Neuroscience and Psychology, University of Glasgow

Kv1.6 is a member of the 'Shaker-like' Kv1 family of voltage-gated potassium channels (VGKCs), which are known to impose a 'brake' on neuronal excitation via delayed outwardly rectifying currents. Genetic and immune-mediated loss of function in these channels and their interacting complex proteins such as CASPR2 is associated with neuronal hyperexcitability and is implicated in the pathophysiology of neuropathic pain. Kv1.6 in particular is required for recovery towards normal mechanical sensitivity thresholds after axotomy-induced hypersensitivity. Here, we describe behavioural, anatomical and physiological characterisation of two Kv1.6 knock-out mice: produced by homologous recombination with an E.coli LacZ reporter cassette (Kcna6-tm1), and via Crispr-mediated deletion with no exogenous reporter (Kcna6-em1). Replacement of endogenous alleles with a LacZ selection cassette has historically been a common approach to generate knock-out animals or report gene expression, yet little attention has been paid to downstream consequences of expressing exogenous β-galactosidase.

Via in situ hybridisation and immunohistochemical assays, we reveal expression of Kcna6 in DRG neurons (particularly small-diameter nociceptors) and satellite glia, as well as in spinal cord cell populations. Behaviourally, Kcna6-tm1 mice are markedly hyposensitive to noxious heat, despite having normal intraepidermal nerve fibre density and DRG neuron responses to noxious temperatures in vitro. A physiological correlate of behavioural hyposensitivity was found in reduced WDR responses to noxious stimuli. However, upon examination of Kcna6-tm1 spinal cord tissue, these LacZ-expressing Kcna6 knock-outs exhibit progressive, gene-dose dependent degeneration of central nociceptor terminals in the superficial dorsal horn (laminae I and II). We observed large (≤35µm diameter) pathological swelling of CGRP- and IB4-positive terminals in Kcna6-tm1 tissue, which are also demarcated by strong staining for the LacZ product β-galactosidase. EM imaging identified gross accumulation of lipid droplets and autophagosome-like structures in these degenerative terminals.Degenerative terminals are not present in Kcna6-em1 mice, which also lack Kv1.6 but do not express exogenous LacZ.

We reason that the inclusion of a LacZ construct commonly employed in mouse knock-out genetics has neurotoxic effects in IB4 and CGRP nociceptors - at least on a Kcna6-null background - potentially exacerbating or masking the underlying phenotype of the knock-out mouse. In the case of Kcna6-tm1 mice, this appears to impair transmission of nociceptive impulses from the periphery to WDR neurons in the CNS. These results should serve as a warning to those using LacZ reporter mice, particularly as many researchers may not be aware that their knock-out line contains a LacZ cassette.
Distinct functions of NO-GC isoforms in the processing of chronic pain

Jonas Petersen 1, Evanthia Mergia 2, Lea Kennel 1, Oliver Drees 4, Rebecca Steubing 4, Catherine Isabell Real 4, Wiebke Kallenborn-Gerhardt 1, Ruirui Lu 1, Andreas Friebe 3, Doris Koesling 2, Achim Schmidtko 1

1 Pharmakologisches Institut für Naturwissenschaftler, Goethe-Universität Frankfurt am Main, Germany
2 Institut für Pharmakologie und Toxikologie, Ruhr-Universität, Bochum, Germany
3 Physiologisches Institut, Universität Würzburg, Germany
4 Institut für Pharmakologie und Toxikologie, Universität Witten/Herdecke, Witten, Germany

Background: A large body of evidence indicates that production of nitric oxide (NO) and activation of NO-sensitive guanylyl cyclase (NO-GC, also referred to as ‘soluble’ guanylyl cyclase, sGC), with subsequent cGMP production essentially contributes to the processing of chronic pain. NO-GC exists in two heterodimeric isoforms and is composed of a catalytic β1 subunit dimerized to a α1 subunit (NO-GC1) or a α2 subunit (NO-GC2). However, the specific function of both isoforms in pain processing remain elusive. Here, we investigated the expression of NO-GC isoforms in dorsal root ganglia (DRG) and in the spinal cord and we evaluated the behavior of mice lacking NO-GC1 or NO-GC2 in various animal models of pain.

Methods: The expression of NO-GC in DRGs and in the spinal cord was investigated by immunohistochemistry, western blot, in situ hybridization and qPCR. The behavior of knockout mice lacking NO-GC1 or NO-GC2 globally (GCα1-/−/− and GCα2-/−/−) or only in dorsal horn neurons (Lbx1-GCα1-/−/− and Lbx1-GCα2-/−/−), and littermate control mice was investigated in animal models of acute nociceptive, inflammatory and neuropathic pain.

Results: We found both NO-GC isoforms to be differently expressed in interneurons of the spinal dorsal horn with NO-GC1 being enriched in inhibitory interneurons. In DRGs, NO-GC1 and NO-GC2 are expressed in non-neuronal cells with NO-GC2 as the major isoform in satellite glial cells. GCα1-/− mice demonstrated reduced hypersensitivity in models of neuropathic pain with normal inflammatory pain behavior. By contrast, GCα2-/− mice exerted increased hypersensitivity in models of inflammatory pain but showed normal neuropathic pain behavior. Cre-mediated deletion of one NO-GC isoform in spinal dorsal horn neurons recapitulated the behavior phenotypes observed in the global knockout.

Conclusion: cGMP produced by NO-GC isoforms mediates distinct functions in chronic pain processing. The downstream signaling mechanisms underlying NO/cGMP-dependent pain processing need to be further elucidated.
Expression of calretinin among different neurochemical classes of interneuron in the mouse superficial dorsal horn

Erika Polgár, Maria Gutierrez-Mecinas, Olivia Davis, David Hughes, Andrew Todd

Spinal Cord Group, University of Glasgow, UK

In the mouse, ~75% of neurons in laminae I-II are interneurons. These form complex neuronal circuits and are involved in pain and itch mechanisms. Our understanding of the neuronal circuitry underlying somatosensory processing remains restricted, due to difficulty in defining functional populations among the interneurons.

We have identified four largely non-overlapping excitatory interneuron populations defined by expression of neuropeptides: neurotensin, neurokinin B (NKB), gastrin-releasing peptide (GRP), and substance P (SP) as well as a fifth population in glabrous skin territory that express dynorphin. The calcium-binding protein, calretinin, is present in many excitatory neurons in this region, but we know little about its relation to these neuropeptide markers. Calretinin-positive cells have been implicated in detecting noxious mechanical stimuli, but are not required for tactile allosthenia after nerve injury. It has been reported that ~30% of lamina I-II neurons are calretinin-immunoreactive, with the great majority being excitatory. Our first aim was to investigate calretinin expression among different neuropeptide-containing excitatory interneurons, to facilitate interpretation of these behavioural findings. Inhibitory calretinin cells correspond to islet cells and are thought to contain mRNA for Tac1, the gene for SP. We have previously shown that some Tac1-expressing neurons in Laminae I-II are inhibitory. Therefore, our second aim was to investigate calretitin expression among inhibitory Tac1 cells.

To detect GRP neurons we used GRP::eGFP mice. To identify neurons that express Tac1 we performed intraspinal injections of the adeno-associated virus, that codes for the Cre-dependent form of eGFP, into Tac1 Cre mice. Tissue from wild-type mice was used to reveal other neuronal markers. All tissue was processed for immunocytochemistry and analysed with confocal microscopy.

We show that in laminae I-II calretinin is expressed by ~40% of neurons, and ~25% of the inhibitory cells. Calretinin was present in the majority of GRP- and NKB-expressing cells, and virtually all of the excitatory Tac1-positive neurons. However, very few of the neurotensin or dynorphin cells were calretinin-immunoreactive. We also found that nearly all viral-labelled inhibitory Tac1 neurons were calretinin-positive.

Our results show that in laminae I-II calretinin is differentially expressed among neuropeptide-containing excitatory interneuron populations. The lack of calretinin in neurotensin and dynorphin excitatory neurons is consistent with the suggestion that these cells are involved in tactile allosthenia.
Sensitization of nociceptive sensory neurons by oxaliplatin

Nurjahan Saleque, Clive Gentry, Nisha Vastani, Stuart Bevan and David Andersson

King’s College London, Wolfson Centre for Age-Related Diseases, London, UK.

Background: Oxaliplatin is a platinum-based chemotherapeutic drug used in the treatment of colorectal cancer. Oxaliplatin is associated with side effects including acute cold induced paraesthesias and chronic neuropathic pain that are regularly dose limiting. This phenomenon of acute pain evoked by cooling is unique for oxaliplatin and the mechanisms responsible for cold hypersensitivity have not been fully explained. Here, we have examined the cold sensitivities of sensory neurons from oxaliplatin-treated mice and the effects of direct applications of oxaliplatin to the receptive fields of intact skin-nerve preparations.

Methods: Animals. 8-10-week-old C57Bl/6J mice received a single intraperitoneal (i.p) injection (6mg/kg). Paw withdrawal latencies were assessed using a 10°C cold plate. [Ca2+]i-imaging. Dorsal root ganglion (DRG) neurons were dissociated enzymatically 3-4 days post final injection. Microfluorometric [Ca2+]i-measurements were made using Fura-2 loaded DRG neurons ~18 hours after dissociation. Cells were superfused with temperature controlled solutions and stimulated with a 35-10°C cold ramp. Intact skin-saphenous nerve preparation. The saphenous nerve with the skin that it innervates was dissected from naïve mice and placed in synthetic interstitial fluid heated to 32°C and bubbled with 95% O₂, 5% CO₂. Receptive fields were isolated and exposed to electrical, mechanical and thermal stimulations to enable recordings from single primary afferent fibers and oxaliplatin was directly applied to receptive fields of single fibers.

Results: A single i.p. injection of oxaliplatin decreased the paw withdrawal latencies to the cold stimulus (10°C) 4-96 hours post-injection. The proportion of cold sensitive DRG neurons isolated from mice treated with oxaliplatin was greater (6.7%) compared to vehicle treated mice (4.9%). Surprisingly, the average temperature threshold for activation was significantly shifted towards colder temperatures in neurons from oxaliplatin-treated (23.88±0.5°C) compared to vehicle-treated mice (26.02±0.5°C). Direct application of oxaliplatin (600µM) to receptive fields of intact skin-nerve preparations made some normally temperature insensitive mechanosensitive afferents highly sensitive to cooling. Furthermore, acute oxaliplatin treatment in vitro sensitized single fibers to mechanical stimulation.

Conclusions: It is likely that the limited recruitment of cold sensitive neurons using [Ca2+]i-imaging failed to account fully for the behavioural sensitization in vivo. However, our findings from skin-nerve preparations demonstrate that oxaliplatin sensitizes nociceptive sensory afferents acutely in vitro. The observed functional abnormalities may represent the cellular basis for acute oxaliplatin induced cold paraesthesias and mechanical hypersensitivity. Identification of the cellular and molecular mechanism(s) responsible for oxaliplatin-induced cold and mechanical hypersensitivities may allow for more effective use of oxaliplatin as a cancer therapy.
Investigating Interactions between the Immune System and Peripheral Nervous System in the Initiation and Maintenance of Neuropathic Pain

Oliver Sandy-Hindmarch, Georgios Baskozos, David L H Bennett, Annina B Schmid

Nuffield Department of Clinical Neuroscience, John Radcliffe Hospital, University of Oxford.

Introduction
Neuropathic pain poses a significant clinical problem and affects a large global population. Despite the great financial and economic burden caused by neuropathic pain, relatively little is known about its causes. Studies in animal models have revealed a role for the immune system and inflammation in the generation and maintenance of neuropathic pain. These studies have proved useful, however further work in human models of neuropathic pain are now required to fully elucidate the role of the immune system in the pathogenesis of neuropathic pain. Carpal tunnel syndrome (CTS) provides a unique human model system that allows the prospective examination of the role of the immune system in disease and recovery.

Methods
A cohort of 55 CTS patients and 21 healthy controls was used to investigate the presence of inflammation and its relationship with symptoms in peripheral nerve injury. Gene expression analysis of 48 selected genes, previously identified as being involved in inflammation or neuropathic pain was conducted with qPCR based TAQMAN card arrays on RNA extracted from participants' blood samples. Investigations were made to determine differences in systemic inflammation between healthy controls and CTS patients as well as in patients before and 6 months after carpal tunnel decompression.

Results
No genes were significantly dysregulated between CTS patients and healthy controls. From pre to post-surgery, IL-6 and IL-9 were significantly dysregulated (P=0.033, P=0.014 respectively, P<0.05), with IL-6 being decreased and IL-9 increased post-surgery. The gene dysregulation of IL-9 negatively correlated with patients' symptom scores including the Boston symptom questionnaire (correlation coefficient = 0.32, P=0.007) and neuropathic pain symptom inventory scores (correlation coefficient = 0.25, P=0.03).

Discussion
The identification of systemically dysregulated genes in CTS patients suggests a role for the immune system in focal nerve injury, which changes after surgery. The increase in IL-6, a prominent pro-inflammatory cytokine involved in neuropathic pain severity, indicates a role for inflammation during active disease. IL-9 is a pleotropic cytokine and has been shown to have anti-inflammatory effects. As IL-9 was increased after surgery and had a negative correlation with a range of patients' symptom scores, a functional role for IL-9 in the resolution of CTS seems likely.
Mid face Toddler Excoriation Syndrome (MiTES) – a second phenotype caused by polyalanine tract expansions in PRDM12

Miss Nivedita Sarveswaran1, Professor Celia Moss2,6, Dr Sahana Srinivas3, Dr Michael Nahorski1, Dr Vykuntaraju Gowda4, Dr Fiona Browne5, Professor Christopher Geoffrey Woods1,7

1Cambridge Institute for Medical Research and 7Department of Medical Genetics, University of Cambridge, Cambridge, U.K.
2Birmingham Women’s and Children’s Hospital, and 6University of Birmingham, Birmingham, U.K.
3Department of Pediatric Dermatology and 4Department of Pediatric Neurology, Indira Gandhi Institute of Child Health, Bangalore, Karnataka, India.
5Department of Dermatology, Our Lady’s Children’s Hospital, Crumlin, Dublin, Ireland.

Mid-face Toddler Excoriation Syndrome (MiTES) is a newly recognised condition as of 2017. Young children present with a strong compulsion to pick the skin around their nose and eyes to the point of forming deep, scarring lesions. This bilateral, mid-facial distribution is distinct from other causes of self-inflicted facial mutilation such as skin infections or trigeminal trophic syndrome. However, a strikingly similar phenotype was observed in a family diagnosed with congenital insensitivity to pain (CIP) caused by mutations in PRDM12, motivating our investigation of this gene.

Genomic DNA was obtained from four families, consisting five affected children and their unaffected parents, for targeted Sanger sequencing of all PRDM12 exons. One child did not possess any exonic mutations in PRDM12, but the remaining four were homozygous for an expansion in the terminal exon of PRDM12 resulting in a polyalanine tract expansion to 17 or 18 alanines. This tract is polymorphic, and in healthy individuals ranges from 7-13 alanines.

All parents were heterozygotes, consistent with an autosomal recessive pattern of inheritance for MiTES, as with PRDM12-CIP.

Homozygous expansion to 19 alanines in PRDM12 has been shown to cause pathogenic intracellular aggregation, which may prevent key transcriptional events in the development of sensory ganglia and differentiation of nociceptive neurons. (Chen Y-C, Auer-Grumbach M, Matsukawa S et al. Transcriptional regulator PRDM12 is essential for human pain perception. Nat Genet 2015; 47:803–8). It is therefore puzzling that a homozygous expansion of just one less alanine yields not a generalised pain phenotype, but an anatomically circumscribed intense itch which resolves in late childhood.

Our findings confirm MiTES as a recognisable clinical entity, which in most cases has a genetic basis of a polyalanine tract expansion of 18 in PRDM12. Why itch insensitivity is so highly localised in MiTES, and why the course of disease dramatically differs from CIP, is yet to be determined.
Molecular and cellular correlates of nerve repair in humans

Annina B Schmid (1), Georgios Baskozos (1), Katherine Windsor (1), Pall Karlsson (2), Oliver Sandy-Hindmarch (1), Gregory Weir (1), Lucy McDermott (1), Joanna Burchall (3), Michael Ng (3), Akira Wiberg (3), Dominic Furniss (3), David LH Bennett (1)

1 Nuffield Department of Clinical Neurosciences, The University of Oxford, UK
2 Department of Clinical Medicine, The Danish Pain Research Center, Aarhus, Denmark
3 Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, The University of Oxford, UK

We only have a rudimentary understanding of the molecular and cellular determinants of nerve repair and neuropathic pain in humans. This cohort study uses the most common entrapment neuropathy (carpal tunnel syndrome (CTS)) as a human model system to prospectively evaluate the cellular and molecular correlates of neural regeneration and its relationship with clinical recovery.

In 60 patients undergoing CTS surgery, we used quantitative sensory testing (QST) and nerve conduction studies to evaluate the function of large and small fibres before and six months after surgery. Clinical recovery was assessed with the global rating of change scale and the Boston carpal tunnel questionnaire. Twenty healthy participants provided normative data. Neural regeneration was determined on repeated skin biopsies from the median nerve territory of the hand by determining intraepidermal nerve fibre density (IENFD) and nodal architecture. RNA sequencing of the skin was performed to identify a molecular signature associated with neural regeneration in target tissues. The regenerative role of genes of interest were studied in human induced pluripotent stem cell-derived sensory neurons.

At 6 months post-surgery, neurophysiological as well as somatosensory recovery of both small and large nerve fibers was apparent. Structural neural regeneration was apparent by a partial recovery of IENFD, whose extent correlated with symptom improvement. Nodal length increased postoperatively and correlated with improved hand function. RNAseq revealed that >500 genes were dysregulated following surgery, with a highly enriched cluster being related to axonogenesis. The most differentially expressed gene is implicated with neural regeneration.

In conclusion, CTS as a model system revealed the time course as well as cellular and molecular correlates of nerve repair in humans.
skInsight: An optical platform for ultrafast and spatially precise evoked and quantified protective pain behaviour

Ara Schorscher-Petcu, Liam E. Browne

Wolfson Institute for Biomedical Research & Department of Neuroscience, Physiology and Pharmacology, University College London

Nociception serves a vital function by alerting an organism to the presence of harmful stimuli and triggering rapid protective withdrawal behaviours. Nociceptive behaviour is classically studied using thermal, mechanical or chemical stimuli that are usually limited to relatively low spatiotemporal precision. More recently, transdermal optogenetic approaches have been developed, allowing for precise light-driven activation of genetically targeted nociceptor populations in freely-behaving mice. Building on our previous work, here we have developed skInsight, a behavioral platform that provides vastly improved spatiotemporal precision and enables fully automated quantification. The system can target a laser pulse of defined duration (down to 100 µs), diameter (down to 150 µm), and intensity to the plantar surface of a mouse paw. Concomitant video recordings at high-speed are subsequently analysed with custom software, allowing for objective detection of nocifensive behaviour at the millisecond timescale. This automated analysis enables us to efficiently dissect and rapidly quantify distinct types of protective behaviours otherwise not discernible by simple observation: from local responses that occur below the withdrawal threshold or vary in timing, up to rapid whole-body repositioning behaviours.

We validated the system in mice expressing ChR2 in a broad class of primary afferent nociceptors. In this animal model, we addressed how varying a single optical stimulation affects the probability, magnitude and diversity of protective behaviour. Next, we investigated how the shape of the stimulus affects withdrawal behaviours. These experiments enabled us to define the minimal input size required to trigger protective behaviours and identify that repositioning behaviours are highly predictive of stimulus intensity.

This platform allows us to probe with high spatial and temporal precision the sensory-motor input-output relationship of genetically-defined cutaneous afferent subpopulations. Combined with other neurophysiological techniques, skInsight can be employed to understand how neural circuits are recruited by both noxious and innocuous sensory inputs and drive a specific adaptive behavioural response. The accurate description of these circuits will be key to understanding the alterations occurring during disease and identifying new therapeutic targets for chronic pain states.
Mechanical force detection in human stem cell-derived pain-sensing neurons requires PIEZO2

Katrin Schrenk-Siemens, Jörg Pohle, Muad Abd El Hay, Charlotte Rostock, Shiying Lu, Jan Siemens

Department of Pharmacology, Im Neuenheimer Feld 366, University of Heidelberg, 69120 Heidelberg, Germany

Mechanosensation - the ability to detect and transduce mechanical stimuli from our inner and outer environment - is important for our wellbeing: it not only mediates the perception of touch and vibration but also permits the detection of painfully noxious mechanical impact. Polymodal nociceptive neurons are equipped to detect and electrically respond to mechanical stimuli, however the molecular basis of mechano-nociception is not fully understood. While the role of PIEZO2 as low-threshold mechanosensor of innocuous stimuli has been well-established in recent years, the protein has also emerged as a candidate molecule to mediate mechano-nociception. In humans, however, proof for this clinically relevant function is lacking, largely because of the difficulty to study discrete nociceptive sensory neuron subtypes in vivo or in vitro. We generated human nociceptive-like neurons from human embryonic stem cells. By varying the differentiation procedure, diverse populations of nociceptor-like cells could be generated. Similar to their native counterparts, the majority of them respond to mechanical stimulation. Strikingly, deleting the PIEZO2 gene completely abolishes any mechanosensitivity in all derived nociceptor-like cells, suggesting that PIEZO2 is critical for mechano-nociception in humans.
Kappa Opioid Receptors mediate TRPA1 analgesia

Evangelia Semizoglou, Clive Gentry, Nisha Vastani, Stuart Bevan, David Andersson

Wolfson CARD, Institute of Psychiatry, Psychology and Neuroscience, King’s College London

The available therapies for chronic pain fail to provide permanent relief to patients and are often associated with severe side effects. Chronic pain has led to over-prescription of opioid analgesics and has contributed to the increasing rates of opioid addiction and deaths. The need for novel analgesics devoid of side effects in the central nervous system is therefore urgent. The aim of this study is to explore the modulation of nociception, mediated by a crosstalk of opioid receptors and TRP (Transient Receptor Potential) channels in the peripheral nervous system. Transgenic mice lacking TRPA1 (Transient Receptor Potential Ankyrin 1) have reduced sensitivity to noxious cold and mechanical stimulation. We discovered that this sensory loss is related to peripheral KOR (Kappa Opioid Receptors) which are engaged in the absence of TRPA1. Administration of KOR antagonist normalizes this phenotype in Trpa1−/− mice but has no effect on nociception in wild type mice. Studies of skin-saphenous nerve preparations show that mechanical stimulation elicits fewer action potentials in Trpa1−/− nociceptors, compared to wild type. Additionally, in vivo calcium imaging experiments of the L4 DRGs (Dorsal Root Ganglia) reveal that less neurons respond to noxious cold and mechanical stimulation in Trpa1−/− mice, compared to wild type. This sensory abnormality is reversed by administration of the opioid receptor antagonist naloxone. In situ hybridization experiments in lumbar DRGs from wild type mice show that approximately 40% of the KOR positive neurons also express Trpa1. Transcriptomic and protein levels of KOR in DRGs from wild type and Trpa1−/− mice are unaltered according to RNA sequencing, qPCR, western blot and ELISA assays. We are currently studying the interactions between KOR and TRPA1, using a stable cell line expressing the opioid receptor, the channel and a biosensor to monitor cAMP levels. To conclude, the sensory deficits of Trpa1−/− mice are not directly related to the loss of TRPA1 but are explained by increased activity of KOR in the periphery. Ongoing studies will identify the molecular mechanisms for TRPA1 modulation of KOR activity. Our results may facilitate identification of molecular targets for new analgesic therapies for chronic pain.
Ample evidence supports the notion that learning processes influence pain perception. Two key learning processes, namely perceptual learning and associative learning, are thought to underlie neuroplastic changes important in the development of chronic pain. It is theorised that aberrant association of innocuous bodily sensations (CS+, conditioned stimuli) with painful events (US, unconditioned stimuli), may result in innocuous sensations attaining aversive value. Furthermore, as these sensations rarely repeat with the exact physical properties with which they were first encountered, the learnt responses to the innocuous bodily CS+ should generalize to similar stimuli. Hence, in theory, stimulus generalisation may result in a range of innocuous bodily sensations acquiring aversive properties, a process potentially important in the pathogenesis of chronic pain, but this theory remains untested. We hypothesise that an initially innocuous somatic CS+ could potentially take on the sensory qualities of pain following conditioning. Furthermore, we hypothesise this is a) modality dependent, only extending to somatic CS+ whilst other modalities of CS+ will simply acquire an unpleasant quality, and is b) temporally mediated, thus a smaller temporal distance between the CS+ and US will increase the likelihood of a CS+ acquiring painful qualities. Additionally, we hypothesise that using initially distinguishable CS+/− will lead to generalisation effects. Here we present a research plan to investigate how the CS-US pairing during aversive associative learning alters the perceptual learning of CS characteristics in pain-free subjects. This will be assessed through measuring the discriminability of learnt CS+/− and their perceived painfulness. We will also explore in what contexts aversive learning might result in the encoding of an otherwise innocuous CS+ as painful, through varying the initial CS+/− discriminability, the CS/US modality, and the temporal delay between the CS and US. Combinations of electrical tactile (for both innocuous and painful somatic stimulation), auditory and visual stimulation will be employed in this approach. We will model reinforcement learning mechanisms underlying pre to post-conditioning changes in discriminability and painfulness using a Bayesian ideal observer model, which will describe how pain could potentially be learnt through dynamic updating of brain representations of the painfulness of the stimulus. We will discuss how the results may contribute towards a better understanding of longstanding pain symptoms that occur despite no enduring tissue injury.
Methylglyoxal, a glycolytic metabolite associated with painful diabetic neuropathy alters C-fibre activity-dependent slowing in a sex-dependent manner

Carole Torsney, Atanaska Velichkova, Amy L. Hall

Centre for Discovery Brain Sciences, University of Edinburgh, United Kingdom

Background
Diabetic neuropathic pain is associated with raised plasma concentrations of the glycolytic metabolite methylglyoxal (MG) that post-translationally modifies voltage-gated sodium channels Nav1.7 and Nav1.8 (Bierhaus et al., 2012). These channels are involved in activity-dependent slowing (ADS) of C-fibre nociceptors (Petersson et al., 2014), whereby repetitive stimulation results in progressive slowing of action potential conduction velocity (Thalhammer et al., 1994), likely influencing spinal pain processing (Dickie et al., 2017). We have recently shown altered C-fibre ADS in a chemotherapy-induced neuropathic pain model (Galley et al., 2017) and the CFA inflammation model in a sex-dependent manner (Dickie et al., 2017).

Objective
To explore whether methylglyoxal alters C-fibre ADS 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.

Results
Chronic MG did not alter threshold, amplitude or conduction velocity of the C-fibre response in both sexes. In females, using both latency and width measures, chronic MG increased the frequency-dependent ADS compared to vehicle treatment. In males, MG decreased or did not affect frequency-dependent C-fibre ADS, as assessed by the latency and width measures, respectively.

Conclusion
Chronic MG application alters C-fibre ADS in a sex-dependent manner.

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Regulation of mRNAs in the axon of nociceptors during chronic pain states in vitro

Asta Arendt Tranholm (1,2), Victoria Chapman (2), Federico Dajas-Bailador (3), Cornelia de Moor (1)

(1) School of Pharmacy, University of Nottingham, Nottingham, UK
(2) Arthritis Research UK Pain Centre, School of Life Sciences, University of Nottingham, Nottingham, UK
(3) Queens Medical Centre, School of Life Sciences, University of Nottingham, Nottingham, UK

Introduction

The differential localisation of mRNAs in cellular compartments and their localised translation are important factors in the variability of protein synthesis in neuronal systems. In addition to their role in normal development and function, differences in mRNA localisation and regulation have been associated with the manifestation of physiological conditions such as chronic pain. It has been found that inhibiting mRNA polyadenylation, a type of mRNA regulation, reduces pain behaviours in rodent models of chronic pain. Cytoplasmic polyadenylation of specific mRNAs localised to the axon of nociceptors is hypothesised to be highly relevant for the manifestation of sensitisation. In this project, the regulation of mRNAs localised to the axon of dorsal root ganglion (DRG) cells in an in vitro model of chronic pain is explored with the potential of finding targets for future therapeutics. An in vitro model of chronic pain is generated by treating cells with the pro-inflammatory and pro-nociceptive mediator PGE2.

Methods

Dissociated DRG cells from E16 C57 BL6 mice were seeded in porous membrane chambers and treated with 10uM PGE2 for 24 hours to induce sensitisation of nociceptors. The method was verified using calcium imaging and qPCRs. RNA was extracted from cell bodies and axons separately, allowing for an assessment of localised mRNA regulation within the axon using qPCRs and RNA sequencing.

Results

Calcium imaging indicates a sensitisation of the cell culture after 24 hours of 10uM PGE2 treatment, illustrated by an increased and prolonged firing of nociceptors when activating with 200nM of capsaicin. The cell culture remains healthy after treatment as indicated by activating with 25mM KCl. RNA was isolated from separate fractions obtained from cell bodies and axons, allowing for assessment of localised changes. RNA isolated from the cell bodies shows a significant increase in NGF expression as measured through qPCR. RNA isolated from axons has been sent for sequencing to evaluate the mRNA regulation in a sensitised culture.

Conclusions

Treatment with PGE2 induces a sensitised state in DRG cultures mimicking that of chronic pain. Dissociated DRG cells grown in porous membrane chambers treated with PGE2 allow for evaluations of localised mRNA changes.
Peer volunteerism: does it help in managing pain?

Dr. Mimi Tse

The Hong Kong Polytechnic University

Chronic non-cancer pain is common among older adults and is often associated with significant physical and psychosocial incapacities. Older adults with pain are more depressed, anxious, and have reduced social interaction. Pain in older adults tends to be constant in nature, moderate to severe in intensity, and years long in duration. Prevalence of pain among nursing home residents is as high as 70%-80%. Nursing home residents are physically frail, live in "closed" nursing home environments, and may have difficulty seeking pain management strategies.

Aim: to recruit and train peer volunteers (PVs) to lead pain management program targeting the older adults living in nursing homes

Design: A pre-post experimental study

Method: A total of 45 peer volunteers were recruited from the Institute of Active Ageing, hosted by the Faculty of Health and Social Sciences of The Hong Kong Polytechnic University in the past few years. They completed the pain management training and visited older adults living in nursing homes that suffered from chronic pain. The pain management education program included physical exercises, interactive teaching and sharing of pain management using non-pharmacological strategies.

Results: The pain management program helped the nursing home residents to learn ways to soothe the pain, reduce the pain intensity, enhance the activity of daily living, and increase happiness. Peer volunteers showed a significant increase in self-rated pain management knowledge.

Conclusion: Findings of this study indicate that education on pain self-management is essential. Due to the limited health care resources and budgets, training of laypersons provides an opportunity for them to transfer pain self-management knowledge to nursing home residents.
Peptide antagonist of P2X3 receptors with compact stable structure and pronounced analgesic effect

Alexander Vassilevski, Peter Oparin, Eugene Grishin

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia;
Future Analgesics Ltd, Moscow, Russia

P2X3 is a member of the ionotropic purinergic receptor family and a validated molecular target in a number of pain syndromes, such as pain in arthritis or cystitis, trigeminal neuralgia or bone cancer. Only a few selective blockers of P2X3 receptors are known today, and most of them suffer from significant limitations in terms of drug development. In this communication, we describe a new peptide named PT6 from the venom of the crab spider Thomisus onustus. PT6 acts specifically against P2X3 (IC50~10 nM) with no activity on P2X2 or P2X2/3 channels. We produced recombinant PT6 in Escherichia coli and established its spatial structure in solution using NMR. The peptide presents a compact disulfide-stabilized structure conforming to the inhibitor cystine knot motif. PT6 is resistant to pepsin, trypsin, chymotrypsin, and elastase; it is stable in human blood plasma and refolds completely after being heated to 80 °C. Intravenous and subcutaneous administration of comparatively low doses of PT6 (0.1 mg/kg, while the maximum tolerated dose exceeds 2000 mg/kg) into mice produced pronounced analgesia in CFA-induced inflammatory pain, acetic acid-induced writhing and formalin tests, surpassing that of conventional analgesics such as diclofenac. Moreover, PT6 restored grip strength in mice with inflamed joints modeling arthritis pain. 125I-labeled PT6 was produced and used in ADME studies in mice. The peptide was found to be cleared from the circulation via renal filtration and no accumulation in organs after multiple dosing was noted. Importantly, due to the P2X3 selectivity, PT6 showed no dysgeusia or ageusia effects in mice. We conclude that PT6 is an attractive hit with potent analgesic effect and may address unmet medical needs. PT6 is being developed by Future Analgesics Ltd as a perspective medication for the therapy of P2X3-mediated conditions.
S100A4 – a functional protein in neuropathic pain processing?

Gesine Wack1, Juliana Heidler2, Ilka Wittig2, Katrin Schröder3, Ralf Brandes3, Achim Schmidtko1, Wiebke Kallenborn-Gerhardt1

1Institute of Pharmacology, Department of Pharmacy, Goethe University, Frankfurt am Main, Germany
2Functional Proteomics, SFB 815 Core Unit, Goethe University, Frankfurt am Main, Germany
3Vascular Research Centre, Frankfurt am Main, Germany

S100A4 is a member of a family of calcium-binding proteins that are widely distributed in the nervous system and appear to be involved in the regulation of neuronal survival and plasticity. In addition to affecting various intracellular processes, S100A4 is secreted into the extracellular space, where it executes additional biological functions. Previous studies suggest that the expression of S100A4 in the nociceptive system is regulated after nerve injury. However, the functional role of S100A4 in pain processing remains elusive. Here we performed immunohistochemistry, western blot, qPCR, and behavioral experiments to analyze the cellular distribution and pain-related function of S100A4 in neuropathic pain caused by peripheral nerve injury in mice. We show that S100A4 is expressed in sensory neurons and non-neuronal cells, and that its distribution considerably changed after nerve injury. Altogether, our data suggest that S100A4 might modulate neuropathic pain hypersensitivity and warrants further study.
Demonstration of significant pain relief in subjects with painful osteoarthritis of the knee with a novel anti-NGF, anti-TNFα bispecific fusion protein.

Fraser Welsh1, Tharani Chessell1, Keith Tan1, Kessia Hammett2, Peter Thornton1, Ian Gurrell1, Stephen McMahon2, Thor Ostenfeld1, Iain Chessell1

1 Neuroscience, IMED Biotech Unit, AstraZeneca, Cambridge; 2 Wolfson Centre for Age Related Diseases, Kings College London

Aim of investigation
Nerve growth factor (NGF) plays a role in pain pathophysiology with clinical studies demonstrating significant efficacy of anti-NGF mAbs. In vitro data suggest that TNFα may synergise with low concentrations of NGF to drive nociceptive gene expression. MEDI7352, an anti-NGF and anti-TNFα bispecific molecule, demonstrates synergy in vivo at the level of NGF and TNFα signaling, with anti-hyperalgesic efficacy being observed in preclinical models using MEDI7352 doses at which the individual components of the protein, when dosed separately, are inactive. We sought to investigate the safety, tolerability, and efficacy of MEDI7352 in a PhI study in patients with painful osteoarthritis of the knee.

Methods
Target engagement modelling of preclinical data suggests that MEDI7352 achieves efficacy by suppressing ≤10% NGF. The same models have been used to predict efficacious clinical doses. A first-in-human (FIH) interleaved single ascending dose (SAD) and multiple ascending dose (MAD) study in patients with painful osteoarthritis (OA) of the knee is ongoing. In the SAD portion, N=53 subjects have received intravenous doses of placebo or MEDI7352 (0.3, 2, 10, 50, 250, and 1000 µg/kg). Safety data includes follow-up MRI imaging of joints.

Results
Single i.v. administration of 0.3 to 1000 µg/kg, MEDI7352 was well tolerated with no serious adverse events reported. PK appeared dose-proportional and consistent with predictions. PD data indicate that target engagement has been achieved and increases in a dose-dependent manner. NRS and WOMAC analysis suggest that statistically significant pain relief is achieved at 50ug per kg or greater.

Conclusions
For the first time, we report that co-sequestration of NGF and TNFα mediated by MEDI7352 produces significant reversal of pain in subjects with painful osteoarthritis of the knee after a single administration.
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<td><a href="mailto:i.beauprie@dal.ca">i.beauprie@dal.ca</a></td>
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<td>Ulrich Beese</td>
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<td>University Medical Center Groningen</td>
<td><a href="mailto:u.beese@umcg.nl">u.beese@umcg.nl</a></td>
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<td>UCLA</td>
<td><a href="mailto:cmcahill@ucla.edu">cmcahill@ucla.edu</a></td>
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<td>Gerard Callejo</td>
<td>University of Cambridge</td>
<td><a href="mailto:gc534@cam.ac.uk">gc534@cam.ac.uk</a></td>
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<td>Wendy Campana</td>
<td>UCSD</td>
<td><a href="mailto:wcampana@ucsd.edu">wcampana@ucsd.edu</a></td>
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<td>Adam Carinci</td>
<td>University of Rochester School of Medicine and Dentistry</td>
<td><a href="mailto:adam.carinci@gmail.com">adam.carinci@gmail.com</a></td>
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<td>Alex Casson</td>
<td>University of Manchester</td>
<td><a href="mailto:alex.casson@manchester.ac.uk">alex.casson@manchester.ac.uk</a></td>
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<tr>
<td>Fernando Cervero</td>
<td>Bristol University</td>
<td><a href="mailto:fernando.cervero@mcgill.ca">fernando.cervero@mcgill.ca</a></td>
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<td>Sampurna Chakrabarti</td>
<td>University of Cambridge</td>
<td><a href="mailto:sc968@cam.ac.uk">sc968@cam.ac.uk</a></td>
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<tr>
<td>Nathaniel Chandra</td>
<td>University of Sydney</td>
<td><a href="mailto:nathaniel.chandra@gmail.com">nathaniel.chandra@gmail.com</a></td>
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<tr>
<td>Fabiana Chaves Dias</td>
<td>King's College London</td>
<td><a href="mailto:fabichavesdias@gmail.com">fabichavesdias@gmail.com</a></td>
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<tr>
<td>Iain Chessell</td>
<td>AstraZeneca</td>
<td><a href="mailto:iain.chessell@azneo.com">iain.chessell@azneo.com</a></td>
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<tr>
<td>Alex Clark</td>
<td>University of Oxford</td>
<td><a href="mailto:alex.clark@ndcn.ox.ac.uk">alex.clark@ndcn.ox.ac.uk</a></td>
</tr>
<tr>
<td>Trevor Coe</td>
<td>Auckland City Hospital</td>
<td><a href="mailto:trevorc333@gmail.com">trevorc333@gmail.com</a></td>
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<tr>
<td>Chantelle Connaughton</td>
<td>Mundipharma Research Ltd</td>
<td><a href="mailto:Chantelle.connaughton@mundipharma-research.com">Chantelle.connaughton@mundipharma-research.com</a></td>
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<td>James Cox</td>
<td>University College London</td>
<td><a href="mailto:j.j.cox@ucl.ac.uk">j.j.cox@ucl.ac.uk</a></td>
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<tr>
<td>Ulku Cuhadar</td>
<td>King's College London</td>
<td><a href="mailto:ulku.cuhadar@kcl.ac.uk">ulku.cuhadar@kcl.ac.uk</a></td>
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<td>Robson da Costa</td>
<td>UFRJ, Brazil and KCL, UK</td>
<td><a href="mailto:rbsndcst@gmail.com">rbsndcst@gmail.com</a></td>
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<tr>
<td>Alexander Davies</td>
<td>University of Oxford</td>
<td><a href="mailto:alexander.davies@ndcn.ox.ac.uk">alexander.davies@ndcn.ox.ac.uk</a></td>
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<tr>
<td>Olivia Davis</td>
<td>University of Glasgow</td>
<td><a href="mailto:o.davis.1@research.gla.ac.uk">o.davis.1@research.gla.ac.uk</a></td>
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<td>AZNeuro</td>
<td><a href="mailto:wessel.degraaf@azneo.com">wessel.degraaf@azneo.com</a></td>
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<td>Quazi Fahm E Deen</td>
<td>University College London</td>
<td><a href="mailto:fahm.deen.15@ucl.ac.uk">fahm.deen.15@ucl.ac.uk</a></td>
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<tr>
<td>Anne Desevre</td>
<td>Bioseb</td>
<td><a href="mailto:adesevre@bioseb.com">adesevre@bioseb.com</a></td>
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<tr>
<td>Anne Desevre</td>
<td>Bioseb</td>
<td><a href="mailto:adesevre@bioseb.com">adesevre@bioseb.com</a></td>
</tr>
<tr>
<td>Carola Di Bella</td>
<td>University College London</td>
<td><a href="mailto:carola.bella.16@ucl.ac.uk">carola.bella.16@ucl.ac.uk</a></td>
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<td>Jon Levine</td>
<td>UCSF</td>
<td><a href="mailto:jon.levine@ucsf.edu">jon.levine@ucsf.edu</a></td>
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<tr>
<td>Shengnan Li</td>
<td>University College London</td>
<td><a href="mailto:shengnan.li.12@ucl.ac.uk">shengnan.li.12@ucl.ac.uk</a></td>
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<tr>
<td>Derungs Louis</td>
<td>Université de Lausanne</td>
<td><a href="mailto:louis.derungs@unil.ch">louis.derungs@unil.ch</a></td>
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<tr>
<td>Ruirui Lu</td>
<td>Goethe University</td>
<td><a href="mailto:Lu@em.uni-frankfurt.de">Lu@em.uni-frankfurt.de</a></td>
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<td>Ana Luiz</td>
<td>UCL</td>
<td><a href="mailto:a.luiz@ucl.ac.uk">a.luiz@ucl.ac.uk</a></td>
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<td>Donald Iain MacDonald</td>
<td>University College London</td>
<td><a href="mailto:domhnall.macdonald@gmail.com">domhnall.macdonald@gmail.com</a></td>
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<td>Duncan Mackenzie</td>
<td>Ormiston</td>
<td><a href="mailto:duncan@ormiston.co">duncan@ormiston.co</a></td>
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<td>Jacquie Maignel</td>
<td>IPSEN Innovation</td>
<td><a href="mailto:jacquie.maignel@ipsen.com">jacquie.maignel@ipsen.com</a></td>
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<td>King's College London</td>
<td><a href="mailto:marilia_manchope@hotmail.com">marilia_manchope@hotmail.com</a></td>
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<td>Flavia Mancini</td>
<td>University of Cambridge</td>
<td><a href="mailto:fm456@cam.ac.uk">fm456@cam.ac.uk</a></td>
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<tr>
<td>Uwe Maskos</td>
<td>Institut Pasteur</td>
<td><a href="mailto:umaskos@pasteur.fr">umaskos@pasteur.fr</a></td>
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<tr>
<td>Dimitra Mazaraki</td>
<td>Max Delbrück Center for Molecular Medicine in the Helmholtz Association</td>
<td><a href="mailto:Dimitra.Mazaraki@mdc-berlin.de">Dimitra.Mazaraki@mdc-berlin.de</a></td>
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<td>Stephen McMahon</td>
<td>King's College London</td>
<td><a href="mailto:stephen.mcmahon@kcl.ac.uk">stephen.mcmahon@kcl.ac.uk</a></td>
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<td>Sara Memarpour Hobbi</td>
<td>University of Sheffield</td>
<td><a href="mailto:smemarh@gmail.com">smemarh@gmail.com</a></td>
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<td>Katharina Metzner</td>
<td>Goethe University Frankfurt</td>
<td><a href="mailto:metzner@em.uni-frankfurt.de">metzner@em.uni-frankfurt.de</a></td>
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<td>Steven Middleton</td>
<td>University of Oxford</td>
<td><a href="mailto:steven.middleton@ndcn.ox.ac.uk">steven.middleton@ndcn.ox.ac.uk</a></td>
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<td><a href="mailto:h.mikaeili@ucl.ac.uk">h.mikaeili@ucl.ac.uk</a></td>
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<td>Mundipharma Research Ltd</td>
<td><a href="mailto:max.mirza@mundipharma-rd.eu">max.mirza@mundipharma-rd.eu</a></td>
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<td>Joana Maria Monteiro Serrao</td>
<td>EMBL Rome</td>
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<td>Mundipharma Research GmbH &amp; Co.KG</td>
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