A MULTIDISCIPLINARY APPROACH FOR STRATIFYING PATIENTS WITH PERIPHERAL NEUROPATHIC PAIN

NEUP7-0379
PAINFUL SMALL FIBRE NEUROPATHY AS THE RESULT OF A SKIN DISORDER - THE CASE OF EPIDERMOLYSIS BULLOSA
M. Calvo¹
¹Biological Sciences Faculty. Pontificia Universidad Catolica de Chile, PhysiologyPain, Santiago, Chile

Small fibres in the skin are vulnerable to damage in metabolic or toxic conditions such as diabetes mellitus or chemotherapy resulting in small fibre neuropathy and associated neuropathic pain (NP). Whether injury to the most distal portion of sensory small fibres due to a primary dermatological disorder can cause NP is still unclear. Recessive Dystrophic Epidermolysis Bullosa, (RDEB) is a rare condition in which mutations of proteins of the dermo-epidermal junction lead to cycles of blistering followed by regeneration of the skin. Damage is exclusive to the skin and mucous membranes, with no known direct compromise of the nervous system. It is increasingly recognised that most RDEB patients experience daily pain, the aetiology of which is unclear but may include inflammation (in the wounds), musculoskeletal (due to atrophy and retraction scars limiting movement) or NP. In this study we investigated the incidence of NP and examined the presence of nerve dysfunction in RDEB patients. Around three quarters of patients presented with pain of neuropathic characteristics which had a length dependent distribution. Quantitative sensory testing of the foot revealed striking impairments in thermal detection thresholds combined with an increased mechanical pain sensitivity and wind up ratio (temporal summation of noxious mechanical stimuli). Nerve conduction studies showed normal large fibre sensory and motor nerve conduction however skin biopsy showed a significant decrease in intraepidermal nerve fibre density. Autonomic nervous system testing revealed no abnormalities in heart rate and blood pressure variability however the sympathetic skin response of the foot was impaired and sweat gland innervation was reduced. We conclude that chronic cutaneous injury can lead to injury and dysfunction of the most distal part of small sensory fibres in a length dependent distribution resulting in disabling NP. These findings also support the use of neuropathic pain screening tools in these patients and treatment algorithms designed to target neuropathic pain.
ADVANCES IN SPINAL CORD STIMULATION: INSIGHTS ON POTENTIAL MECHANISMS

NEUP7-0435
EFFECTS OF HIGH FREQUENCY SPINAL STIMULATION ON NOCICEPTIVE PROCESSING IN THE RAT SPINAL CORD.
M. Jones¹, M. Smith¹, S.B. McMahon¹
¹Wolfson CARD- King’s College London, Neurorestoration Group, London, United Kingdom

Chronic pain is not merely a repetition of acute. It is associated with the recruitment of a number of pathophysiological changes which serve to amplify the processing of noxious information. The biology and pharmacology of these processes has been particularly well studied in the peripheral nervous system and in the spinal cord. Here we will review these mechanisms and the evidence that they contribute to chronic pain states in humans. We will then consider how chronic pain mechanisms might be modified by repetitive electrical stimulation of the spinal cord, considering both ‘conventional’, relatively low frequency epidural stimulation at 50-100 Hz, and also the more recently introduced high frequency stimulation. We will describe some of the experiments that we have undertaken using 10 kHz stimulation on the processing of sensory information by both peripheral axons and spinal cord neurons.

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ADVANCES IN SPINAL CORD STIMULATION: INSIGHTS ON POTENTIAL MECHANISMS

NEUP7-0381
SPINAL MODULATORY MECHANISMS OF SPINAL CORD STIMULATION IN EXPERIMENTAL MODELS OF NEUROPATHIC PAIN
R. Srinivasa¹, Y. Guan¹
¹Johns Hopkins University, Anesthesiology and Critical Care Medicine, Baltimore- MD, USA

The management of neuropathic pain has been a challenge for clinicians. Pharmacotherapy has been limited in efficacy and associated with significant side effects. The relative safety and reversibility of electrical neuromodulation strategies have made it an attractive strategy for managing patients with refractory neuropathic pain. Considerable technological advances and clinical studies have led to the use of newer neurostimulation parameters in recent years. However, the spinal neurophysiologic and neurochemical mechanisms underlying the therapeutic actions of these treatments are not well understood.

In preclinical models of neuropathic pain, we have examined the effects of low-intensity electrical stimulation of large myelinated, Ab-fibers on neuropathic pain behavior and response properties of spinal dorsal horn neurons, using both behavioral and neurophysiologic approaches. We have reported that pain inhibition by spinal cord stimulation (SCS) involves suppression of nociceptive transmission in spinal, deep wide-dynamic range neurons in an intensity and frequency dependent manner. In addition, our observations suggest that kilohertz stimulation and conventional 50 Hz stimulation may attenuate mechanical hypersensitivity through different peripheral and spinal segmental mechanisms. In more recent studies, using powerful mouse transgenic tools and spinal cord slice preparations, we have demonstrated that Aβ-fiber stimulation induces frequency-dependent, sustained depression of synaptic response to C-fiber inputs in superficial (lamina II) dorsal horn neurons. Furthermore, the suppression of spinal nociceptive transmission and inhibition of neuropathic pain-related behavior by SCS may be mediated by activation of cannabinoid CB1 receptors.

An improved understanding of the biological basis of neurostimulation, especially SCS, from preclinical studies can potentially enable clinicians and biomedical engineers to develop novel techniques and strategies to enhance the clinical utility of neuromodulation therapies for pain.

References:

Chemotherapy-induced peripheral neuropathy (CIPN) is the most commonly reported neurotoxic and dose-limiting side effect of paclitaxel thereby affecting survival in many cancer patients; and its persistence in survivors negatively affects quality of life and rehabilitation. The mechanism of paclitaxel-related CIPN remains to be fully defined. Hyperexcitability of sensory neurons and its most extreme form, spontaneous activity (SA), are key cellular-level drivers of neuropathic pain. A recent study using real-time polymerase chain reaction ion channel micro-array revealed a significant increase in expression of a number of these that were increased following paclitaxel treatment. This seminar will present data that reveals important potential roles for Cav3.2, Na1.7 and HCN channels in generating SA and hyperexcitability in animal models of CIPN. As well, translational data using human DRG neurons from patients with and without pain arising in the sampled dermatome will also be presented that further underscore the potential importance of these channels in human pain.
Chemotherapy induced peripheral neuropathy (CIPN) is a common and disabling side effect of many commonly used anti-neoplastic drugs. The incidence depends on the drug used, with up to 85% of patients having some form of CIPN with platinum based drugs, and around 60% with paclitaxel. It is important for patients (and their oncologists) to have some indication of the level of risk associated with different chemotherapies.

If CIPN develops during treatment it can lead to dose reduction or cessation, with resultant impact on survival. At 6 months after finishing potentially neurotoxic chemotherapy, around 1/3 of patients will have evidence of CIPN. It is important to understand the underlying mechanisms and risk factors in order to inform treatment choices, develop preventive measures, and be able to treat effectively.
Dr. Flatters will discuss recently published preclinical data examining the contribution of oxidative stress, mitochondrial dysfunction and glycolysis in sensory neurons, in vivo, to the pathogenesis of CIPN. The temporal interplay between these different contributory factors in the development, maintenance and resolution of CIPN will be discussed, along with the potential of specific pharmacological modulation to alleviate CIPN.
The human C-tactile afferent system: observations in healthy subjects and denervated patients

Human C-tactile (CT) afferents do not mediate pain under normal conditions but instead seem to play a critical role for the hedonic aspects of touch perception. Single unit microneurography recordings show that CTs are most reliably activated by a mechanothermal stimulus with the characteristics of a human caress (stroking velocity 1–10 cm/sec; temperature 32 deg C) with correlation between CT firing and pleasantness perception. Brain imaging studies in healthy subjects as well as in patients with selective denervation of large myelinated or unmyelinated afferents suggest that CT information is processed in a network that includes the insular, orbitofrontal and anterior cingulate cortices. Thus, a picture has emerged where CTs are tuned to respond to a human caress, project to limbic related brain network, and contribute to the maintenance of well-being. Further, there is recent evidence suggesting extensive interactions between the CT and pain systems in humans.
C-Low Threshold Mechanoreceptors (C-LTMRs) represent a unique population of primary sensory neurons. Although discovered almost a century ago, C-LTMRs’ functional specialization is still under intense investigation. During my presentation, I'll discuss how we used a combination of mouse genetics, FACS sorting and RNA deep sequencing to show that C-LTMRs share many molecular features of Ad- and Ab-LTMRs. Using targeted neuronal ablation we also found that C-LTMRs play a critical role in modulation of injury-induced mechanical sensation as well as formalin-evoked pain. Our results suggest that in addition to their well described role in sensing pleasant touch, C-LTMRs can also play a critical role in modulation of injury-induced pain sensation.
C-TACTILE AFFERENTS IN NEUROPATHIC PAIN: FROM MOLECULAR TO CLINICAL INSIGHTS

NEUP7-0448
C-TACTILE AFFERENT INPUTS: DYNAMIC CONVERSION BETWEEN PLEASURE AND PAIN

D. Mahns¹, S. Nagi²
¹Western Sydney University, School of Medicine, Penrith, Australia
²Center for Social and Affective Neuroscience, Department of Clinical and Experimental Medicine, Linköping, Sweden

The peripheral sensory substrates subserving the perceptions of pain have been known for over a 100 years, but it is only in the last two decades that we have begun to understand the sensory substrates subserving pleasant or affective touch. Pleasant touch has been attributed to C-tactile (CT) afferents that have very low thresholds to mechanical stimulation. In recent work we have demonstrated that the line separating pleasant touch and pain is not static, but can be reconfigured by varying forms of acute or chronic muscle pain. We have shown that stimulation of CT afferents can produce allodynia, in which a normally pleasant stimulus becomes painful. Furthermore, repeated episodes of muscle pain, produced by physical activity or intramuscular injection of hypertonic saline, leads to gentle touch and innocuous cooling being perceived as painful – a phenotype consistently linked to the activation of C-TLMRs afferents.
CHRONIC NEUROPATHIC PAIN IN IMMUNE-MEDIATED NEUROLOGICAL DISEASES

NEUP7-0439
CHRONIC NEUROPATHIC PAIN IN IMMUNE-MEDIATED PERIPHERAL NEUROPATHIES
A. Hietaharju
1Tampere University Hospital, Neurology and Rehabilitation, Tampere, Finland

The main subtypes of autoimmune neuropathies include Guillain-Barré syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), Lewis-Sumner syndrome (LSS), paraproteinæmic neuropathies and vasculitic neuropathies. GBS is considered as a prototype of an acute immune-mediated peripheral neuropathy, whereas the others are predominantly chronic disorders. Their pathogenesis is related with the breakdown of the immunologic tolerance to myelin or axonal antigens. Even though the importance of immune system has been recognized in the pathogenesis of neuropathic pain, there are only few studies, which have focused on pain in immune-mediated neuropathies.

Studies have shown that two-thirds of GBS patients report pain during the acute phase of the disease and more than one-third of patients suffer from severe chronic pain one year after the onset of disease. What is interesting is that pain is also experienced by the GBS patients with mild disease or pure motor involvement. Chronic pain in GBS may be related with demyelination leading to prominent changes in sodium channel distribution and ectopic discharges. In addition, there is evidence that autoantibodies to GD2 gangliosides cause sensitisation of sensory afferent nerves, reflected by mechanical allodynia and spontaneous pain.

Patients with chronic immune-mediated neuropathies may also be debilitated by persistent pain. According to a study by Goebel et al, pain was moderate or severe in 39 % of CIDP patients. In a Dutch study, the proportion of patients with CIDP reporting severe pain was twofold higher than in patients with GBS. A recent study showed that auto-antibodies to a paranodal protein, contactin-associated protein I, is associated with rare painful subtypes of CIDP.

Overall, up to 50 % of patients with immune-mediated neuropathy may report pain. In an Italian population of 93 patients with autoimmune neuropathies, the most painful neuropathy was anti-myelin-associated glycoprotein (MAG) neuropathy, which is a form of paraproteinæmic neuropathy. It was followed by vasculitic neuropathy. Even though the number of studies on pain in autoimmune neuropathies is scarce, they have succeeded in highlighting the frequency and characteristics of chronic pain in these disorders. Further research focusing on the pathogenetic mechanisms of pain in immune-mediated neuropathies will definitely elucidate the role of inflammation and immune response in neuropathic pain.
Background and Aims:

Infarction in the territory of the anterior spinal artery classically manifests with bilateral segmental lower motor neurone and pyramidal signs, loss of pain and thermal sensation and preservation of touch and proprioception below the level of injury.

Methods:

We describe two patients presenting with acute ipsilateral upper limb pain associated with flaccid paralysis and contralateral loss of thermal and pain sensation.

Results:

Two women, 22 and 24 year-old, had an acute unilateral pain associated with weakness of the right upper limb. Contralateral thermoalgesic sensory loss became evident later. Both patients were initially diagnosed as inflammatory myeloradiculopathies. MRI showed abnormal signal in anterior spinal artery territory unilaterally at C3-C5, and predominantly unilateral at C2-C5, respectively. Neurophysiological testing revealed hypoesthesia to innocuous temperature and thermal hypoalgesia contralateral to the pain and motor deficit, and denervation on the side of paralysis. Somatosensory evoked potentials were preserved. Contact heat evoked potentials were absent from the arm contralateral to the paralysis.

Conclusions:

The acute upper limb pain could not be attributed to a lesion of the ipsilateral dorsal root, or of the fibres crossing in the anterior commissure, since its large and small diameter afferents were largely spared both anatomically and functionally. Ischaemia of small diameter afferents entering through the affected ventral root is more likely. These afferents may supply cutaneous or visceral structures from the limb, or constitute the nervi nervorum. A map of the spinal artery territory shows its relation with this clinical presentation. Funding: Grant Fondecyt 1 120339.
Background and Aims:

Hyperalgesia and allodynia are typical signs of neuropathic pain. Quantitative sensory testing (QST) is a validated tool to clinically assess these phenomena. However, whether QST reveals findings that are reported by the patients is unclear. The aim of this study was therefore to investigate self-reported symptoms assessed with the painDETECT® questionnaire (PDQ) with results of validated QST.

Methods:

PDQ and QST data of 96 patients with chronic neuropathic pain were analysed. Questions upon presence of painful light touch, painful cold or heat, light pressure triggering pain as well as numbness upon PDQ were compared to findings of mechanical allodynia (DMA), increased sensitivity to heat, cold or pressure pain as well as loss of detection upon QST, respectively.

Results:

At least one PDQ question was rated clinically relevant (≥ 3, i.e. moderately, strongly or very strongly) and at least two pathological QST values were present in each patient. Self-reported pain symptoms upon PDQ showed only a small to moderate concordance with corresponding signs assessed upon QST. The positive and negative likelihood ratio to predict QST values with PDQ scores did not reach convincing values.

Conclusions:

Results demonstrate that self-reported PDQ symptoms cannot predict abnormal QST values. The poor predictive power of the PDQ may depend on several factors based on possibility of comparison between PDQ and QST and also on methodical issues. PDQ can therefore not replace QST in the clinical assessment of signs in neuropathic pain.
Background and Aims:

Background  By definition NeuP requires a lesion to any part of the somatosensory system. But it is unknown if injury to the most distal portion of sensory small fibres due to a primary dermatological disorder can cause NeuP. Recessive Dystrophic Epidermolysis Bullosa, (RDEB) is a rare condition in which mutations of proteins of the dermo-epidermal junction lead to cycles of blistering followed by regeneration of the skin. Damage is exclusive to the skin with no known direct compromise of the nervous system. RDEB patients experience daily pain that is usually refractory to conventional treatment.

Aims  we investigated the incidence of NeuP and examined the presence of nerve dysfunction in 29 RDEB patients.

Methods:

Adult patients were recruited from Debra charity.

Results:

Three-quarters of patients presented with pain of neuropathic characteristics that had a length dependent distribution. QST of the foot revealed impairment in thermal detection thresholds combined with an increased mechanical pain sensitivity and wind up ratio. Skin biopsy showed a significant decrease in intraepidermal nerve fibre density. Autonomic C fibres were functionally intact except those in the skin, where the sympathetic skin response was impaired and sweat gland innervation was reduced. Nerve conduction studies showed normal large fibre sensory and motor nerve conduction.

Conclusions:

Chronic cutaneous injury can lead to dysfunction of the most distal part of small sensory fibres in a length dependant distribution resulting in disabling NeuP. These findings support the use of NeuP screening tools and targeted treatment in these patients.
Chronic pain is a complex phenomenon and two patients with an apparent similar clinical tableau could in fact result from totally different mechanisms that will not respond to the same treatment. The most important challenge is to find a way to better target patients who will respond to a specific treatment. Being able to phenotype chronic pain patients based on the mechanisms involved in their pathology seems to be the best way to reduce the trial-error process of current pain treatment algorithms. Recent studies have highlighted that patients presenting an increased excitatory mechanism (central sensitization by temporal summation) versus a reduced efficacy of inhibitory mechanisms (Conditioned Pain Modulation—CPM) will respond differently to different treatments.

Clinical tests for central sensitization (hyperalgesia, allodynia…) are available, while testing for CPM in a clinical environment is difficult and rarely performed and standardization is needed. During this talk, we will discuss the potential implication of the autonomic nervous system and the affective component of pain in modulating CPM. Finally, we will see that these endogenous mechanisms are very variable among healthy subjects and patients suffering from chronic pain and how we could include this information in a treatment plan. Potential clinical consequences and future needs will be discussed.
CONDITIONED PAIN MODULATION: LESSONS LEARNED, UNANSWERED QUESTIONS, FUTURE DIRECTIONS

NEUP7-0455
TECHNICAL PARAMETERS OF CPM METHODOLOGY; IMPORTANT CONSIDERATIONS FOR IMPROVING RELIABILITY AND REPORTING

D. Kennedy¹
¹Imperial College London, Pain Research- Department of Surgery & Cancer, London, United Kingdom

Conditioned pain modulation (CPM) is a psychophysical experimental measure of the endogenous pain inhibitory pathway in humans; the “pain inhibits pain” phenomena. CPM paradigms consists of the evaluation of a painful test stimulus followed by a second evaluation either at the same time as a distant, painful conditioning stimulus (parallel paradigm) or in series after the stimulus has been withdrawn (sequential paradigm). Pain inhibition is not universal but in most subjects the pain intensity experienced with the test stimulus will be reduced with exposure to the conditioning stimulus. To date, studies in healthy volunteers have investigated the magnitude of CPM effect for various test and conditioning stimuli and the reliability of numerous CPM paradigms. While CPM is emerging as an important prognostic factor in pain medicine the testing and reporting of CPM is not standardized. At present, there are no published normative data for CPM effect, there are inconsistencies in how CPM effect is determined and it is unclear what qualifies as a “normal range” effect. This talk will explore technical aspects of CPM testing and important considerations for the implementation of CPM methodology and reporting of results. Improving transparency and reducing risk of bias in CPM studies will strengthen the evidence for the reliability of CPM. Improvements in reporting will enable the comparison of findings between studies and facilitate the translation of healthy volunteer study findings to clinical populations.
Budapest Criteria – Not sufficient for CRPS diagnosis

The Budapest Criteria have been an important step forward for the diagnosis of CRPS in clinical practice and scientific studies. Unfortunately, they have been validated only against clinical entities which are easily discriminated from CRPS, like polyneuropathy or peripheral nerve injury. No validation, however, has been done against typical posttraumatic pain of another origin e. g. after limb fracture or persistent pain after limb surgery, neither against patients with somatoform or dissociative disorder or long lasting disuse, respectively. Additionally, the Budapest Criteria focus on neurological signs and symptoms and neglect the CRPS-associated alterations of joints, tendon and other deep structures, which are highly important for the clinical outcome. Joint contraction is not the same as disturbed motor skills! Also, the typical distal spread of signs and symptoms beyond the area of lesion has been ignored by the Budapest Criteria. Last but not least, the diagnosis is reduced to clinical exploration, the very important radiologic findings, particularly the 3-phase-szintigraphy (the assessment with the highest specificity) are ignored or only superficially described. In consequence the majority of reference to the German pain clinics are patients with pseudo-CRPS, always with reference to the Budapest criteria after knee arthroscopy, in children with longer disuse and so on.
EMERGING CELL-BASED THERAPY FOR NEUROPATHIC PAIN

NEUP7-0371
MESENCHYMAL STEM CELLS PREVENT AND REVERSE OPIOID TOLERANCE AND OPIOID-INDUCED HYPERALGESIA
J. Cheng¹
¹Cleveland Clinic, Pain Management Department, Cleveland, USA

More than 240 million opioid prescriptions are dispensed annually to treat pain in the US. The use of opioids is commonly associated with opioid tolerance (OT) and opioid-induced hyperalgesia (OIH), which limit efficacy and compromise safety. The dearth of effective way to prevent or treat OT and OIH is a major medical challenge. We hypothesized that mesenchymal stem cells (MSCs) attenuate OT and OIH in rats and mice based on the understanding that MSCs possess remarkable anti-inflammatory properties and that both OT and chronic pain are associated with neuroinflammation in the spinal cord. We found that the development of OT and OIH was effectively prevented by either intravenous or intrathecal MSC transplantation (MSC-TP), which was performed before morphine treatment. Remarkably, established OT and OIH were significantly reversed by either intravenous or intrathecal MSCs when cells were transplanted after repeated morphine injections. The animals did not show any abnormality in vital organs or functions. Immunohistochemistry revealed that the treatments significantly reduced activation level of microglia and astrocytes in the spinal cord. We have thus demonstrated that MSC-TP promises to be a potentially safe and effective way to prevent and reverse two of the major problems of opioid therapy.
Recent studies have demonstrated many commonalities in the mechanisms underlying nerve injury-induced neuropathic pain and itch. In a series of preclinical studies, we reported that intraspinal transplantation of precursors of embryonic cortical GABAergic interneurons from the medial ganglionic eminence (MGE) reduced mechanical hypersensitivity in both traumatic nerve injury and chemotherapy-induced models of neuropathic pain in the mouse. The same approach is effective against scratching and resolved skin lesions in the Bhlhb5 mutant mouse model of neuropathic itch. By electron microscopic and spinal cord slice physiology we find that the transplanted cells and the host spinal cord are remarkably plastic, allowing for extensive integration of the transplanted cells into host circuits. Importantly, optogenetic stimulation of transplanted cells demonstrated that the inhibitory effects are GABA-A receptor mediated. We now show that increasing GABAergic signaling, either by spinal cord transplant of MGE cells or pharmaco logically, not only reduces scratching produced by a variety of pruritogens, but also alleviates itch in a model of a non-neuropathic, inflammatory skin disease. Specifically, transplantation of progenitor cells dramatically resolved skin lesions and reduced spontaneous scratching in a transgenic mouse model of atopic dermatitis, which is driven by overexpression of the Th2 cell-associated cytokine, IL-31. Systemic injections of GABA-A (muscimol) or B (baclofen) agonists recapitulated the transplant effects against both acute and chronic itch. Finally, demonstrating a pharmacological approach to management of pruritis, we show that subthreshold doses of baclofen and muscimol synergize in their antipruritic effects against both acute and chronic itch, without enhancing sedation.
NEUP7-0383
BONE MARROW STROMAL CELLS INHIBIT NEUROPATHIC PAIN VIA TGF-BETA SECRETION
R.R. Ji1, G. Chen1, Y. Huh1
1Duke University Medical Center, Anesthesiology, Durham, USA

Neuropathic pain after nerve injury and chemotherapy remains a pressing clinical problem. Here we report an effective way to control neuropathic pain in animal models via stem cell therapy. A local, intrathecal injection of bone marrow stromal cells (BMSCs) following lumbar puncture alleviates early and late phase neuropathic pain symptoms, such as allodynia and hyperalgesia, for several weeks in mouse models of neuropathic pain after chronic constriction injury (CCI), spared nerve injury (SNI), and chemotherapy-induced peripheral neuropathy (CIPN). Moreover, intrathecal BMSCs reduced CCI-induced spontaneous pain and axonal injury of dorsal root ganglion (DRG) neurons, and inhibited CCI-evoked neuroinflammation in DRG and spinal cord tissues. Remarkably, BMSCs secreted TGF-β1 into the cerebrospinal fluid, and neutralization of TGF-β1, but not IL-10, reversed that the analgesic effect of BMSCs. Conversely, intrathecal administration of TGF-β1 potently inhibited neuropathic pain. TGF-β1 acted as a powerful neuromodulator and rapidly (within minutes) suppressed CCI-evoked spinal synaptic plasticity and DRG neuronal hyperexcitability via TGF-β receptor-1-mediated non-canonical signaling. Finally, intrathecally injected BMSCs migrated to the injured DRGs via a chemotaxic axis. Nerve injury upregulated CXCL12 in lumbar L4-L6 DRGs, and this up-regulation caused migration of intrathecally injected BMSCs to DRG through the CXCL12 receptor CXCR4, which was expressed on BMSCs. BMSCs that migrated from the injection site survived at the border of DRG for several months. Our findings support a paracrine mechanism by which intrathecal BMSCs target damaged DRGs to elicit neuroprotection and sustained neuropathic pain relief via TGF-β1 secretion.
Pain is the most variable manifestation of carpal tunnel syndrome (CTS) with some patients experiencing subjectively severe pain when other measures would suggest that the disease is mild while, at the other extreme, patients with objective evidence of severe median nerve impairment may experience no pain. Nor is this a simple inverse correlation, the severity and frequency of pain in CTS appear not to be correlated with other aspects of the disease in smaller studies, and the distribution of pain does not follow the standard anatomy of the median nerve. Even though CTS is considered a nerve disorder it is unclear whether CTS pain is primarily of peripheral neurogenic origin or arises in other tissues at the wrist, and the role of central mechanisms in modifying the presentation is uncertain. Notwithstanding the lack of utility of pain as a diagnostic entity in the individual patient, in very large samples (24613) of CTS patients there are patterns in the occurrence of pain which may help us to explore its causation. There are some confounding variables. Subjective pain scores tend to be higher in women and in the dominant hand. They decrease with age. Pain increases significantly with increasing severity of neurophysiological impairment, though the correlation is weak, but in the most severely impaired median nerves there is a decrease in average pain, predominantly due to a reduction in night pain and tingling while daytime pain does not show this effect. Pre-treatment pain may be a valid prognostic indicator.
Entrapment neuropathies are commonly believed to exclusively affect the large fibre population, which explains the clinical reliance on large fibre tests (e.g., electrodiagnostic studies) in the assessment of patients with suspected entrapment neuropathies. These beliefs were recently challenged by findings from both an animal model of mild chronic nerve compression as well as from translational work in patients with lumbar or cervical radiculopathy and carpal tunnel syndrome. I will present the compelling evidence for small fibre dysfunction and structural degeneration and their clinical relevance in the diagnostic evaluation of patients with entrapment neuropathies. I will also discuss our most recent work which uses carpal tunnel syndrome as a unique model system that allows the prospective evaluation of neural regeneration capacity following surgical decompression. Our data suggest that somatosensory nerve function largely recovers (e.g., quantitative sensory testing), whereas structural regeneration of small fibres as determined in skin biopsies remains incomplete. Using RNA sequencing, we are currently determining whether a molecular signature is associated with neural regeneration.

A better understanding of neural dysfunction and its regeneration capacity in patients with entrapment neuropathies will not only assist the assessment of these patients, but will also facilitate the development of novel treatment strategies.
Persistent post-surgical pain following carpal tunnel decompression is not uncommon however there is little evidence for the prediction of surgical outcome in this group of patients. Patients with carpal tunnel may suffer with chronic pain and paraesthesia prior to undergoing surgery therefore it is possible that pain processing may become altered or maladaptive. We hypothesized that peripheral sensitization and deficient endogenous pain inhibition pre-surgically were associated with persistent postsurgical pain. Novel to an entrapment neuropathy, we used quantitative sensory testing (QST), conditioned pain modulation (CPM), neuropathic pain symptoms, pain intensity and interference to describe pre-surgical pain phenotype and investigated the association of phenotype with surgical outcome and persistent postsurgical pain. The identification of potentially modifiable targets in patients with entrapment neuropathies may in the future enable pre-surgical management to improve postsurgical outcome.
Determining small fibre rarefaction - is skin punch biopsy (in)dispensable?
It is becoming clear that effective treatments have perhaps failed not due to a lack of efficacy, but because of problems with the end points to detect improvement in clinical trials of diabetic neuropathy. Organizations such as the ADA and PNS as well as the FDA continue to advocate symptoms/signs and electrophysiology as primary endpoints and skin biopsy with assessment of intra epidermal nerve fibre density (IENFD) as an end point for assessing small fibres. Yet trial after trial has failed using symptoms and signs and neurophysiology and even though IENFD is an invasive procedure requiring sophisticated laboratory techniques for assessment, it has also failed as an end point in a number of clinical trials of a range of peripheral neuropathies. We and others have shown that corneal confocal microscopy (CCM), a rapid non-invasive ophthalmic technique can quantify early small nerve fibre damage with good sensitivity and specificity in a range of peripheral neuropathies including diabetic neuropathy, Fabry disease, CMT1A, Friedreich’s ataxia, Amyloid neuropathy, HIV neuropathy, CIPN and CIDP. We have shown that corneal nerve damage assessed using CCM has a better diagnostic sensitivity and specificity than IENFD for diabetic neuropathy. Of significant relevance arguing for CCM as a FDA end-point we have shown corneal nerve fibre regeneration within 6 months of pancreas transplantation in diabetic patients even though IENFD does not change. We have also shown corneal nerve regeneration following treatment with ARA290, a novel derivative of erythropoietin, with no change in IENFD. More recently we have shown an improvement in CCM abnormalities after 28 days of treatment with ARA290 which was associated with an improvement in GAP-43 IENF morphology, but not IENFD. CCM therefore represents a simple, non-invasive measure which fulfills all the FDA criteria for a surrogate end-point.
EVOKED POTENTIALS FOR DIAGNOSING SMALL FIBER DISEASE: COOL OR OUTDATED?

NEUP7-0390
(Wh)Y SHOULD WE COMBINE CONTACT HEAT (CHEPS) AND PINPRICK EVOKED POTENTIALS (PEPS)?

U. Baumgärtner

1Heidelberg University, Department of Neurophysiology Center for Biomedicine and Medical Technology Mannheim CBTM, Mannheim, Germany

Brief noxious heat stimuli excite thinly myelinated type II A-delta fibers (AMH II), which was demonstrated in monkey skin using laser and contact heat stimuli. For excitation of these fibers, heat thresholds are relatively low and the discharges adapt rapidly. A second class of A-fiber nociceptors has higher temperature thresholds and is adapting less. The latter are termed type I A-fiber-mechano-heat nociceptors (AMH I) and mainly respond to mechanical (pinprick) stimuli. By using specific stimuli, like noxious heat or pinprick stimuli, the function of these subclasses of A-fiber nociceptors can be addressed separately with, e.g., contact heat evoked potentials (CHEPS) or pinprick evoked potentials (PEP). Depending on the individual symptoms of a given patient, differentiation between involvement of one – or both – class(es) of A-fibers could bring additional information in the diagnosis of small fiber neuropathy. Since the innervation of hairy and glabrous skin is not the same (AMH II fibers seem to be missing in glabrous skin), neurophysiologic testing with PEPs might prove useful especially in glabrous skin.
Because most studies attribute neuropathic pain to nociceptive system damage, the best tools for investigating patients with neuropathic pain are diagnostic tests selectively assessing the nociceptive system.

According to the guidelines issued by the International Federation of Clinical Neurophysiology, and the Neuropathic Pain Special Interest Group of the IASP, laser evoked potentials (LEPs) related to Ad-fibre activation are the easiest and most reliable neurophysiological technique for assessing nociceptive system function.

Laser-generated radiant heat pulses selectively excite free nerve endings in the superficial skin layers and activate Aδ and C nociceptors. Although laser stimuli activate both Aδ and C fibres, scalp potentials related to C-fibre activation (C-LEPs), can be obtained only with dedicated techniques that have not yet been standardised for clinical application. Hence the commonly studied LEPs are those related to Aδ-fibres activation. LEPs consist of a lateralized component (N1) and a vertex potential consisting of a N2–P2 complex.

In diseases associated with nociceptive-pathway damage, LEPs can be absent, reduced in amplitude or delayed in latency. Previous studies have shown that in patients with neuropathic pain related to peripheral and central nervous system diseases (postherpetic neuralgia, carpal tunnel syndrome, polyneuropathy and multiple sclerosis) the severity of ongoing burning pain is related to LEP and amplitude. This relationship—although only an indirect finding in some instances—indicates that ongoing burning pain is strongly associated with damage to the nociceptive system.
Nerve injury leads to devastating and often untreated neuropathic pain. While acute pain perception (nociception) is conserved across phyla, much less is known about the evolution of neuropathic pain. Here we show that after peripheral nerve injury, the fruit fly *Drosophila melanogaster* exhibits sensory neuropathy leading to a long-lasting neuropathic sensitization. While loss of acute nociception in healthy animals extended lifespan, after injury sensitization-resistant animals die much faster, suggesting that nociception could be both beneficial and detrimental depending on animal health. Mechanistically, excitotoxic output from damaged peripheral nociceptive neurons triggered a loss of central GABAergic interneurons via the conserved basic helix-loop-helix transcription factor *twist*, resulting in a global sensitization of the nociception escape circuit. This is the first molecular insight into neuropathic sensitization in invertebrates, and the mechanisms governing neuropathic pain appear to be conserved from flies to mammals.
GENETIC AND EPIGENETIC REGULATION OF NEUROPATHIC PAIN

NEUP7-0422
EPIGENETICS AND PAIN - WHAT DO WE ACTUALLY KNOW

F. Denk
King’s College London, Biomedical Health and Sciences, London, United Kingdom

This talk will introduce attendees to the study of pain epigenetics. Why is it interesting? How has it furthered our understanding of neuropathic pain development? Will it aid the search for new treatments? And what are some of the common problems associated with this fledgling field? This session will seek to provide the answers and equip the audience with the necessary knowledge to keep up with what is a rapidly evolving area of research.
HOW CAN WE ACCELERATE CLINICAL ADVANCES FROM NEUROPATHIC PAIN RESEARCH?

NEUP7-0316
WASTE IN PRE-CLINICAL NEUROSCIENCE RESEARCH WITH A FOCUS ON NEUROPATHIC PAIN

M. MacLeod¹
¹, United Kingdom

There is increasing focus on shortcomings in the design, conduct, analysis and reporting of in vivo experiments testing the efficacy of candidate drugs; and how these shortcomings may go some way to explaining the failure of efficacy which has been observed in animal studies to translate to clinical trials.

Problems include risks of bias arising from suboptimal study designs; underpowered studies; publication bias; flexibility in data analysis; primary outcome switching and hypothesising after results are known (“HARKing”).

A hierarchy of approaches to improve the reliability of in vivo studies might include:

Level 1: Study reports comply with existing guidelines such as the ARRIVE guidelines, so that there is transparency in what was done

Level 2: Studies are conducted taking appropriate measures to reduce the risk of bias, such as randomisation, blinded conduct of the experiment and blinded assessment of outcome; and are planned on the basis of a coherent sample size calculation

Level 3: Study protocols, including statistical analysis plans, are determined in advanced and are archived such that research users can check where the study as executed deviated from the study as planned

Level 4: The existence of a study is asserted through some system of registration, to address the issue of publication bias

Level 5: The study is planned to have an appropriate positive predictive value, based on the likelihood of refuting the null hypothesis, the statistical power and the chosen Type 1 error; and this is asserted in advance, to avoid misinterpretation

Level 6: Formal strategies to assess the burden of evidence in favour of efficacy are developed, including but not limited to systematic review and meta-analysis of existing evidence and a GRADE-like approach to assess the strength of evidence

Level 7: Where the in vivo data appear promising, to develop tools for multicentre animal studies to confirm effects in "preclinical phase 3 studies"

While these approaches may at first sight appear burdensome, and difficult to enable even in a pre-competitive commercial environment, there are simple measures which could lead to substantial improvements. Ethical imperatives relating both to the use of animals in research and to the exposure of human study subjects to potential harms dictate that as a community we need to do our best to increase value and reduce waste in research involving animals.
Although not unique to neuropathic pain, the poor track record of successful translation of pre-clinical research into new therapies is complex, multifactorial, and an area of active discussion. The Lancet has recently commenced a high-profile initiative on scrutinising the efficiency of current biomedical research “Research: Increasing Value, Reducing Waste” (http://www.thelancet.com/series/research). This initiative asserts that 85% of biomedical research funding is not used efficiently (or “wasted”) and posed a number of questions in order to elucidate potential solutions:

- Are research questions relevant to researcher users and knowledge users?
- Is research appropriately designed, conducted and analysed?
- Is research regulation efficient and effective?
- Are full reports of research accessible?
- Are research reports unbiased and useable?

Dr. Gilron, an expert in clinical neuropathic pain research, will reflect on this topic from the clinical research perspective.
Patients suffering from neuropathic pain complain of sensory deficits and different types of pain. Neuropathic pain may be ongoing, such as burning pain, or paroxysmal, such as electrical-shock-like sensations, or provoked by various stimuli, e.g. gentle brushing (i.e. dynamic mechanical allodynia). Although in some neuropathic pain condition specific types of pain may predominate, none of them are aetiologic specific. Clinical and neurophysiological studies indicate that the different types of pain arise from distinct mechanisms. In patients with peripheral and central nervous system diseases the severity of ongoing burning pain is related to laser evoked potential amplitude changes, thus indicating that this type of pain is strongly associated with damage to the nociceptive system. Conversely in patients with paroxysmal electrical shock-like pain neurophysiological studies show damage to the non-nociceptive large myelinated fibre pathways. Dynamic mechanical allodynia has been associated with a relative preservation of nociceptive system as assessed with laser evoked potentials.

Neurophysiological techniques can be therefore useful for dissecting the mechanisms underlying the different neuropathic pain symptoms.
Neuropathic pain arises as a consequence of a lesion or disease affecting the somatosensory system [1]. The diagnosis of a neuropathy is, based on tests demonstrating only the sensory deficits. Confirming sensory loss to one of the sensory modalities (e.g. light touch, cold or warm temperature etc.) and delineation of the area affected by the negative sensory phenomena are central to the determination as to whether a nervous system lesion is the cause of the sensory disturbance (i.e., whether it is compatible with neuropathy) [1]. Nerve conduction studies and somatosensory evoked potentials assess only the function of thick-myelinated fibers. In contrast, the function of the unmyelinated and thinly myelinated afferent nerve fibers can be assessed by quantitative sensory testing (QST), whereas morphological alterations and a rarefication of the nerve fibers can be depicted by analysis of the intraepidermal nerve fiber density in skin biopsies and more recently also by confocal microscopy of corneal nerve fibers (CCM, corneal confocal microscopy) [2].

QST is a psychophysical method used to quantify somatosensory function in healthy subjects and patients, based on measurements of responses to calibrated, graded innocuous or noxious stimuli (generally mechanical or thermal). As a psychophysical method, the validity and reliability of QST can be strongly influenced by different factors. For performing QST in healthy subjects and in patients, it is recommended to use predefined standardized stimuli and instructions, validated testing algorithms, and reference values corrected for anatomical site, age, and gender. Furthermore, interpretation of results should always take into account the clinical context, and possible limitations (language and cognitive difficulties, anxiety, litigation, no differentiation between peripheral and central nervous system damage) should be considered [3].

QST has been initially developed for research purposes to explore the underlying mechanisms of different pain and neuropathy states. Several studies, e.g. [4], have demonstrated different combinations of sensory changes in neuropathic pain states, indicating different mechanisms, independent of the underlying etiology. A recent study was able to identify three distinct subgroups with characteristic sensory profiles, corresponding to specific neuropathic pain mechanisms [5]. Stratifying patients according to the sensory profile can help in treatment decision, as in a recent prospective, randomized, double-blind trial and in most retrospective group analyses, certain sensory findings and especially the presence of hyperalgesia prior to treatment was shown to be a predictor for better treatment response [6], for review see [7]. Furthermore, the stratification of patients based on the sensory phenotype can improve the outcome of clinical trials [7]. Additionally, QST can be used for treatment monitoring, e.g. of topically applied medication or other specifically acting drugs.

The present lecture will discuss current studies on the practical use of QST in the clinical routine and on its application in the diagnostic procedures and in the treatment decisions in neuropathic pain.


Skin punch biopsies have become an important tool in the diagnostic assessment of neuropathic pain conditions. The minimally-invasive technique is well tolerated by patients and can be performed easily and repetitively without relevant side effects. Particularly the quantification of intraepidermal nerve fibers using the pan-axonal marker protein gene product-9.5 may give important hints on the involvement of small caliber nerve fibers in pain conditions. In the meantime, further techniques have been developed e.g. for the assessment of inflammation and potential demyelination that extend the diagnostic possibilities of skin punch biopsies. With the increasing number of studies investigating painful and also painless conditions using skin punch biopsies, data on diverse skin biopsy findings is increasing. Cautious interpretation of these results is necessary, since intraepidermal nerve fiber density usually does not correlate with pain and the pathomechanisms underlying alterations in skin innervation in diverse painful conditions need to be elucidated. In this presentation, current knowledge on skin biopsy findings in focal and generalized painful conditions will be summarized and potentials and limitations of skin biopsy assessments will be discussed.
It has been proposed that Complex Regional Pain Syndrome (CRPS) is a post-traumatic autoimmune disease, and we have previously observed that antibody producing B cells are required for the full expression of CRPS-like changes in a mouse tibia fracture/cast immobilization CRPS model. The current studies used the mouse model to evaluate the progression of post-fracture nociceptive and inflammatory changes in wildtype (WT) and muMT fracture mice lacking B cells. The pronociceptive effects of injecting WT fracture mouse serum antibodies into muMT fracture mice were also evaluated as were the effects of CRPS patient’s serum. Serum-derived IgM antibodies from WT fracture mice and CRPS patients had pronociceptive effects in the fracture limb when injected into muMT fracture mice. IgM antibody levels in model mice gradually increased in the fracture limb hindpaw skin, sciatic nerve, and ipsilateral lumbar cord, peaking at about 12 weeks post-fracture and then declining. Immunohistochemistry localized post-fracture IgM antibody binding to antigens in the fracture limb hindpaw dermal cell nuclei. The use of liquid chromatography-mass spectroscopy helped to identify several autoantigens reactive with mouse and human CRPS sera. We postulate that fracture induces the expression of novel antigens in the fracture limb skin, sciatic nerve, and spinal cord, which trigger B cells to secrete IgM autoantibodies that bind those antigens and initiate a pronociceptive response. Autoimmunity plays a key role in the progression of nociceptive and vascular changes in the mouse fracture model, and we postulate that it is also a crucial contributor to the CRPS disease process.
IMMUNOLOGICAL MECHANISMS SUPPORTING CRPS: BASIC, TRANSLATIONAL AND CLINICAL EVIDENCE

NEUP7-0402
IMMUNOGLOBULIN G MEDIATED NON-INFLAMMATORY AUTOIMMUNITY IN LONGSTANDING CRPS

A. Goebel¹, Z. Helyes²

¹Translational Medicine/University of Liverpool- Walton Centre NHS Foundation Trust, Pain Medicine, Liverpool, United Kingdom
²Pharmacology, University of Pecs, Pecs, Hungary

IMMUNOGLOBULIN G MEDIATED NON-INFLAMMATORY AUTOIMMUNITY IN LONGSTANDING CRPS

Background: That immunoglobulin G autoantibodies can cause disease through non-inflammatory pathways has long been recognised in other medical disorders, such as Grave’s disease, but the concept that regional autoimmunity can predominantly cause a symptom, such as chronic pain, is new. Most patients with CRPS experience some improvement during the first 18 months after the CRPS-inciting event, however in about 20% high pain intensity persists, and the causes for this, as for the condition in general, have been unclear. We hypothesised that these patients may continue to produce pathogenic immunoglobulin G autoantibodies. Our earlier work had suggested that the passive transfer of patient serum-immunoglobulin G elicits strictly unilateral, enhanced mechanical hyperalgesia and swelling in hind paw injured (skin-muscle incision) mice, suggesting a pathogenic role for these proteins¹. However, we had limited serum available for these experiments, and had only injected on days -1, 0, 4, and 5 (day 0 = day of the injury). The observed effects were modest and short-lasting, and the mechanisms underpinning them were unknown.

Methods: We purified immunoglobulin G off waste-plasma from patients (n=6) who underwent plasma exchange treatment², and from healthy controls. Having thus abundant serum immunoglobulin G available, we now injected patient-, or control-IgG (non-pooled), or saline intraperitoneally, starting with the morning of the hind paw injury, and then daily. Following behavioural measurements, we sacrificed the animals in successive experiments starting with day 1, up to day 14 post-injury, and harvested paws and spinal cord/brain tissues. We measured local cytokines and neuropeptides, and astrocyte/microglia cell activation/numbers in the spinal cord dorsal horn, periaqueductal grey, or somatosensory cortex. For some experiments, we treated the animals with steroids.

Results: Repeated, daily injection of CRPS immunoglobulin G invariably caused profound, augmented hind paw mechanical hyperalgesia compared with healthy, which persisted through the experimental period and appeared to deepen at the later time points. In contrast, initially enhanced paw swelling in the CRPS groups normalised over time, although there was variability between the preparations. There was no correlation between the degree of hyperalgesia and the degree of swelling, suggesting that more than one type of autoantibody might be involved. Brain glial cell activation was sensitive to the incision injury, however the CRPS injections caused strong additional activation, compared with healthy, which in magnitude far exceeded the incision-induced changes. There was no evidence that CRPS-IgG caused an enhanced inflammatory response in the paws, when compared with healthy/saline. Steroid treatment over five days initially suppressed the augmented behavioural response, but this effect appeared to be only temporary.
Discussion: Our results suggest that an enhanced immunoglobulin G passive transfer-trauma model is reliable and valid for use in mechanistic and therapy-related research about persistent CRPS. Circulating CRPS immunoglobulin G cause strictly unilateral paw sensitivity and glial cell activation, which is stable over time, without eliciting enhanced peripheral inflammation. A two-hit phenomenon applies, whereby the paw/limb trauma, perhaps through its associated inflammation, changes the local environment rendering circulating immunoglobulin G auto-antibodies pathogenic, however steroid therapy does not completely appear to suppress this process. Given the established involvement of the central nervous system in patients with CRPS the observed strong central glial cell activation in the animals points towards an opportunity to apply this model to study these features. Non-inflammatory CRPS immunoglobulin G may be pathogenic either through direct binding to sensory peripheral nerves, consequently changing transduction or transmission properties, e.g. through receptor cross-linking. Alternatively nearby cells might be stimulated to produce signalling mediators which can activate these nerves. Whether there is also direct binding to central nervous system tissues should also be investigated.

Bibliography

Basic, translational and clinical evidence for innate immune mediators in CRPS

Mice with impaired innate immune system activation (TLR 2 and TLR 4⁻/⁻ mice) develop less inflammatory symptoms (edema, warmth) after fracture in the animal fracture model as model of CRPS. However, nociceptive behavior (mechanical allodynia and weight bearing of the affected paw) developed similar to the wild type animals. These data indicate that the innate immune system seems to be involved in the development of the initial inflammatory changes in the mouse fracture model, but are less important for the development of nociceptive behavior.

These data will be compared with data from human skin biopsies showing an evidence of the involvement of the innate immune system in CRPS. Keratinocytes and mast cells are both cells of the innate immune system and significantly increased in CRPS. The expression of pro-inflammatory cytokines like TNFα and IL-6 was enhanced in keratinocytes. The role of the innate versus adaptive immunity in the development of CRPS and the implications for the therapy of CRPS are discussed.
We often think that the optimal result of a randomized clinical trial (RCT) is a “positive” one. However, the best results are those that accurately represent the true treatment effect. RCTs are considered the gold standard when evaluating causal treatment effects, but the accuracy of the results depends a great deal on the execution of the trial. Important methodological features that should be considered when designing RCTs for pain treatments include: (1) the choice of primary outcome measure, (2) strategies to improve accuracy of pain reporting, (3) prespecification of primary outcome measures and analyses, and (4) interpretation of confidence intervals. This presentation will focus on the ways in which careful consideration of these features can improve assay sensitivity (i.e., the ability of the trial to demonstrate a treatment effect if one truly exists). It will also cover how greater attention to RCT methodology may advance the field of pain by avoiding false results, thereby preventing both premature abandonment of clinically promising treatments and the misuse of time and financial resources on treatments that will ultimately fail more rigorous testing or be used clinically without actual benefit. References for further reading regarding these and other design issues will be highlighted.
CASPR2 is a neurexin-like molecule important for cell to cell interactions within the nervous system. It can be found at the juxtaparanode of myelinated fibres where it associates with and clusters certain voltage gated potassium channels for example kv1.1 and 1.2; two channels known to regulate sensory neuronal excitability. There are a number of neurological conditions caused by autoantibodies (auto-Abs) targeting proteins within the voltage gated potassium channel complex (VGKC-complex) and some of these conditions are associated with peripheral nerve hyperexcitability and neuropathic pain. CASPR2 is one such antigenic target and the presence of auto-Abs against this protein positively correlates with the prevalence of pain which can be relieved by immunotherapy. To investigate the role of CASPR2 in regulating sensory function we initially studied mice lacking CASPR2 (CNTNAP2−/−) and found that they displayed increased pain-related behaviour, particularly to mechanical stimuli. DRG neurons from CNTNAP2−/− mice were hyperexcitable and also exhibited a reduced Kv current. Immunostaining found a decrease in membrane Kv1.2 in DRG neurons from CNTNAP2−/− mice, suggesting that CASPR2 can regulate neuronal excitability via the trafficking of VGKCs. Passive transfer of patient CASPR2 auto-Abs to mice resulted in mechanical pain-related hypersensitivity. There was no evidence of any gross inflammatory response or structural damage to the nervous system caused by these auto-Abs. Instead however, when applied in culture CASPR2 auto-Abs bound DRG neurons and induced hyper-excitability due to the internalisation of VGKCs. These data show the passive transfer of an autoimmune pain disorder and highlight the role of CASPR2 in regulating the sensory neuronal excitability.
KV1 CHANNELS AS MODULATORS OF HYPEREXCITABILITY IN NEUROPATHIC PAIN

NEUP7-0378
ALTERED POTASSIUM CHANNEL DISTRIBUTION AND COMPOSITION IN MYELINATED AXONS SUPPRESSES HYPEREXCITABILITY FOLLOWING INJURY
M. Calvo

Biological Sciences Faculty. Pontificia Universidad Catolica de Chile, Physiology/Pain, Santiago, Chile
Altered shaker-type Kv channel composition and distribution in myelinated axons suppresses hyperexcitability in neuropathic pain

Margarita Calvo, Natalie Richards, Alejandro Barroso, Lan Zhu, Dinka Ivulic, Ning Zhu, Philipp Anwandter, Manzoor A. Bhat, Stephen B McMahon, and David LH Bennett

Aim of Investigation

Neuropathic pain (NP) following peripheral nerve injury is associated with hyperexcitability in damaged myelinated sensory axons, which begins to normalise over time. Understanding the mechanisms that suppress hyper-excitability will potentially provide insight as to why in certain patients such mechanisms fail leading to chronic pain states. Shaker type voltage-gated potassium channels (Kv1 channels) are key determinants of neuronal excitability. However, in normal conditions they do not contribute to axon conduction properties, as they are located in the juxtaparanode where they are electrically insulated from the node of Ranvier. But, during development and following primary demyelination Kv1 channels are redistributed into the paranode, and can act to suppress excitability. Here we sought to determine if Kv1 channels play any role in the adaptive mechanisms to suppress hyperexcitability that follows nerve injury.

Methods

We investigated the composition and distribution of Kv1 channels within the nodal complex of myelinated axons in a neuroma model and the SNT model in rats using immunofluorescence, western blots, and electron-microscopy. In the same way we investigated the expression of Kv1 channels in patients with Morton neuroma and in control subjects. The functional role of Kv1 channels was studied in the rat neuroma model using electro physiology and behavioral experiments by applying a Kv1 channel blocker (αDTX).

Results

At the site of neuroma in the rat, expression of Kv1.1 and 1.2 (normally localised to the juxtaparanode) was markedly decreased. In contrast Kv1.4 and 1.6, which were hardly detectable in the naïve state, showed increased expression within juxtaparanodes and paranodes following injury. Within the dorsal root (a site remote from injury) we also noted a redistribution of Kv1 channels towards the paranode. In humans healthy control subjects Kv1.2 was normally localised to the juxtaparanode, while nerves from neuroma patients had very low expression of Kv1.2 and de novo expression of Kv1.4 and 1.6, which were present both in paranode and juxtaparanode. Blockade of Kv1 channels with αDTX after injury reinstated hyperexcitability of A-fibre axons and enhanced mechanosensitivity.

Conclusions

Changes in the molecular composition and distribution of axonal Kv1 channels, therefore represents a protective mechanism to suppress the hyperexcitability of myelinated sensory axons that follows nerve injury.
Cold allodynia, or painful hypersensitivity to innocuous cold, is a common symptom of neuropathic and inflammatory pain following peripheral nerve injury. The mechanisms underlying this disabling sensory alteration are not entirely understood. In primary somatosensory neurons, cold-sensitivity is mainly determined by a functional counterbalance between cold-activated TRPM8 channels and Shaker-like Kv1.1-1.2 channels underlying the excitability brake current $I_{KD}$. Here we studied the role of $I_{KD}$ in nerve damage-triggered cold allodynia. We found that cold allodynia induced by chronic constriction injury (CCI) of the sciatic nerve in mice, was related to both an increase in the proportion of cold-sensitive neurons (CSNs) in dorsal root ganglia contributing to the sciatic nerve, and a decrease in their cold temperature threshold. $I_{KD}$ current density was reduced in high-threshold CSNs from CCI mice compared to sham animals, with no differences in cold-induced TRPM8-dependent current density. The electrophysiological properties and neurochemical profile of CSNs revealed an increase of nociceptive-like phenotype among neurons from CCI animals compared to sham mice. These results were validated using a mathematical model of CSNs including $I_{KD}$ and TRPM8, showing that a reduction in $I_{KD}$ current density shifts the thermal threshold to higher temperatures, and that the reduction of this current induces cold-sensitivity in former cold-insensitive neurons expressing low levels of TRPM8-like current. Taken together, our results suggest that cold allodynia is largely due to a functional downregulation of $I_{KD}$ in both high-threshold CSNs and in a subpopulation of polymodal nociceptors expressing TRPM8, providing a general molecular and neural mechanism for this sensory alteration.

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The pathophysiology of neuropathic pain is incompletely understood. A crucial role of neuro-immune interactions is known from animal and human studies and here particularly the role of microRNA (miRNA) is increasingly focused on. miRNA are small non-coding RNA sequences that are involved in the regulation of multiple cellular processes including the cross-talk between the immune and the nervous system. Evidence mostly from experimental animal studies is increasing that distinct miRNA may be involved in the pathophysiology of neuropathic pain. Human studies investigating miRNA profiles in patients' biomaterial are also giving first promising results. The hope is to find a diagnostic and prognostic biomarker for neuropathic pain and new druggable targets for more efficacious novel treatment options. In this presentation current data on miRNA profiles in patients with different focal and generalized pain conditions including pain after peripheral nerve injury will be summarized and the diagnostic value of potential biomarkers will be discussed.
MicroRNAs AS BIOMARKERS AND REGULATORS OF NEUROPATHIC PAIN DISORDERS

NEUP7-0411
MIRNA CONTAINING EXOSOMES FOR NEUROIMMUNE COMMUNICATION IN THE NERVOUS SYSTEM

M. Malcangio1, R. Simeoli1, G. Olivero1
1King’s College London, Wolfson CARD, London, United Kingdom

Exosomes are extracellular vesicles which are secreted by all types of cells, including immune cells and neurons, and mediate intra- and inter-cellular communication. Exosomes derive from multivesicular bodies (MVBs) and secretory exosomes contain a specific cargo composition including a variety of microRNAs (miRs). Recent evidence indicates a significant dysregulation of miRs in the dorsal root ganglia (DRG) and spinal cord after peripheral nerve injury.

We evaluated whether sensory neuron cell bodies in the DRG and dorsal horn (DH) nerve terminals released exosomes and miRs under depolarizing- or noxious like-conditions.

Incubation with KCl resulted in accumulation of exosomes and miRs (miR-21, Let-7b and miR-124) in culture media of both DRG neurons and DH synaptosomes. Similarly, stimulation with capsaicin induced significant release of exosomes and miRs in a time-dependent fashion in media collected from cultured DRG neurons and DH synaptosomes.

However, whilst both DRG neurons- and DH synaptosomes-derived exosomes expressed TSG101 and Flotillin-1, only DRG neurons expressed the opsonin MFG-E8 which acts as eat-me signal for phagocytic cells. We know that macrophages contribute to the mechanisms underlying neuropathic pain, at the site of nerve damage in the periphery and in the DRG whereas microglia are critical players in the dorsal horn of the spinal cord. Therefore, we are currently assessing whether synaptomes and/or sensory neuron-derived exosomes-containing miRs are phagocytosed by macrophages and/or microglia and mediate cellular communication under neuropathic pain conditions.

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miRNAs associated with the painful diabetic neuropathy

Diabetes is one of the leading causes of peripheral neuropathy. It is estimated that 10-20% of diabetic patients have ongoing pain while 50-60% of diabetics with peripheral neuropathy have ongoing pain. The mechanisms specific to pain in diabetic neuropathy have not been identified which is directly reflected in the unavailability of specific treatment options for painful diabetic neuropathy (PDN). Therefore, it is a very important open question about the factors leading to PDN in only a subset of diabetic individuals. Owing to their capacity to regulate a set of disease-associated transcripts, microRNAs (miRNAs) constitute a novel class of therapeutic targets. In the current study, we performed a genome-wide screen throughout the somatosensory cortex - dorsal root ganglion (DRG), spinal dorsal horn (SDH) and cerebral cortex (Brain) - to identify miRNAs associated with the hypersensitivity developing and establishing phases in the diabetes conditions. Following an extensive in silico analyses, an enriched list of 55 miRNAs was prioritized for further investigation and the regulation of several of them was confirmed by quantitative PCR. Fluorescence In situ Hybridization for miRNA combined with immunofluorescence for protein markers (FISH-IF) was performed to investigate cellular localization of prioritized miRNAs. In order to facilitate long-term overexpression or inhibition of selected miRNA to investigate its impact on diabetes-mediated hypersensitivity, we developed novel viral tools. Current efforts are dedicated to elucidate functional relevance of prioritized miRNA candidates and to characterize their mechanism of action by identifying their precise mRNA targets.
MOLECULE TO MAN APPROACHES TO INVESTIGATE PATHOPHYSIOLOGICAL MECHANISMS AND TREATMENT OF NEUROPATHIC PAIN

NEUP7-0032
PAINFUL AND PAINLESS SODIUM CHANNELOPATHIES AND STRATEGIES FOR PERSONALIZED TREATMENT

S. Dib-Hajj

Center for Neuroscience and regeneration Research, NeurologyYale Sch Med., USA

A monogenic link of SCN9A, the gene that encodes sodium channel Na_{1.7}, and SCN11A, the gene encoding Nav1.9, to pain disorders in humans has provided compelling evidence that these channels are important contributors to the pathophysiology of pain in humans. Direct involvement of Nav1.7 in pain disorders is confirmed by the discovery of dominant gain-of-function mutations in two severe pain syndromes, inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD), while recessive loss-of-function mutations have been found in patients with congenital insensitivity to pain (CIP). Recently, gain-of-function mutations in Nav1.9 were identified in patients with familial episodic pain (FEP) as well as patients with painless injuries. While IEM, PEPD and FEP and painless injuries are rare disorders, they identified critical pathways for pain in humans, and suggested that more common variants might also be linked to peripheral neuropathies. We now provide genetic and functional studies which link Nav1.7, Nav1.8 and Nav1.9 to more common pain disorders. Electrophysiological characterization of mutations in these genes has now elucidated the molecular basis for altered excitability of dorsal root ganglion neurons that express these mutant channels, establishing a mechanistic link to human pain conditions. Approaches including atomic structural modeling and pharmacological testing in vitro have also proven useful to predict the response of neurons expressing specific Nav1.7 mutant channels to existing drugs, and the successful implementation of this strategy in a personalized clinical application. These findings validate peripheral sodium channels as targets for development of new pain therapeutics.
Genetic approach to painful peripheral neuropathies

Among painful neuropathies, small fiber neuropathy (SFN) is a distinct entity, in which predominantly the small thinly myelinated and unmyelinated A\(\delta\) and C nerve fibers are affected. SFN characteristically leads to severe neuropathic pain, and may be accompanied by autonomic dysfunction. Recent studies showed mutations in several sodium channels, though also other pain-related genes may be involved in the pathogenesis of painful small fiber neuropathy. Therefore, we need a reliable technique to rapidly and accurately re-sequence multiple genes at low cost, and we use a targeted enrichment kit to capture the coding and exon-flanking intron sequences of nine sodium channel genes encoding a-subunits (SCN3A, SCN8A-SCN11A) and b-subunits (SCN1B-4B) expressed in nociceptive neurons using the Molecular Inversion Probes- Next generation sequencing (MIPs-NGS) methods. MIP-NGS is a reliable, flexible, and inexpensive method to detect genetic variations in thousands of patients. Genomic sequencing is important to establish a diagnosis, and in the future might support individualisation of therapeutic approaches. However, the results from genomic sequencing can only be appropriately interpreted in the context of an extensive functional assessment, or family segregation analysis of phenotype and genotype.
Increasing evidence suggests that spinal microglia regulate neuropathic pain in male animals. For example, spinal cord inhibition of microglial signaling using microglial inhibitor minocycline and TLR4 and P2X4 inhibitors only reduced nerve injury-induced mechanical allodynia in male mice. We also found that nerve injury primarily activates p38 in spinal microglial cells of male mice. Furthermore, intrathecal but not peripheral inhibition of p38 reduced inflammatory and neuropathic pain in male mice. We also investigated the effects of several astroglial modulators on nerve injury-induced neuropathic pain in male and female mice following intrathecal injection. These astroglial modulators include L-a-aminoadipate (astroglial toxin), carbenoxolone (connexin 43 inhibitor/gap junction blocked), U0126 (ERK kinase inhibitor), and D-JNKI-1 (JNK inhibitor). We found that spinal administration of astroglial toxin, gap-junction blocker, U0126, and D-JNKI-1 effectively reduced neuropathic pain in both sexes. Nerve injury also caused identical upregulations of astroglial markers GFAP and Connexin 43 in both sexes. Collectively, our data have confirmed male-dominant microglial signaling in neuropathic pain. Our data also suggest that spinal astroglial signaling contributes to neuropathic pain in both sexes.
Chemotherapy-induced painful neuropathy (CIPN) is a distressing, dose-limiting side-effect of chemotherapeutic agents, which is poorly treated by analgesics at present. The development of novel therapies, which requires a deeper understanding of the mechanisms underlying CIPN, is therefore needed. Although initially thought to be neurocentric, extensive preclinical data has recently indicated that chemokine-mediated neuron/non-neuronal communication plays a crucial role in several models of CIPN. Indeed, in a preclinical model of Vincristine-induced neuropathic pain, we have recently observed that chemokine signalling in monocytes, which are recruited from the circulation to the sciatic nerve, is a potential therapeutic target. Specifically, the inhibition of signalling between endothelial-derived fractalkine (FKN) and CX3CR1 expressed by monocytes shows potential for prophylactic treatment of Vincristine-induced pain, with CX3CR1 knock-out mice experiencing a significantly delayed onset of vincristine-induced pain. CX3CR1 is expressed by both patrolling and inflammatory monocytes, however, only inflammatory monocytes express the chemokine receptor CCR2. Current research in my lab is using transgenic and pharmacological tools to investigate the role of CCR2-mediated neuron/monocyte crosstalk, enabling us to differentiate between the roles of patrolling and inflammatory monocytes and thus elucidate the mechanisms underlying chemotherapy-induced pain in more detail. Our most recent data suggests that whilst FKN/CX3CR1-mediated neuron/monocyte communication plays a role in the induction of vincristine-associated pain, CCR2-mediated signalling is more pertinent during established pain and thus provides a potential target for therapeutic as opposed to prophylactic treatment. Targeting specific chemokines could therefore provide a versatile strategy for treating pain in patients at different stages of chemotherapy.

Supported by the MRC
In the last decade, substantial evidence from preclinical studies has increased the recognition of the role of neuroimmune signaling as an important component in the pathogenesis of persistent pain. Despite this evidence, the occurrence and clinical significance of immune activation in human chronic pain conditions still remains uncertain. In my talk, I will discuss evidence from recent brain and spinal imaging studies from our group showing that immune activation can be indeed observed in humans suffering from various pain disorders. I will show that patients with chronic back pain and lumbar radiculopathy exhibit elevated $[^{11}\text{C}]$PBR28 signal, indicating increased levels of the translocator protein (TSPO), at multiple levels of the nervous system. In these patients, TSPO elevations were observed in the brain (Loggia et al., Brain 2015) and, more recently, spinal cord and neuroforamen (which contains nerve roots and dorsal root ganglia) (Albrecht et al, in preparation). As TSPO upregulation is a marker of activated glia and macrophages, these data implicate immunoactivation as a potentially important and clinically relevant mechanism in human pain disorders, as proposed by the results of many preclinical studies.
Many of the treatments for neuropathic pain are drugs that mimic or enhance the actions of the endogenous noradrenergic system. There is good evidence that noradrenaline, acting at a spinal level, can exert potent analgesic effects. The locus coeruleus projects to and releases noradrenaline on both the spinal cord and throughout much of the brain. This organisation may explain why the beneficial actions of treatments mimicking or augmenting noradrenergic actions are often compromised by side effects that are a consequence of actions at a supraspinal level (e.g. anxiety, confusion, sedation, mood changes, sleep disturbance). To obtain better analgesia requires investigation of the neural organisation of the central noradrenergic descending pain control system. This work has been enabled by strategies to selectively target these neurones with viral vectors allowing genetic manipulation of their activity. These spinally projecting neurones are a discrete subset of the locus coeruleus (<15%). They mediate analgesic effects in naïve animals and this action appears to fail in neuropathic pain models. Optogenetic stimulation of the LC shows that there are subsets of neurones mediating both pro and anti-nociceptive actions. Using retrograde chemogenetic approaches enables a dissociation of the beneficial analgesic actions of the descending LC neurones from the stress and anxiety and pro-nociceptive effects of activating the ascending noradrenergic projections to the forebrain. These findings demonstrate a dichotomous ‘modular’ functional and anatomical organisation of the locus coeruleus and provide a template for strategies to selectively engage the descending noradrenergic module for analgesic benefits with fewer cognitive side effects.
Anxiety and depression are frequently observed in patients suffering from chronic pain, which dramatically adds to the patients' pain burden. On the other hand, the activation of the descending noradrenergic pathway has been implicated in the analgesic effects of antidepressants and anticonvulsants. As the noradrenergic-locus coeruleus has been involved in pain, anxiety and depression disorders, it is likely that the noradrenergic system is involved in the comorbidity of these diseases.

During this Workshop, novel data will be presented providing new insights into the noradrenergic system and the amygdala circuits mediating the effects of neuropathic pain, and pain-induced anxiety. I will show that the noradrenergic-LC system is a critical hub for the development of the anxio-depressive consequences of long-term neuropathic pain. Furthermore, the LC can increase but also decrease pain hypersensitivity in neuropathic pain. Finally, the noradrenergic circuit that mediates neuropathic pain-related anxiety will be described. These data stimulate interest in novel therapeutic options that modulate the noradrenergic and amygdalar circuits.
Individualized pain therapy based on phenotyping: are we there yet?

Prof. Dr. Ralf Baron

Division of Neurological Pain Research and Therapy, Department of Neurology, University Hospital, Kiel, Germany

Neuropathic pain represents a major medical problem and treatment has been unsatisfactory. Therefore, a new hypothetical concept was proposed in which pain is analyzed on the basis of underlying mechanisms and sensory abnormalities rather than on the basis of the causing etiology. If a systematic clinical examination of the pain patient and a precise phenotypic characterization is combined with a selection of drugs acting at those particular mechanisms, it should ultimately be possible to design optimal treatments for the individual patient.

To achieve these goals several international consortia (German Research Network on Neuropathic Pain, IMI-Europain, Neuropain) established a large data-base that includes epidemiological and clinical data as well as standardized symptom questionnaires and quantitative sensory testing. More than 2000 patients with different neuropathic pain states have been examined. Furthermore, epidemiological and clinical data on the symptomatology of several thousand patients from a cross sectional survey (painDETECT) are available.

Using a subgroup analysis, in all peripheral neuropathic etiologies three different somatosensory profiles could be identified. Thus, clear phenotypic subgroups exist in neuropathic pain.

Several recent clinical trials using sensory profile-based classification techniques could already identify a differential treatment effect in subgroups of patients. Patients with peripheral neuropathic pain were treated with topical 8% capsaicin patches. Treatment responders and non-responders were retrospectively analysed based on their baseline QST-profile. Capsaicin responders had more severe cold- and pin-prick hyperalgesia but did not differ significantly from non-responders regarding the other QST parameter. The sodium channel blocker oxcarbazepine was evaluated in a cohort of patients with peripheral neuropathic pain who were prospectively stratified into two groups by QST. Patients in the first group (irritable nociceptor phenotype) had hypersensitivity and preserved small nerve
fiber function, patients in the second group signs of cutaneous deafferentation (non-irritable nociceptor). The number needed to treat to obtain one patient with more than 50% pain relief was 6.9 in the total sample, 3.9 in the irritable, and 13 in the non-irritable nociceptor phenotype.

In summary, data exist that patients with different sensory profiles will respond differently to treatment. Consequently, cohorts in clinical trials should be stratified and potentially enriched with patients who likely respond to the study drug based on the sensory profile rather than on the underlying etiology. This approach has the potential to minimize pathophysiological heterogeneity within the groups under study and to increase the power to detect a positive treatment result. In clinical proof-of-concept trials the study population can be enriched prospectively on the basis of “a priori” defined entry criteria. This enrichment with patients who potentially require a specific treatment will increase the likelihood for positive trial outcomes. In clinical practice it will be possible to establish an individualized therapy, i.e. to identify the right patients who require a specific treatment option.

References


GLOBAL BURDEN OF DISEASE - THE IMPACT OF NEUROPATHIC PAIN

F. Blyth

University of Sydney, Australia

From the mid-twentieth century onwards, global health has been shaped by the powerful forces of population growth, population ageing, and the rising burden of non-communicable diseases relative to that of communicable diseases. Burden of disease concepts and measures have developed in the last two decades as a means of assessing the relative impact of different health conditions, injuries and risk factors on the health and wellbeing of populations. Impact has been defined as the loss of expected healthy years of life due to death and/or disability.

Burden of disease studies – global and national – are used by government and non-government organisations to inform the allocation of limited healthcare resources. The Global Burden of Disease (GBD) Study, under the auspices of the World Health Organisation, has been in existence since the 1990s (1). As the methods and scope of the GBD Study have evolved over time, the huge global health burden of pain conditions has become more apparent. In GBD 2010, pain conditions were the leading cause of disability (measured as Years Lived with Disability), with low back pain the leading condition-specific cause of disability (2).

To date, the focus of the GBD has been on painful musculoskeletal conditions, and the contribution of neuropathic pain conditions to the global burden of disease has not been directly and fully assessed (3). The implications of this for research, policy and practice are significant, and need to be understood and addressed to reduce the global burden of neuropathic pain.
Primary burning mouth syndrome (BMS) is defined as an “intraoral burning or dysaesthetic sensation, recurring daily….more than 3 months, without clinically evident causative lesions” (IHS 2013). In addition to pain, taste alterations are frequent (dysgeusia, xerostomia).

Although lacking clinical signs of neuropathy, more accurate diagnostic methods have shown neuropathic involvement at various levels of the neuraxis in BMS: peripheral small fiber damage (thermal quantitative sensory testing, electrogustatometry, mucosal nerve fiber density), trigeminal system lesions in the periphery or the brainstem (brainstem reflex recordings, trigeminal neurography, evoked potentials), or signs of decreased inhibition within the central nervous system (deficient brainstem reflex habituation, positive signs in QST, neurotransmitter-PET findings indicative of deficient striatal dopamine function).

Abnormalities in electrogustatometry indicate involvement of the small Aδ taste afferents, in addition to somatosensory small fibers. According to these findings, clinical entity of BMS can be divided into two main subtypes resembling either peripheral or central neuropathic pain, which may overlap in individual patients. The central type does not respond to local treatments, and associates often with psychiatric comorbidity (depression or anxiety), whereas the peripheral type responds to peripheral lidocaine blocks and topical clonazepam.

BMS is most prevalent in postmenopausal women, having led to a hypothesis that BMS is triggered as a consequence of nervous system damage caused by neurotoxic factors affecting especially vulnerable small fibers and basal ganglia in a setting of decrease in neuroprotective gonadal hormones and increase in stress hormone levels, typical for menopause.
Inflammation in the pathophysiology of neuropathic pain

The concept that the inflammatory response associated with damage in the nervous system, namely neuroinflammation, may contribute to a variety of pain states, has been widely accepted. Peripheral nerve injury results in neuronal sensitization and local inflammatory reactions.

Peripheral nerve injuries and diseases often lead to pain persisting beyond the resolution of damage, indicating an active disease-promoting process, which may result in chronic pain. This is regarded as a maladaptive mechanism resulting from a process (neuroinflammation) that originally serves to promote regeneration and healing. Knowledge on these physiological and pathophysiological mechanisms has accumulated over the last few decades, and has started to yield potential therapeutic targets. Key players are macrophages, T-lymphocytes, cytokines, and chemokines. In the spinal cord and brain, microglia and astrocytes are involved. Recently, data are emerging on the regulation of these players.

As an example, clinical research has shown an important role of anti-inflammatory cytokines in neuropathic and other chronic pain states. In addition, there is ample evidence of an analgesic action of anti-inflammatory cytokines in animal models. The interplay of anti-inflammatory cytokines and the nociceptive system provides possibilities and challenges concerning treatment strategies based on this concept. Why an organism tends to a more pro-inflammatory or a more anti-inflammatory reaction is presently being studied. Genetic factors, early life events, and epigenetic mechanisms all appear to play a role, also infections acquired over life-time, and the gut-microbiome.

Chemosensitive or sensitized nociceptors increase their activity upon stimulation with the appropriate chemical or combination of chemicals. Neuronal activity increases in in-vitro preparations and in experimental animals after application of algesic mediators like prostaglandins and pro-inflammatory cytokines. In humans, researchers have to decide which body fluid or tissue to investigate to correlate levels of such mediators with measures of pain. If such a correlation is found, the question of a causal relationship remains. Longitudinal studies and in studies with interventions directed at the mediator in question may give evidence toward one or the other. Our group could show such correlations in some but not in all conditions tested. Patients with chronic widespread pain and fibromyalgia had systemic pro-inflammatory cytokine profiles with reduced blood levels of anti-inflammatory cytokines. Unexpectedly, in patients with post-herpetic neuralgia, systemic cytokine profiles were not different from controls, and local cytokine levels in affected skin were not different from the unaffected side. We found systemic elevation of pro-inflammatory cytokines in patients with complex regional pain syndrome (CRPS), with painful versus painless peripheral neuropathy, and in length-dependent versus non-length dependent small fiber
neuropathy. In one longitudinal study, analyzing cytokine profiles in local blister fluid from patients with CRPS, we found bilaterally increased pro-inflammatory tumor necrosis factor-alpha and macrophage inflammatory protein-1beta and decreased anti-inflammatory interleukin-1 receptor antagonist protein levels compared to non-CRPS patients. After 6 months of analgesic treatment, the increased cytokine protein levels in CRPS patients had returned to the level of non-CRPS patients. Such findings, similar to those reported by others, showing an increase in systemic interleukin-8 in patients with fibromyalgia that was normalized after a course of multidisciplinary therapy, encourage an interpretation of a pathophysiological importance. However, comparison between studies is hampered by the wide range of methods and the differences in quality. Intervention studies directed at the respective mediators or unbiased modern methods like proteomics may provide solutions.

MicroRNAs and other non-coding RNAs have been discussed as potential master switches that may link nerve injury, pain and inflammation. As an example, the microRNA miR-let-7d was found to be dysregulated in patients with fibromyalgia syndrome (FMS), and its expression also correlated with mean pain intensity over the last 4 weeks and with a reduction of skin innervation in FMS patients. MiR-let-7d is one of the microRNAs regulating the insulin-like growth factor -1 (IGF) /IGF-1 receptor (IGF-1R) pathway. In skin biopsies of FMS patients we found an increased miR-let-7d expression and a reciprocal decrease in IGF-1R mRNA, selectively in patients with reduced skin innervation. Taken together, these data provide evidence for small nerve fiber impairment in a subgroup of patients suffering from FMS, who have a more “neuropathic” phenotype. This is possibly linked to altered miRNA expression and concurrent decreased cutaneous IGF-1R signaling, implying a connection between nerve degeneration, neuroinflammation and pain.
The implementation of optogenetic tools to manipulate neuronal activity with light is transforming our ability to investigate neural systems. By expressing light-sensitive proteins, or opsins, in genetically defined neuronal populations, optogenetic approaches permit new experimental questions that span from the specific properties of defined synaptic connections to their roles in complex behaviors. Implementation of optogenetic strategies typically requires remote light sources and fiber-optic delivery schemes that impose considerable physical constraints on natural behaviors. The stress to the animals confounds interpretation, particularly in studies of pain circuitry, as stress is a well-known regulator of pain and analgesia. This presentation will describe our work to develop micro-scale optoelectronic devices with wireless powering schemes allowing for fully wireless operation, and permanent integration of light sources with the central or peripheral nervous system. A case study is presented for use of wireless optogenetic manipulation of peripheral neurons to manage evoked and spontaneous pain.
Numerous studies have shown associations between genetic variants and neuropathic pain disorders. Rare monogenic disorders are caused by mutations of substantial effect size in a single gene, while common disorders are likely to have a contribution from multiple genetic variants of mild effect size, representing different biological pathways. In addition to neuropathic pain disorders themselves, association studies have uncovered the role of genetic variants in modulating therapeutic effects of drugs used to treat them. In this review we summarize the state of knowledge about genetic contributors to neuropathic pain and its management and glean possible implications for personalized and targeted treatment of neuropathic pain conditions.
Classical drug development involves the identification of molecular targets; synthesis of novel compounds which interact with those targets; safety testing; testing the effects of those compounds in pre-clinical (in vitro and in vivo) models; followed by clinical trials in the target population. Factors associated with successful navigation of this pathway are not well understood, and success may be incorrectly attributed to a particular approach or strategy rather than to good luck.

Causes of disappearing efficacy (largely but not always driven by an understandable desire to get a new drug to patients, and to market, as quickly as possible) include:

- researchers may confuse statistical significance with positive predictive value – that is, assume that if p<0.05 then their finding is “true”.
- for whatever reasons, the effects observed in one laboratory may disappear when tested elsewhere, even when the circumstances of testing are “identical” – this is the so called replication crisis.
- the pre-clinical effects may be true, and replicable, but only observed in such a narrow set of circumstances as to make their application to necessarily diverse human populations fruitless.

These issues are tractable, and we have proposed a three stage pre-clinical pipeline comprising (1) exploratory curiosity driven research, free of inferential statistics, to allow the efficient development of hypotheses in which the investigator has high confidence; (2) a rigorous hypothesis testing experiment with pre-specification of the primary outcome measure and the statistical analysis plan, powered to deliver a positive predictive value greater than 95%; and (3) multicenter preclinical animal studies with deliberate heterogeneity between sites to ascertain the robustness of the experimental findings to different situations.

Only when these three steps have been completed should a clinical trial be initiated.

Ethical animal and human research rests on the balance between harms and potential benefits arising from the research: maximizing benefits by increasing the value of research outputs improves the ethical status, and the usefulness, of the research which we do.

Disclosure statement:
MRM is a member of the UK Home Office Animals in Science Committee, the UK MHRA Commission for Human Medicines and Vice Chair of the CHM Expert Advisory Group for Neurology, Pain and Psychiatry
There are two aspects of animal models relating to neuropathic pain which require scrutiny when assessing their clinical relevance: internal and external validity. External validity (models and outcome measures) will be discussed in another workshop; so after summarising the important points of external validity the speaker will focus on internal validity.

Internal validity is the extent to which the design, conduct, analysis and reporting of an experiment is susceptible to the detrimental effects of experimental bias. The presentation will use data from meta-analyses to demonstrate the extent to which preclinical evidence in the neuropathic pain field is susceptible to bias. The discussion will then move to introduce methods by which this impact could be mitigated by applying the generic concepts espoused in “Good Laboratory Practice” to the design and conduct of experiments. In this context, the audience will be introduced to the online Experimental Design Assistant (www.eda.nc3rs.org.uk). The benefits of transparent reporting of neuropathic pain animal research using the ARRIVE guidelines as an example format will then be described. This will be followed by an example of how sharing raw data at the individual animal level in an openly accessible format can facilitate external scrutiny and replication of data.

The discussion will then move to describe the benefits of using systematic review to assess preclinical neuropathic pain evidence to prevent waste and improve efficiency, by for instance guidance as to the optimal choice of model or outcome measure for a particular experiment. The SyRF tool for conducting preclinical systematic reviews will be showcased (www.syrf.org.uk).

Finally, some of the challenges which pre-clinical systematic review poses will be covered—most notably coping with the large volume of publications and the rapid accrual of new data. The presentation will finish with a description of how the speaker and his collaborators have been developing and validating text mining and machine learning techniques as a first step to creating “living systematic reviews” of the preclinical neuropathic pain evidence base.
Much has been learned about the mechanisms of acute and chronic pain through modern neuroimaging studies. There is also a growing effort to develop brain biomarkers of pain and to develop neuroimaging prognosticators of treatment efficacy. To do this, brain imaging studies of chronic pain have mostly examined both structural and functional abnormalities that are identified when comparing data between patients and healthy control subject. This approach is typically used to identify fundamental group differences or to link findings in the patients to the quality or intensity of their pain, to a measure of their pain sensitivity, to their ongoing or evoked pain, and/or to a behavioural indicator (trait or state). As a clinical tool, the challenge has been to obtain information at the individual patient level and to be able to use this information reliably for prognostication. Thus, this talk will provide an overview of the brain imaging technologies that are commonly used in chronic pain research. I will then discuss the technical capabilities and limitations of these technologies. Finally, I will discuss whether the information gleaned from these approaches and chronic pain models pertain to the realities of chronic pain conditions.
This presentation will focus on how behavioral studies in animal models might be adapted to align with the requirements of precision medicine.

To date, animal modelling around neuropathic pain has been dominated by homogeneity in both models and outcomes. The emerging recognition of clinical heterogeneity in pain mechanisms and thus treatment responses requires that pre-clinical methods evolve appropriately. Two aspects of such experiments should be considered when discussing their clinical relevance: internal and external validity. Since internal validity will be discussed in other workshops, the focus here will be on external validity.

Advances in modelling of clinical disease: for many years animal modelling relating to neuropathic pain focused entirely on traumatic nerve injury; a condition examined in only ~8% of clinical trials. However, recently other relevant disease models have been developed and validated. Examples will be given as to how scrutinizing a portfolio of diverse disease models reveals heterogeneity at multiple levels.

A major area where the pre-clinical realm has long been misaligned with clinical neuropathic pain research and practice is in the challenging field of pain outcome measures: to date the vast majority of outcomes reported in neuropathic pain-relevant animal models involve easily measured limb withdrawal responses to sensory stimuli. Such threshold outcomes are very rarely assessed as efficacy outcomes in neuropathic pain clinical trials. However, they are increasingly employed to create individual patient sensory profiles through which heterogeneous tractable pain mechanisms can be identified. Furthermore, those animal models that do exist are only profiled for the broad sensory gain phenotype of neuropathic pain and not the more prevalent sensory loss profile. Therefore, the speaker will posit that measurement of multi-modality sensory thresholds in animal models should be “rebadged” as profiles rather than outcomes and a systematic sensory profiling of existing models undertaken. Such a shift in ethos will require alternative measures of the impact of pain in rodents - the approach of measuring ethologically relevant, pharmacologically tractable, behaviours will be advocated. The use of such measures is relevant to both sensory gain and sensory loss phenotypes. The use of multi-centre studies to rapidly validate such new outcomes will be mentioned.

The extensively validated multimodal German Neuropathic Pain Network clinical sensory profiling method has recently been used to define sensory clusters and elucidate heterogeneity in patients. The adaptability of this method to profiling of animal models is thus an important consideration. A discussion of which measures are already achievable in animal models, which will be reasonable targets on which to expend effort and which will be most challenging.
Patients with neuropathic pain present with different symptoms and signs that may represent different underlying mechanisms. Recent studies have also shown that patients can be grouped based on specific clusters of sensory signs allowing separation of patients into groups with presumed different underlying pain mechanisms. There is increasing evidence that stratifying patients based on symptoms and sensory signs can improve the predictive value of some pharmacological treatments. However, systematic reviews of the predictive value of symptom and sensory profiling are challenging because they are often based on secondary data from studies that are not powered to study predictors of treatment response, use of different questionnaires and sensory testing, publication and reporting bias, and either lack of placebo control or difficulty in incorporating placebo control in the interpretation of the results. Randomized controlled trials suggest that preservation of small-fiber function and the presence and severity of allodynia are associated with better pain-relieving effect to injections with botulinum toxin type A, while the results for topical lidocaine and capsaicin patches are conflicting. Previous studies have suggested that preserved nociceptors and evoked pain predict the response to sodium channel blockers, and a recent phenotype-stratified study found a better effect of oxcarbazepine in patients with peripheral neuropathic pain and the so-called irritable nociceptor phenotype with evoked pain and preserved small nerve fiber function, as determined by detailed quantitative sensory testing. There is also some evidence that certain pain descriptors, in particular paroxysmal pain, predict the response to treatment.
The clinical pain experience is a consequence of a combination of the tissue changes inflicted by the causative factor, construct of the peripheral nerves that transduct and conduct the nociceptive message, and the modulation function of this message that takes place in the central nervous system. Each of these three factors can be a target for therapeutic intervention, and therefore their functional state is of much interest, since it is likely to affect the result of such intervention. For example, patients with a modulation dysfunction that overly enhances pain perception should respond better to agents that reverse this dysfunction than patients expressing well-functioning non-enhancing modulation, who are less likely to benefit from that agent. Tools readily available for assessment of central pain modulation are conditioned pain modulation (CPM) and temporal summation (TS) which measure the capacity for pain inhibition and for pain facilitation respectively. This talk will briefly mention the methodology of these tests, describe the known changes in modulation during painful disorders, and whether these tests can stratify patients into subgroups of presumed pathophysiological mechanisms. We will then review the limited data available on relevance of pain modulation parameters in prediction of treatment effects, and suggest future directions.
This presentation reviews recent findings from animal models providing insights into neural and non-neural processes that contribute to neuropathic pain following trigeminal nerve injury. Rodent models of trigeminal neuropathic pain may display spontaneous pain-like behaviour and mechanical or thermal hypersensitivities outside as well as within the site innervated by the injured afferent fibres. Underlying mechanisms include the hyperexcitability that may develop in the injured afferents and also alterations in the cell bodies of these and adjacent afferents in the trigeminal ganglion that involve intracellular signalling changes modulated by extracellular chemical mediators and non-neural (eg glial) cells. The resulting afferent input into the brainstem and the central sensitisation that it induces in neurons of trigeminal nociceptive pathways in the central nervous system (CNS) contribute to the spontaneous pain and mechanical or thermal hypersensitivity (hyperalgesia, allodynia) manifested in many trigeminal neuropathic pain states. Trigeminal central sensitisation and associated nociceptive behaviour are modulated by several intrinsic CNS circuits operating through endogenous chemical mediators and receptor mechanisms that represent targets by which many analgesic drugs and procedures exert their pain-relieving effects. The expression of trigeminal central sensitisation and nociceptive behaviour is also dependent on the functional integrity of non-neural cells (e.g. astroglia and microglia) in the CNS. This expression varies across genetically diverse rodent strains, consistent with reports that genetic factors contribute to the variability between patients in pain expression following trigeminal nerve injury and in the analgesic effectiveness of therapeutic approaches used to manage the pain.

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This presentation will review findings from studies on human experimental and clinical chronic orofacial pain that reveal the continued promise of tests of orofacial somatosensory function and other biomarkers for improved clinical diagnosis of orofacial pain. The use of qualitative and quantitative sensory tests as well as neurophysiological tests in studies using human experimental pain models and clinical orofacial pain will be described. The possible correlation between sensory findings and neurophysiological findings will be reviewed with a special focus on the agreement between chairside clinical qualitative sensory tests and the more sophisticated qualitative sensory tests. Recommendations for evaluation techniques in patients with chronic orofacial pain in the extraoral as well as the intraoral region will be provided.
Trigeminal neuropathic pain – mechanisms and management

Substantial progress has been made in understanding the underlying mechanisms and diagnostic characteristics of trigeminal neuropathic pain (TNP). Nevertheless, it remains a challenge to evaluate the balance between peripheral and central nervous system factors. A simple, but feasible approach could be to infiltrate or inject local anesthetics in the painful area around the involved nerve fiber. The lecture will review and discuss recent studies with local anesthetic blocks in neuropathic pain conditions, the applicability of this approach in the trigeminal system and consequences on normal somatosensory function in addition to clinical studies in probable TNP conditions.
Botulinum toxin type A (BTX-A) is widely used to treat muscle hyperactivity, based on its ability to inhibit synaptic exocytosis, and therefore to disable neural transmission. A number of experimental studies have indicated that BTX-A may have analgesic activity independent of its effect on muscle tone. This presentation will cover newer clinical studies of BTX-A in NP and potential mechanisms of action. In particular data concerning efficacy, safety and responder profiles of repeated administrations of BTX-A in patients with peripheral neuropathic pain (NP) will be presented. BTX-A is now generally proposed as last choice in the therapeutic arsenal of patients with focal peripheral neuropathic pain.
RECENT ADVANCES IN THE PHARMACOTHERAPY OF NEUROPATHIC PAIN: FOCUS ON BOTULINUM TOXIN

NEUP7-0428
MECHANISMS OF ACTION OF BOTULINUM TOXIN IN PERIPHERAL NEUROPATHIC PAIN
B. Davletov¹
¹University of Sheffield, Biomedical Science, Sheffield, United Kingdom

BOTOX® and related botulinum neurotoxins (BoNTs) efficiently trigger local neuromuscular paralysis for several months, but can also provide partial relief for sufferers with severe migraine and intractable neuropathic pain. Our recent data show botulinum-specific targeting of subpopulations of sensory neurons. It would be highly advantageous to develop new BoNTs which do not paralyse muscles but still possess their anti-nociceptive properties. Unfortunately BoNTs are the most lethal paralytic toxins known and working with these proteins presents a great danger. We developed new technology that solves the safety issues and also allows production of new non-paralytic botulinum products for long-lasting neuronal silencing. We now developed a unique panel of Neuronal Targeting and Blocking molecules that can silence specific neurons for months allowing reliable management of chronic pain. We demonstrated a defined targeting of distinct subpopulations of nociceptive neurons by tailor-made botulinum molecules and tested them in well-characterized pain models. Novel botulinum molecule, Bitox, efficiently alleviates mechanical allodynia in a rodent model of neuropathic pain offering a significant advancement in the therapeutic options for patients. The novel synthetic botulinum molecules have the potential to underpin treatment of different types of neurological disorders including neuropathic pain, migraine and neurodegenerative disorders with minimal side-effects.
RECENT ADVANCES IN THE PHARMACOTHERAPY OF NEUROPATHIC PAIN: FOCUS ON BOTULINUM TOXIN

NEUP7-0468
PERINEURAL INJECTIONS OF BOTULINUM TOXIN IN NEUROPATHIC PAIN

C. Maier
1Berufsgenossenschaftliches Universitätsklinikum Bergmannsheil Bochum, Pain Medicine, Bochum, Germany

Perineural applied botulinum toxin A for neuralgia – novel approach

Christoph Maier1, Lynn Eitner1, Carla Avila Gonzalez2

1 BG-University Hospital Bergmannsheil gGmbH, Department of Pain Management, Ruhr-University Bochum
2 BG-University Hospital Bergmannsheil gGmbH, Department of Anesthesiology, Intensive, Palliative Care and Pain Medicine, Ruhr-University Bochum

Most responder to subcutaneous applied botulinum toxin A (BONT-A) demonstrate mechanical allodynia or thermal hyperalgesia (1). However, alldynia/hyperalgesia and preserved thermo reception is present only in 20-40% of patients with focal peripheral neuropathic pain (2,4). The majority of patients with peripheral nerve injury (PNI) present hypoesthesia or no relevant changes of thermal perception in the painful area (4). An alternative approach might be in these cases the perineural injection of BONT-A (p-BONT). But hitherto there are only some case reports (5).

We present an open study including 60 patients with PNI after failure of medical treatment, who got strictly perineural injections (50/100 IE BONT-A) using high-solution ultrasound. 70% reported a 30% pain relief after the first injection up to 8–16 weeks, the long term response of >30% pain relief after repeated applications (follow-up 3….30 months) was good in 63%. No severe adverse effects occurred, except one case of moderate temporary muscle paresis. In some patients the Quantitative Sensory Testing (QST) revealed a significant increase of thermal or mechanical detection threshold, but in most cases QST was unchanged (1,3).

Due to the lack of placebo controlled studies and long term observations the place p-BONT is unclear. However, it might be a promising new approach in treatment of therapy-resistant patients with peripheral neuralgia.


Persistent post-surgical pain (PPSP) is common, with variable etiologies and a plethora of contributing mechanisms and risk factors recently identified in the literature. Depending on the type of surgery, between 10 and 70% of PPSP patients suffer from neuropathic pain.

Only few perioperative interventions have shown important reductions of PPSP incidence and severity, most of these focusing on perioperative regional anesthesia techniques. Systemic pharmacological interventions, until now, have not yielded large reductions in PPSP incidence, highlighting the importance or risk stratification to allow targeted interventions in patients who are most likely to develop PPSP.

The lecture will briefly summarize the existing literature on sensory processing in PPSP, and will present the results of three ongoing / recently completed studies aimed at understanding: 1) The role of cognitive factors (e.g. the extent of cognitive flexibility) in the development of PPSP; 2) The association between patient expectations about post-surgical pain and their experience of PPSP; and 3) The role of PPSP in the overall picture of post-surgical outcomes and undesired complications.

In addition, findings from a recent systematic review on PPSP risk factors will be highlighted to facilitate a discussion on the implications of presented data in further clinical research in the field.
Neuropathic pain (NeuP) prevalence in the general population is around 8% and 10% of people with chronic pain have “possible NeuP”, generally associated with poorer quality of life (Torrance, Pain 2013). Depending on the type of surgery, chronic postsurgical pain involves NeuP in 30% of the cases (6-54%) including minor surgical procedures (Haroutiunian, Pain 2013; Dualé, J Pain 2014). NeuP is also a prominent factor in severe chronic pain after surgery in children (Batoz, Br J Anaesth 2016). For IASP, 2017 is the ‘Global Year against Pain after Surgery’. The control of acute postoperative pain is far from optimal and severe postoperative pain stands as a major risk factor for the development of persistent pain after surgery. NeuP often considered as a chronic pain condition has once been “acute”. Thereby, presence of a NeuP component in acute postoperative pain and its relationship with severe persistent post-surgical pain deserves attention. In postoperative pain setting, acute NeuP represents a special challenge. First, its incidence is still unknown although it seems to be associated with severe pain (Martinez, Pain 2012). Presence of inflammatory components in postoperative pain makes the detection of NeuP more difficult. Also, there is no consensus regarding adequate tools for correct diagnosis and to differentiate. Second, the course of postoperative NeuP remains largely undetermined and may have a delayed onset (subacute pain) often neglected (Borsook, Ann Surg 2013). More, the response to diagnostic tools fluctuates from time to time (Dualé, J Pain 2014; Johansen, Acta Anaesthesiol Scand 2016). Finally, adequate treatment of acute NeuP has not been established but might be different from established chronic NeuP (Xu & Yaksh, Curr Opin Anesthesiol 2011).
SENSORY, PSYCHOLOGICAL AND COGNITIVE FACTORS THAT SHAPE THE TRAJECTORY OF PERSISTENT POST-SURGICAL PAIN (PPSP)

NEUP7-0454
PREDICTIVE BIOPSYCHOSOCIAL PHENOTYPES ASSOCIATED WITH PPSP, AND DEVELOPMENT OF A RISK ALGORITHM FOR CLINICAL DECISION-MAKING

R. Edwards
1Brigham & Women's Hospital, Anesthesiology, Chestnut Hill, USA

Dr. Edwards will present data from ongoing studies of the multidimensional predictors of pain-related outcomes 6-12 months after total knee replacement and mastectomy. Significant pre-operative risk factors include psychosocial processes, sensory/psychophysical variables, health behaviors, pain characteristics and history, sleep, etc. Many of these risk factors are inter-related, forming "clusters" of risk-relevant variables. Collectively, Dr. Edwards' presentation will explore biobehavioral mechanisms that contribute to the chronification of post-surgical pain, and ongoing efforts to develop algorithms that maximize our ability to identify (pre-surgically) the highest-risk patients in order to provide tailored treatments to those individuals with the greatest need and potential benefit.
Peripheral and central neuropathic types of pain both show signs of sensory loss and hyperexcitability. In peripheral neuropathic pain there is abnormal spontaneous and increased evoked activity from damaged nerve fibres and from regenerating sprouts, while in central pain conditions loss of input is associated with deafferentation hyperexcitability. The clinical translation of such neuronal hyperexcitability includes pain in the injured innervation territory, a lowered pain threshold to one or several modalities, pain induced by non-noxious stimuli, altered summation, extraterritorial spread of pain etc.

The neuronal hyperexcitability has either a peripheral or a central component or a combination of such mechanisms. A series of tests are being used to test for such neuronal hyperexcitability. Examination of thresholds to mechanical, thermal and chemical stimuli alone are generally insufficient to determine whether a specific response is peripherally or centrally mediated. But there are other possibilities: i) the use of various blocks or combination of quantitative sensory testing with pharmacological modulation of neuropathic pain may aid in distinguishing between peripheral and central sensitisation; ii) tests that have a specific peripheral or central organization may assist to determine if an effect is peripherally or centrally determined. For example abnormal temporal summation or measurement of nociceptive reflexes are tests that are organized centrally; iii) compounds acting specifically on sodium channel isoforms expressed either in the PNS or in the CNS may also aid to distinguish between peripheral and central mechanisms. Finally, by studying patients with exclusive central nervous system disorders it is possible indirectly to distinguish between a peripheral or a central effect of a sodium channel blocking compound.
A variety of drugs used in neuropathic pain work presumably by targeting voltage-gated sodium channels (NaVs), which have a critical role in neuronal excitability. While drugs such as opioids, tricyclic antidepressants and calcium channel α2δ subunit ligands seem to have somewhat consistent effects across the spectrum of neuropathic pain conditions, new evidence suggests that the response to NaV blocking drugs may depend on the mechanisms that lead to nerve injury, as well as on the presentation and characteristics of the pain syndrome in an individual patient.

This presentation will review the emerging evidence and present data from ongoing/recently completed studies on NaV blocking approaches for the treatment and the prevention of neuropathic pain. It will also highlight pharmacokinetic considerations regarding drug distribution and their NaV-blocking sites of action. Finally, it will discuss neuropathic pain conditions and patient populations in whom pharmacological targeting of NaV channels may be particularly beneficial.
THE CONTRIBUTION OF THE PLACEBO RESPONSE IN PHARMACOLOGICAL, PHYSICAL- AND SURGICAL INTERVENTIONS: STATE OF ART, PREDICTION AND ETHICAL CONSIDERATIONS.

NEUP7-0437
CAN KNOWLEDGE OF PLACEBO MECHANISMS HELP IMPROVE THE RCT?
L. Vase

1Department of Psychology and Behavioral Sciences, Aarhus University, Aarhus, Denmark

The RCT is currently facing several challenges. One of these challenges is that the placebo response appears to be increasing in RCTs, thereby making it difficult to prove an effect of putative new treatments over placebo. This problem has primarily been approached by using stable factors to predict the magnitude of the placebo response and/or by developing complex designs aimed at reducing the placebo response, in the hope that it would improve the test of the active treatment. Still, the success of this approach has so far been limited.

Based on placebo mechanism studies, a new approach is proposed. The magnitude of placebo effects is large and highly variable. Patients’ perception of the treatment, verbal suggestions given for pain relief, as well as patients’ expectations towards pain relief contribute to the magnitude of the placebo effect across different types of chronic pain. A recent study has shown that it is possible to make approximations of patients’ expectations towards the treatment and hence predict the magnitude of the placebo response in RCTs. Also, by directly asking patients about their perceptions and expectations towards the treatment it may be possible to account for the contribution of the placebo component to the overall treatment in future studies.

Thus, by interfacing insights from placebo and nocebo mechanism studies, it may be possible to enhance the information that can be obtained from RCTs and to account for the variability in the placebo component of the overall treatment effect in ecological valid and ethically appropriate ways. This approach has the potential to improve the scientific test of treatments as well as to illustrate how the effect of treatments can be optimized in clinical practice.
As surgical procedures are inherently associated with risk, it is important to demonstrate that the surgical intervention is effective. As many surgical procedures are performed to improve function and quality of life and to reduce pain, their outcomes are subjective and prone to bias, which may require a placebo-controlled design to demonstrate the true efficacy of surgery. Performing interventional trials with a placebo arm is possible, at least for less invasive procedures. The biggest challenge is to find sufficient number of suitable patients in a reasonable period of time. Placebo-controlled surgical trials are useful to identify procedures that are not better than placebo. Such trials are not free from adverse events, but the harms can be minimised and trials can be carried out in an ethical way.
The transition to a chronic pain state is widely viewed as one of the key questions in pain neuroscience but it is still poorly understood. The hyperalgesic priming model provides insight into these mechanisms because this model has a clearly defined “priming” stimulus that then changes the performance of the nociceptive system such that previously subthreshold stimuli become powerful producers of pain behavior. A large body of work supports the hypothesis that the initiation of the hyperalgesic priming requires changes in gene expression in nociceptors that are largely governed by translation regulation pathways. The first part of this talk will provide evidence that a key factor for this initiation is ERK/MNK-mediated phosphorylation of eIF4E, the 5’ cap binding protein. Mechanisms that maintain hyperalgesic priming once it has transitioned to a chronic phase have been harder to pinpoint. The second part of this talk will focus on potential sex differences in these mechanisms. I will present evidence that in male mice, the maintenance of hyperalgesic priming involves the dopaminergic system with a specialized role for spinal D5 receptors. In females mice the dopaminergic system is likewise involved but seems to operate independently of D5 receptors. A prominent feature of hyperalgesic priming and its maintenance in females is that it strongly attenuated by antagonists of CGRP receptors whereas these same antagonists lack effect in male mice. Hence, the initiation of hyperalgesic priming critically involves nociceptor plasticity but the maintenance of priming depends on plasticity in the CNS that has distinct mechanisms in male and female mice. These findings may have broad implications for understanding the transition to chronic pain.
Persistent forms of pain continue to be a significant clinical problem due to their high prevalence, marked negative impact on quality of life and limited treatment options. The most common treatments such as opioid analgesics and non-steroidal anti-inflammatory drugs are frequently ineffective or have serious side effects. The development of more effective, well-tolerated pain treatments will require a more complete understanding the neural networks that mediate pain. Knowledge of this type will then support the identification of key synaptic and molecular mechanisms that have remained elusive. Work presented in this lecture will address new findings on the neural circuits underlying mechanical allodynia, a condition in which light touch or movement becomes painful in the setting of injury or disease. We have identified dorsal horn neuronal populations required for mechanical allodynia. Interestingly, the requirement for these populations differs depending on the nature of the injury. This concept has important implications not only for understanding at a basic level how the nervous system encodes mechanical allodynia, but it also highlights the need to consider etiology in the design and implementation of therapeutic strategies. Lastly the lecture will also address neural circuits required for mechanical allodynia induced by diabetic neuropathic pain models. Neural circuits for heat hypersensitivity, which is also induced in these models, will also be discussed.
BACKGROUND AND AIMS:

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system causing different symptoms including neuropathic pain. Because in classical experimental autoimmune encephalomyelitis (EAE) mouse models, the development of severe motor defects strongly limits the assessment of pain, an optimized EAE-mouse model of recurrent-remitting (RR) MS has been recently developed. Our aim was to better characterise this model and to perform a pharmacological validation.

METHODS:

We first evaluated paw and facial sensitivity to mechanical and thermal stimuli. We then evaluated for some sensitive behaviours their response to relevant drugs for neuropathic pain. Finally, we evaluated in the lumbar spinal dorsal horn the cellular changes which could be associated with the behavioural defects.

RESULTS:

We showed that RR-EAE mice developed mechanical and thermal hypersensitivity when stimuli are applied to the paw while they developed no thermal hypersensitivity when stimuli are applied to the face. We also showed that pregabalin was more effective than amitriptyline to reduce somatic mechanical allodynia and that both drugs were unable to reduce somatic thermal hyperalgesia. Finally, we showed that these behavioural abnormalities could be related to excessive oxidative stress in spinal cord.

CONCLUSIONS:

To our knowledge, we are the first laboratory to replicate the RR-EAE mouse model developed by Khan et al in 2014, validating its reproducibility. We also demonstrated that sensitive behaviours were not restricted to the extracephalic level and were reduced by pregabalin, a reference medicine for neuropathic pain management, validating its predictivity for new drugs development.

[i] Khan et al., Pharmacology, Biochemistry and Behavior 2014
Background and Aims:

Patients suffering from neuropathic pain secondary to spinal cord injury (SCI) experience not only pain sensation but also some affective disorders. Therefore, research in new animal models has emerged as a need to assay promising therapeutic strategies to treat both neuropathic pain and potential behavioral disturbances associated to SCI. The present study aimed to characterize these alterations in the acute and chronic phase of a SCI mice model.

Methods:

Mechanical allodynia and thermal hyperalgesia were evaluated weekly until twenty-eight days post injury (dpi) (acute phase) and at sixty and nineteen dpi (chronic phase). Emotional alterations such as depression-like behavior, social interaction and anhedonic-like behavior were assessed at twenty-eight and ninety dpi.

Results:

In the acute phase of the disease, mechanical allodynia and thermal hyperalgesia were evidenced until twenty-eight dpi, and at that time, only a mild social interaction alteration was observed, suggesting that hypersensitivity can induce mood alterations resulting in a depression-like behaviour manifestation. In the chronic period of the lesion, mechanical allodynia was not detected, while thermal hyperalgesia was only observed until sixty dpi. Additionally, social defeats and anhedonic-like behaviors were detected at ninety dpi despite the absence of pain responses.

Conclusions:

This data may suggest that chronic central neuropathic pain may cause emotional disturbances that could be persistent over time despite pain responses subside. The present animal model may be suitable for further pharmacological studies to relieve SCI related pain and emotional symptoms that are a major source of suffering and requires special clinical care.
Background and Aims:

Antiretroviral (ARVs) drugs are successful in controlling HIV infection, but some are neurotoxic, causing a peripheral neuropathy in 40% of patients. Here we investigate study quality and its impact on findings from animal models of ARV-induced neuropathy.

Methods:

We systematically searched 3 databases in September 2012, and again in November 2015, to identify original research papers using animal models of ARV-induced neuropathy and reporting pain-related behavioural outcomes. We extracted meta-data (including risks of bias; 7-item checklist) and outcome data and analysed these using stratified meta-analysis.

Results:

We included twenty-seven papers (17 in 2012, 10 in 2015). Administration of ARV was associated with increased pain-hypothesised behaviours (-2.125 SMD (95% CI= -2.551 to -1.698). In studies testing interventions intended to moderate ARV-induced pain, pain-hypothesised behaviours improved to 1.366 SMD (95% CI= 1.122-1.611)

In these studies reporting of randomisation (0.979 SMD (95% CI= 0.670-1.289) vs 1.814 SMD (95% CI= 1.441-2.187)) and blinding (1.120 SMD (95% CI= 0.824-1.417) vs 1.814 SMD (95% CI= 1.390-2.239)) were significantly associated with smaller effect sizes.

Reporting of measures to reduce the risk of bias was higher in more recent publications (Randomisation 70% vs 41%; blinding of outcome assessment 90% vs 41%; allocation concealment 20% vs 0%; sample size calculations 100% vs 0%; reporting of exclusions 40% vs 24%. Median quality score increased from 2 (IQR 1-4) to 4.5 (IQR 3.25-5).

Conclusions:

We found associations between effect sizes and reported risks of bias; and that reporting of measures to reduce the risk of bias has increased.
Background and Aims:

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.

Methods:

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.

Results:

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.

Conclusions:

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.
Background and Aims:

The orofacial pain caused by trigeminal nerve damage is more severe and debilitating than other type of neuropathic pain. However, the studies on trigeminal neuropathic pain including trigeminal neuralgia are lacking. This is largely due to both the lack of proper animal models and the complexity of the major surgical procedure currently being used in these models. Our approach makes the chronic constriction injury of infraorbital nerve (IoN-CCI) rodent model more accessible for the study of neuropathic pain.

Methods:

The facial surface between the eye and whisker pad of the rat was gently shaved. A 0.5 cm incision parallel to the mid-line was made starting at the caudal end of the third row of whisker line towards the ipsilateral orbit (Fig 1). The superficial fascia was bluntly separated to expose the IoN trunk at its distal segment outside the orbital cavity (Fig 2). Two chronic catgut ligatures were loosely tied around the IoN (2 mm apart) (Fig 3). The incision was closed with a polyester suture.
**Results:**

IoN-CCI rats exhibited a significant decrease in threshold toward mechanical stimuli in von Frey test on the ipsilateral side, which started at day 1 after injury and lasted for at least 60 days compared with sham rats. The IoN-CCI rats also displayed an increase in asymmetric face-grooming episodes compared with sham rats.

**Conclusions:**

Our improved IoN-CCI surgical procedure makes this animal model more accessible, which would help facilitate the study of the cellular and molecular mechanisms of trigeminal neuropathic pain including trigeminal neuralgia.
Background and Aims:

Immune response against sensory nerve-derived antigens and complex neuro-immune interactions are suggested responsible for pain and autonomic signs in some CRPS, but the pathophysiological mechanisms are unclear. We established and characterized an enhanced passive-transfer trauma model for CRPS.

Methods:

Small plantar skin-muscle incision was performed in female C57Bl/6 mice daily treated i.p. with purified serum-IgG from CRPS patients or healthy volunteers (n=4-6; 6 mice/group) for 3-13 days. Hindpaw mechanonociceptive threshold was measured with aesthesiometry, paw volume with plethysmometry, myeloperoxidase activity with luminescence in vivo imaging, paw sensory neuropeptides and inflammatory cytokines with immunassays, glia markers in pain-related brain regions with immunochemistry.

Results: CRPS IgG significantly increased and prolonged swelling, and induced stable hyperalgesia of the incised paw compared to healthy IgG. The strongest hyperalgesic effect was observed towards the end of the study, whereas in the control groups mechanical hyperalgesia, and in all groups swelling and post-incision paw-inflammation had fully resolved by that time. CRPS IgG treatment significantly increased the density of astrocyte-related glial fibrillary acidic protein (GFAP) and microglia-staining Iba1 in L4-L5 spinal dorsal horn, periaqueductal gray and somatosensory cortex compared to controls.

Conclusions:

In an enhanced passive-transfer-trauma model for CRPS, daily serum-IgG injection induces stable, strictly unilateral hyperalgesia in rodents over at least two weeks, but this effect does not appear to be related to peripheral inflammation. Astrocyte and microglia activation accompany this process, and might contribute to sustain the enhanced hyperalgesia alongside the presumed regional non-inflammatory autoantibody activity.

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BASIC SCIENCE (ANIMALS): ANIMAL MODELS - PART 1

NEUP7-0049
THE THERAPEUTIC EFFECT OF VITAMIN C IN AN ANIMAL MODEL OF COMPLEX REGIONAL PAIN SYNDROME PRODUCED BY PROLONGED HINDPAW ISCHEMIA-REPERFUSION IN RATS

O. In su¹, K. Jae Hun¹, L. Pyung Bok², K. Young Chul³, N. Francis Sahngun²
¹Konkuk university, anesthesiology, Seoul, Republic of Korea
²Seoul National University Bundang, Anesthesiology, Seould, Republic of Korea
³Seoul National University, Anesthesiology, Seoul, Republic of Korea

Background and Aims:
It is known that increased free radicals from oxidative stress are one of the major causes of complex regional pain syndrome (CRPS). In this study, we tested the hypothesis that vitamin C has a dose-related treatment effect in a chronic post-ischemic pain (CPIP)

model.

Methods:

A total of 49 male rats weighing 250 to 350 g were used. The 4 treatment groups were control (no medication), group 1.0 (administration of 1 mg/day for vitamin C for 5 days), group 2.5 (2.5 mg/day vitamin C for 5 days), and group 7.5 (7.5 mg/day vitamin C for 5 days). The 50% mechanical withdrawal threshold and total blood antioxidant status (TAS) were measured before and after administration of vitamin C.

Results:

Twenty-eight CPIP model rats were generated from 49 rats. The 50% mechanical withdrawal threshold of group 2.5 was higher than that of the control group and group 1.0 ($P < 0.05$). At 1 day of the administration of vitamin C, the 50% mechanical withdrawal threshold of group 1.0 was higher than that of the control group and the blood levels of TAS in groups 2.5 and 7.5 were higher than that in control group ($P < 0.05$). Twelve days after the administration of vitamin C, the blood levels of TAS in groups 2.5 and 7.5 were lower than that of the control group ($P < 0.05$).

Conclusions:

The administration of a proper dose of vitamin C can reduce oxidative stress, increase antioxidants, and recover the threshold for mechanical allodynia in the CPIP model.
BACKGROUND AND AIMS:

Since pain and sleep are so relevant, their improvements are considered to be important. We analyzed the effects of suvorexant on sleep quantity and quality in neuropathic pain model.

METHODS:

We produced the neuropathic pain model mice by partial sciatic nerve ligation (PSNL). After the administration of suvorexant (30mg/kg) or vehicle for 7 days, we recorded the Electroencephalogram and Electromyogram for 24 hours. The recorded data were scored as wake, REM sleep or non-REM sleep with SleepSign®, and the amount time and the number of each episode were calculated. We also analyzed the power spectral densities for sleep states. For statistical analysis, one-way ANOVA was used.

RESULTS:

In the PSNL mice compared to sham mice, the amount of REM sleep and non-REM sleep was significantly decreased, and the amount of wakefulness was significantly increased during light phase. The amount of non-REM sleep was increased (p<0.001), and the amount of wakefulness was decreased (p<0.001) in the PSNL-suvorexnat group as compared with the PSNL-vehicle group during early light phase. In addition, Suvorexant decreased the number of long awake episodes (≥15 min) during light phase (p<0.01). In REM sleep, the power density of θ waves (4-9Hz) significantly decreased in the PSNL mice treated with suvorexant during late light phase (p<0.01) and dark phase (p<0.05).

CONCLUSIONS:

Suvorexant recovered the reduced amount of sleep under neuropathic pain state. However, suvorexant could interfere with the ability to sustain wakefulness and be affecting the quality of REM sleep.
THE ROLE OF MIR-21 IN NEUROPATHIC PAIN AND AFFECTIVE BEHAVIOR AFTER PERIPHERAL NERVE INJURY IN B7-H1 KNOCKOUT MICE

F. Karl, N. Üçeyler, C. Sommer

University Hospital Wuerzburg, Department of Neurology, Würzburg, Germany

Background and Aims:

miR-21 is a promising candidate that may link the cross-talk between the immune system and pain mediators. To further investigate the pathophysiological role of miR-21 in neuropathic pain we assessed mice deficient of the B7 homolog 1 (B7-H1), a major inhibitor of inflammatory response.

Methods:

Young (8 weeks), middle-aged (6 months), and old (12 months) B7-H1 ko mice and wildtype littersmates (WT) received a spared nerve injury (SNI) of the sciatic nerve. We assessed thermal withdrawal latencies and mechanical withdrawal thresholds and investigated anxiety-like and cognitive behavior. Quantitative real time PCR was used to determine miR-21 expression in peripheral nerves after SNI.

Results:

We found age-dependent mechanical hyposensitivity in naive B7-H1 ko mice compared to WT mice (p<0.01). Mice of both genotypes developed mechanical and heat hypersensitivity (p<0.05) after SNI and showed a higher miR-21 expression in the tibial and common peroneal nerves 7 days after SNI (p<0.05); middle-aged and old WT mice displayed a miR-21 upregulation in the sural nerve (p<0.01). Naive young WT mice showed more anxious behavior than B7-H1 ko mice (p<0.05), whereas SNI had no effect on anxiety and cognition.

Conclusions:

Our results suggest that increased miR-21 expression in peripheral nerves after SNI may be associated with mechanical hypersensitivity and that miR-21 may play a pathophysiological role in neuropathic pain, while affective behavior and cognition seem to be spared.

Acknowledgments: Research received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement 602133 (ncRNAPain).
Background and Aims:

Amylin (AMY) belongs to the calcitonin family of peptides. The existence of binding sites for AMY in the dorsal spinal cord and the localization of AMY in the neurons of dorsal root ganglion suggests that this peptide may participate in nociception. We sought to investigate AMY for its potential role to reverse the established tolerance to the antinociceptive actions of morphine.

Methods:

Tolerance was induced by administration of intrathecal (i.t) morphine (22 nmol) once daily from days 1-10. Tolerance induced by the administration of single morphine injections from days 1-5 was reversed when AMY (60 pmoles) was co-administered with morphine from days 6-10. Nociceptive testing was performed daily by tail – flick test.

Results:

Morphin tolerance was established by day 6. Co-administration of AMY (60 pmoles) with morphine on the day six, prevented the decline in the antinociceptive effects of morphine. Rats tolerant to it morphine showed increased mRNA expression of the BDNF gene in their lumbar spinal cord. This increase was blocked when morphine was co-administered with AMY from day 6-10.

Conclusions:

These results suggest that the activation of spinal AMY receptors not only abolished morphine tolerance, but also reduced the morphine induced increase in the BDNF mRNA expression in the lumbar spinal cord.
Background and Aims:

Treatment of neuropathic pain with opiates remains controversial because it typically requires much larger doses than those used to treat acute pain, which potentially increases the risk of abuse and dependence. However, most of the knowledge about opioid neurotransmission is acquired in naïve conditions and studies in animal models of chronic pain are necessary to know the effect of opiates in these modified systems. As the noradrenergic locus coeruleus (LC) has been widely implicated in physical opiate dependence, we will explore LC opioid neurotransmission modifications in a chronic constriction injury (CCI) sciatic nerve model of neuropathic pain at 7 and 30 days after nerve injury.

Methods:

CCI was used as neuropathic pain model at 7 and 30 days after surgery. Behavioral, electrophysiological, biochemical, immunohistochemical, and Western blot assays were performed.

Results:

CCI-30d leads to an LC up-regulation of cAMP, pCREB, protein kinase A, tyrosine hydroxilase and electrical activity; in addition to an increase expression of c-fos. Morphine treatment become less effective producing acute mu-opioid receptor desensitization when evaluating the inhibitory effect of DAMGO onto the forskolin-stimulated adenylyl cyclase activity and outward current produced by saturating concentrations of met-enkephalin in patch-clamp studies. Furthermore, electrophysiological bursting LC activity, cAMP and c-fos rebound was lower in CCI-30d after naloxone precipitation. Behavioural manifestation of opiate withdrawal was reduced in neuropathic animals.

Conclusions:

These data support the hypothesis that the efficacy of opiates in stimulating the noradrenergic system is reduced in rats with long-term neuropathic pain.
Introduction and Aims:

Opioids play a key role in the control of descending pain modulation but how they modulate descending pain facilitation remains understudied. This is important since descending pain facilitation is increased during chronic pain. The dorsal reticular nucleus (DRt) is a descending pain facilitatory area and local neurons express µ-opioid receptors (MOR). Here we evaluated the role of opioidergic modulation of the DRt in naïve and neuropathic animals (spared nerve injury-SNI- model).

Methods:

Naïve male Wistar rats were used for the injection of a lentiviral vector knocking down MOR or the implantation of a guide cannula at the DRt. Neuropathic-animals, two weeks after SNI-induction, were also implanted with a guide cannula into the DRt. One week after stereotaxic procedures, naïve and SNI animals were injected DAMGO or CTAP, MOR agonist and antagonist, respectively, through the guide cannula or submitted to microdialysis. Pain behaviour was assessed by the von Frey test. MOR expression was evaluated by immunohistochemistry.

Results:

In naïve animals, MOR knock down induced a decrease of mechanical thresholds, DAMGO increased mechanical thresholds and CTAP produced no effects. In SNI animals, Met- and Leu-enkephalin significantly increased while MOR expression significantly decreased compared to naïve animals. DAMGO produced a dose-dependent increase of mechanical thresholds in naïve animals and no effects in SNI animals.

Conclusions:

The DRt is under inhibitory opioidergic modulation which is impaired during neuropathic pain. It remains to ascertain if this accounts to enhance descending pain facilitation.

Acknowledgements: FCT/COMPTE project PTDC/SAU-NSC/110954/2009, IASP Early Career Research Grant
Background and Aims:

Fabry disease (FD) is an X-linked inherited lysosomal storage disorder with intracellular accumulation of globotriaosylceramide (Gb3) due to α-galactosidase A (α-Gal A) deficiency. We used the α-Gal A knock-out mouse (Fabry KO) as a model for pain in FD.

Methods:

We investigated thermal withdrawal latencies and mechanical withdrawal thresholds of young (ca. 3 months) and old (≥18 months) naïve and Complete Freund’s Adjuvant (CFA) treated Fabry KO mice and wildtype littermates (WT). Gene expression of pain associated ion channels of dorsal root ganglia (DRG) was analyzed by qRT-PCR, and DRG neurons were characterized by patch-clamp analysis.

Results:

Naïve young Fabry KO mice displayed heat hypersensitivity compared to WT mice (p<0.01), while old Fabry KO mice were less sensitive to heat (p<0.05). Both Fabry KO age-groups showed mechanical hyper- and cold hyposensitivity compared to WT (p<0.001; p<0.01; p<0.05). After i.pl. CFA injection old Fabry KO mice did not develop heat hypersensitivity in contrast to WT littermates (p<0.01). No differences were found in ion channel gene expression in DRGs of old Fabry KO and WT mice. Old Fabry KO mice showed loss of sodium- and decrease of Ih-currents compared to young Fabry KO and old WT mice (p<0.01; p<0.001).

Conclusions:

Similar to Fabry patients, Fabry KO mice show age-dependent sensory deficits. Loss of sodium- and reduction of Ih-currents seems to protect Fabry KO mice from heat hypersensitivity, which may be due to functional ion channel impairment.

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NEUP7-0227
EFFECT OF SPINAL CORD STIMULATION AMPLITUDE ON MECHANICAL HYPERSENSITIVITY IN A PRECLINICAL MODEL OF CHRONIC NEUROPATHIC PAIN.

K. Meuwissen¹, J.W. Gu², T.C. Zhang², E. A. J. Joosten³

¹Maastricht University Medical Centre+, School for Mental Health and Neuroscience, Maastricht, The Netherlands
²Boston Scientific, Neuromodulation Research and Advanced Concepts Team, Maastricht, USA
³Maastricht University Medical Centre+, Department of Anaesthesiology and Pain Management- Pain Management and Research Centre- Maastricht University Hospital- Maastricht- The Netherlands., Maastricht, The Netherlands

Background and Aims:

Today, various spinal cord stimulation (SCS) paradigms are applied for the treatment of chronic neuropathic pain disorders. Two well-known stimulation paradigms, Conventional and Burst stimulation, are hypothesized to exert their analgesic effects through different underlying mechanisms. To acquire more insight we studied the relation between amplitude of Conventional and Burst stimulation in the context of charge delivery, in a rat model of neuropathic pain.

Methods:

Animals (n=12 rats) received a unilateral partial sciatic nerve ligation, and were implanted with quadripolar electrodes in the epidural space at vertebral level T13. Mechanical hypersensitivity was assessed, with Von Frey monofilaments, at 15, 30, 45 and 60 minutes after stimulation onset and at 30 minutes post stimulation. Conventional or Burst stimulation was applied at 66%, 50%, and 33% motor threshold (MT).

Results:

For Conventional stimulation (n=5), increase of paw withdrawal thresholds was correlated positively and directly with stimulation amplitude. For Burst stimulation (n=6), all tested amplitudes significantly differed from baseline (p < 0.05) at 30, 45, and 60 min, but only at 50% MT stimulation did paw withdrawal thresholds return to pre-nerve injury levels, suggesting a non-monotonic effect of amplitude on analgesia for burst SCS. The data further suggests that the effects of burst stimulation are delayed as compared with tonic stimulation.
Conclusions:

Overall, our data suggest that efficacy of SCS is closely dependent on stimulation amplitude. While Conventional and Burst stimulation have the ability to be equally effective, their efficacy showed a different dependence on amplitude.

Fig 1. The effect of Conventional and Burst stimulation on the paw withdrawal threshold for the various amplitudes (percentages of MT) used. WT was assessed 15, 30, 45, and 60 mins. After onset of stimulation and 30 mins post stimulation. The dotted line represents the average paw withdrawal threshold prior to partial sciatic nerve ligation.
Peripheral nerve injury (PNI) is believed to cause maladaptive changes at synaptic level, leading to neuropathic pain which is difficult to treat with common analgesic drugs. Noradrenergic locus coeruleus (LC) neurons have a crucial role in neuropathic pain modulation. In this study we examined whether chronic constriction injury (CCI) could affect glutamatergic synaptic transmission in LC neurons.

CCI was performed on P10 to P12 Sprague Dawley pups. Seven days after CCI, horizontal slices of brainstem (300 µm thick) were prepared and whole-cell patch clamp recording was performed. Evoked and spontaneous excitatory postsynaptic currents (eEPSC and sEPSC) were recorded from LC neurons at a holding potential of -70 mV, in the presence of bicuculline (20 µM).

The sEPSCs recorded from LC neurons of neuropathic rats showed a significant increase in amplitude, but not in frequency. The eEPSC amplitude in neurons of rats undergone CCI was significantly increased compared to the control group (P<0.05). The paired pulse ratio (PPR) elicited with different inter-stimulus intervals (50-250 ms) did not show any difference between neurons of CCI and control pups.

This study shows that PNI increases excitatory synaptic transmission in LC neurons 7 days after chronic constriction injury. The observed synaptic potentiation is mainly due to postsynaptic mechanisms.
Loss of delayed rectifier potassium channel Kv1.6 results in hyposensitivity to noxious heat

L. Peck¹, J. Dawes¹, D. Bennett¹
¹University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom

Background and Aims:

Kv1.6 (encoded by KCNA6) is a member of the Kv1 family of Shaker-like voltage-gated potassium channels. Current evidence declares that Kv1 channels act as a ‘brake’ on excitation in peripheral neurons and members of this family have been implicated in the pathophysiology of neuropathic pain. Here, we present for the first time a comprehensive sensorimotor phenotypic analysis of transgenic KCNA6 knock-out mice.

Methods:

KCNA6 +/+, +/− and −/− male and female mice of mixed genetic background (BL/6;129S5) were used throughout. Behavioural assays included: hot plate, Hargreaves, Von Frey, Rota-rod and open field. Naïve behaviour was assessed in two separate cohorts, with data combined here. Spared nerve injury (SNI) was performed on the second cohort following baseline behaviour assays.

Results:

KCNA6−/− mice had significantly elevated withdrawal thresholds on hot plate (50°C and 53°C, p<0.0001) and Hargreaves (p=0.0001) assays; a similar phenotype was seen in heterozygotes (p=0.0002) on the Hargreaves assay only. Responses to mechanical stimuli were not significantly different; motor behaviour was also normal. Following SNI, we identified no further differences in post-operative ‘pain’ thresholds between genotypes beyond the described thermal hyposensitivity.

Conclusions:

KCNA6 knock-out mice are hyposensitive to noxious heat. This is a curious finding – our current understanding suggests deletion of Kv1 channels would induce hypersensitivity. The specificity of this sensory deficit to the thermal domain is also intriguing and further experiments will investigate whether this effect is mediated by sensory afferents or at spinal level.
IS THERE A REASONABLE EXCUSE FOR NOT PROVIDING POST-OPERATIVE ANALGESIA WHEN USING ANIMAL MODELS OF PERIPHERAL NEUROPATHIC PAIN FOR RESEARCH PURPOSES?

S. Hestehave¹,², G. Munro², T. Brønnum-Pedersen³, K. S.P. Abelson¹
¹University of Copenhagen, Department of Experimental Medicine, Copenhagen, Denmark
²H. Lundbeck A/S, Neurodegeneration In Vivo, Valby, Denmark
³H. Lundbeck A/S, Non-Clinical Safety Research, Valby, Denmark

Background and Aims:

The induction of neuropathic pain in rodents often requires surgical intervention, eliciting acute pain and stress post-operatively, which from a welfare perspective demands peri-operative analgesic treatment. However, many researchers avoid providing such care based largely on anecdotal opinions that it might interfere with model pathophysiology. **Aim:** To investigate effects of various peri-operative analgesic regimens encapsulating different mechanisms and duration of action, on the development of post-operative stress/welfare and pain-like behaviors in the Spared Nerve Injury (SNI)-model of neuropathic pain.

Methods:

Starting on the day of surgery, male Sprague-Dawley rats were administered either vehicle (s.c.), carprofen (5.0mg/kg, s.c.), buprenorphine (0.1mg/kg s.c. or 1.0mg/kg p.o. in Nutella®), lidocaine/bupivacaine-mixture (local irrigation) or a combination of all analgesics, with coverage from a single administration or up to 72 hours. Postoperatively during 28 days animal welfare-parameters, body-weight, food-consumption, and hindpaw mechanical allodynia were followed.

Results:

None of the analgesic regimes compromised the development of mechanical allodynia. Unexpectedly, the combined treatment with 0.1mg/kg s.c. buprenorphine and carprofen for 72 hours and local lidocaine/bupivacaine, caused severe adverse effects with peritonitis. This was not observed when the combination included a lower dose of buprenorphine (0.05mg/kg, s.c.), or when buprenorphine was administered alone for 72 hours.

Conclusions:

Post-operative analgesia does not appear to affect established neuropathic hypersensitivity outcome in the SNI model. However, further studies are needed to determine which treatment is most beneficial, and to investigate possible effects on the neurobiological mechanisms in the development of neuropathic pain.
Background and Aims:

Spinal Cord Stimulation (SCS) is an established last resort treatment modality for patients with painful diabetic polyneuropathy (PDPN). However, one third of PDPN patients who receive SCS are not successfully treated. Evidence for the working mechanisms of SCS is mainly provided by experimental studies with short stimulation paradigms. The lack of long-term or continuous SCS models limits clinical translation and understanding of its working mechanisms. In this study, we established an experimental model of long-term SCS in PDPN and present data on functional behavioral outcome, peripheral blood perfusion and nerve conduction.

Methods:

Male Sprague Dawley rats (n=60) underwent von-Frey measurements, Laser-Doppler-Imaging, and nerve conduction assessment of the tail nerve. Subsequently, rats received a single i.p. injection of 65 mg/kg streptozotocin (n=50) or Saline (n=10). Four weeks post-injection, measurements were repeated and diabetic rats showing mechanical hypersensitivity were allocated to Sham or SCS treatment. A quadripolar paddle lead was implanted at spinal levels L2-L5, and connected to an Interstim II implantable neurostimulator. Rats were stimulated 12 hours/day for a period of four weeks and behavioral outcome, peripheral blood perfusion and nerve conduction velocity were monitored.

Results:

First data indicate that the model of long-term SCS in PDPN animals is operational and results in pain relief. Data is currently being collected and will be presented.

Conclusions:

A model for long term SCS in experimental PDPN has successfully been established. Data obtained from the present study will provide further insight in functional and structural effects of long term SCS in PDPN.
Background and Aims:

The high incidence of osteoarthritis (OA) emphasizes the need to optimise preclinical models of OA for investigation of the pathobiology and to assess the disease-modifying effects of potential novel therapeutics. Our aim was to establish and pharmacologically characterise an optimised rat model of monoiodoacetate (MIA)-induced OA pain.

Methods:

This study was approved by The University of Queensland Animal Ethics Committee. Groups of male Sprague-Dawley rats (160-180g) received a single intra-articular injection of 2, 2.5 or 3mg of MIA or saline (50 µL). General animal health, gait analysis, weight bearing and paw withdrawal thresholds (PWTs) were assessed. The efficacy of gabapentin (30, 70mg/kg), morphine (1, 2, 3mg/kg), meloxicam (3, 10, 30mg/kg) and amitriptyline (3, 10mg/kg) were assessed. Statistical analysis was performed using GraphPad™ Prism.

Results:

There was rapid development of altered gait that was sustained throughout the experimental period following intra-articular injection of MIA in rats without significant differences (p>0.05) between the 3 MIA-groups. Mechanical allodynia was fully developed in the ipsilateral hindpaws from day 12 to day 35 post-MIA injection. In rats with PWTs <=6g, single bolus doses of gabapentin and morphine produced relief of mechanical allodynia whereas meloxicam and amitriptyline lacked efficacy in the doses tested.

Conclusions:

Intra-articular injection of MIA at 2, 2.5 or 3mg induced sustained mechanical allodynia and altered gait parameters for at least 35-days, and is suitable for investigation of the pathobiology of OA and for identification of new molecules with potential to alleviate this condition.
LITTER DEPENDENT EFFECTS OF EARLY LIFE STRESS ON THE BEHAVIOURAL RESPONSE TO NERVE INJURY IN ADULTS

A. Giles¹, P. Arnold¹, K. Keay¹
¹The University of Sydney, Anatomy and Histology, Sydney, Australia

Background and Aims:

Despite triggering the same degree of increased sensitivity (thermal & mechanical) in all rats, chronic constriction of the sciatic nerve (CCI) evokes changes in social behaviour and emotional coping style in only a subgroup (30%) of injured animals. One possibility for this heterogeneity of complex behavioural responses to injury may be related to early life events. We sought to investigate this possibility by examining, in an animal model of neuropathic pain, whether an early-life stress (ELS) might increase the incidence of altered social behaviour and emotional coping style after nerve injury.

Methods:

On post-natal days 3 and 5, six litters of male, Sprague-Dawley rats received i.p., injections of lipopolysaccharide (0.05mg/kg: ELS). Five control litters received equi-volume pyrogen-free saline injections. In adulthood, all of the rats underwent social-interactions testing 6 days before, and for 6 days after CCI. Thermal sensitivity was evaluated in all rats using cold-plate testing.

Results:

All rats showed increased thermal sensitivity. In 80% (4/5) of control litters the incidence of behavioural change within litters was identical to that reported in our earlier studies. In contrast, ELS evoked substantial changes in the distribution of behavioural effects between litters, resulting in a third of litters in which all rats showed altered social behaviour and emotional coping style and a third of litters in which none of the rats showed altered social behaviours.

Conclusions:

Early life stress appears to exert a litter dependent effect, driving the response to nerve injury to each extreme of the behavioural phenotype.
CHARACTERIZATION OF TRAUMATIC SPINAL CORD INJURY MODEL IN RELATION TO NEUROPATHIC PAIN IN THE RAT

Background and Aims:

Neuropathic pain after spinal cord injury (SCI) is a severe condition that responds poorly to usual medications. The establishment of neuropathic pain model after SCI is substantial to better understand the pathophysiology involved and for the development of new therapy. Here we standardize the traumatic SCI model in relation to neuropathic pain.

Methods:

Wistar rats were submitted to traumatic SCI of mild or moderate intensity (drop height 12.5mm and 25mm, respectively) using the NYU Impactor. Behavioral assessments were performed during 8 weeks and after euthanasia the spinal cord adjacent to injury were processed for immunohistochemistry/imunofluorescence.

Results:

Moderate injury group (MDG) and mild injury group (MLG) recovered bladder function 14 and 7 days, respectively, after injury. BBB test showed a motor deficit of 70.07% in the MDG and 44.71% in the MLG. Mechanical allodynia evaluation, by von Frey filaments, showed a progressive pain condition in the MDG (70.82% of impairment), while the MLD demonstrated partial spontaneous improvement in the last weeks (56.07% of impairment). No difference was observed in the tail flick test. H&E staining revealed a more extensive lesion in the MDG (0.65cm) compared to MLG (0.5cm). Stereological quantification of NeuN revealed a neuronal loss of 75.79% in the MDG and 55.24% in the MLG at the lesion area.
Lesion Extension

Distance (mm)

Mild Injury vs. Moderate Injury

Images A-D show different sections of tissue samples for comparison.
Conclusions:

SCI of moderate intensity is a good model for the study of neuropathic pain. This model presented motor deficit and a progressive painful condition, which remained for at least 8 weeks.

Acknowledgement: FAPESP/CNPq.
EFFICIENCY OF COCARNIT IN THERAPY OF DIABETIC NEUROPATHY

S. Berehovy1, N. Nikitina1, N. Skochko1, T. Beregova1, L. Ostapchenko1

1Taras Shevchenko National University of Kyiv, Educational and Scientific Centre Institute of Biology, Kyiv, Ukraine

Background and Aims:

The aim of the study was to investigate the influence of medicine “Cocarnit” on heat-induced tail flick and electromyographic (EMG) recordings of the tibial nerve (TN) and tibialis anterior muscles (TAM) in rats with diabetic neuropathy (DN).

Methods:

The study were conducted on 30 white laboratory rats, which were divided into 3 groups of 10 animals each. Rats of group 1 were used as control. In rats of the second and third groups were induced type I diabetes by administration of streptozotocin (65 mg/kg, i/p). Diabetes in rats was confirmed by the presence of hyperglycemia. At the 30th day of experiment to the rats of 2nd and 3rd groups saline and Cocarnit (1 mg/kg, i/p) were administered, respectively. Cocarnit is complex of nicotinamide, thiamine pyrophosphate, cyanocobalamin and adenosintriphosphate sodium (company “World Medicine”). Saline and Cocarnit were injected during 9 days once a day. Heat-induced rat tail-flick latency was determined as measure for nociceptive pain. EMG recordings of the TN and tibialis anterior muscles were performed intraoperatively in diabetic rats.

Results:

Rats with diabetes on 28 day of experiment develop polyneuropathy which was manifested in the increase of tail-flick latency by 41.8% (p<0.01) in comparison with the control. The increase of tail-flick latency correlated with an increase in the delay time of the nerve impulse to the muscle. Cocarnit restored the pain threshold and EMG recordings of the TN and TAM in rats.

Conclusions:

The current investigation demonstrates that Cocarnit is a potential candidate for the treatment of DN.
Background and Aims:

Patients suffering chronic pain are at high risk of suffering long-lasting affective disorders. Preclinical studies demonstrating that prolonged experimental pain leads to depressive-like and anxiety-like behavior in rodents. Locus coeruleus (LC) is a major source of noradrenaline in the CNS and although its role in the descending inhibition of acute pain is well established, it has been reported a contribution to the facilitation of chronic pain. In the present study, we have evaluated the time-dependent changes in the descending and ascending pathways of LC during the development of neuropathic pain model.

Methods:

Sprague-Dawley and Lon Evans-Tg(TH-CRE)3.1.Desis male rats were used. CCI was used as neuropathic pain model (2, 7 and 30 days after surgery). A combination of behavioral pharmacology and chemogenetics techniques were used for testing pain threshold and anxio-depressive-like behaviors.

Results:

Blockade of ipsilateral LC activity produced a decrease of nociceptive threshold in CCI-2d group. Also, it was observed when descending LC pathway within the spinal cord was inhibited. Inhibition of contralateral LC activity produced an increase of the nociceptive threshold in CCI-7d and CCI-30d groups. Activation of ipsilateral LC produced an increase of the nociceptive threshold in all groups. Blockade both ipsilateral and contralateral LC activity reversed the depressive-like behavior in CCI-30d group.

Conclusions:

At early time of neuropathy there is an activation of the noradrenergic descending pain pathway that contribute to endogenous analgesia. At late time of neuropathy there is a LC functional sensitization that may involve the ascending LC pathways implicate in pain-related depression.
Background and Aims:

It was conducted a study with type 1 diabetic rats induced by streptozotocin for better evaluation of the efficacy of low level laser therapy in treating painful diabetes and in protecting nerve damage. It was also analyzed the possible role of laminin in peripheral fibers regenerative processes after laser therapy.

Methods:

induction of type 1 diabetes: A single intraperitoneal injection of streptozotocin (85 mg/kg) was administered in male Wistar rats. Assessment of Allodynia: The von Frey filaments were used to assess nociceptive thresholds. Laser therapy: Rats were irradiated with GaAs Laser (Gallium Arsenide, Ibramed, Brazil) emitting a wavelength of 904 nm and an output power of 45mWpk. Transmission electron microscopy: sciatic nerve was collected after transcardial perfusion with Karnovsky’s fixative solution. Sciatic nerve was collected for laminin analyses by western blot.

Results:

The application of four sessions of low level laser therapy was sufficient to reverse allodynia and protect peripheral nerve damage in diabetic rats.

Conclusions:

The results of this study indicate that low level laser therapy is feasible to treat painful diabetic condition in rats using this protocol. Although its efficacy in reversing painful stimuli and protecting nerve fibers from damage was demonstrated, this treatment protocol must be further evaluated in biochemical levels to confirm its biological effects.
Background and Aims:

Neuropathic pain treatment is problematic because of the limited efficacy of first-line drugs such as Gabapentin/Pregabalin and antidepressants (tricyclics and serotonin-noradrenaline reuptake inhibitors) and the inefficacy of selective serotonin-reuptake inhibitors (SSRIs). The present study investigates whether blocking 5-HT6 receptor-elicited mTOR signalling (i) attenuates mechanical and thermal allodynia and co-morbid cognitive symptoms induced by traumatic neuropathy in rats, and (ii) improves the weak analgesic efficacy of Fluoxetine, an SSRI.

Methods:

Fourteen days after L5 spinal nerve ligation (SNL), allodynic rats were treated with the following treatments: SB258585 (5 µmol/kg i.p.), PZ1388 (5-25 µmol/kg i.p.), 5-HT6 receptor inverse agonists; PZ668 (2 nmol i.t.), a 5-HT6 receptor antagonist; Rapamycin (0.3-3-10 nmol i.t.), an mTOR inhibitor; Fluoxetine (time release pellets, 28 mg/kg/day for 5 days, s.c.), followed or not by an injection of SB258585 (5 µmol/kg i.p.) or vehicle on day 5. Then, we assessed their tactile and thermal sensitivity (von Frey hair and paw immersion at 15°C), and their cognitive performance (social interaction and novel object recognition tests).

Results:

SNL induced tactile and thermal allodynia associated with cognitive dysfunction. Rapamycin, SB258585 and PZ1388 reduced tactile/thermal allodynia and improved cognitive performance. The effects of these last two compounds involved spinal 5-HT6 receptors because they were abolished by i.t. administration of PZ668. Both fluoxetine and SB258585 alone exerted partial anti-allodynic and pro-cognitive effects. Fluoxetine-SB258585 combination suppressed tactile allodynia and rescued cognitive performance.

Conclusions:

This work clearly identifies new targets for future development of drugs and/or for repositioning 5-HT6 receptor ligands and mTOR inhibitors for management of neuropathic pain.
Background and Aims:

Pain arising from HIV-related sensory neuropathy (HIV-SN) is very difficult to manage as drugs commonly used for neuropathic pain are usually not effective. The aim of this study was investigate the possible antinociceptive effect of intrathecal (i.t.) ziconotide and CTK01512-2, two a N-type voltage-gated calcium channel (VGCC) blockers, in mice models of HIV-SN.

Methods:

HIV-SN was induced by the injections of gp120 (100 ng/site, i.t. on days 0, 3 and 6) and/or stavudine (50 mg/Kg, intravenously, on days 0 and 4) in both female and male C57Bl/6 mice. Before and after treatments, von Frey test was performed and adverse effects were investigated. Animals were also i.t. treated with CTK01512-2 and ziconotide (100 nmol/site) 14 days after HIV-SN induction. N-type VGCC mRNA was detected by qRT-PCR in dorsal root ganglion.

Results:

The treatment with gp120, stavudine and gp120+stavudine, but not boiled gp120 and/or saline, produced hyperalgesia both in female and male mice. At 14th day after induction, we detected the hyperalgesia peak and increased levels of N-type VGCC mRNA splicing variants e37a and b. I.t. CTK01512-2 or ziconotide was able to fully reverse gp120-, stavudine- or gp120+stavudine-induced hyperalgesia. Ziconotide, but not CTK01512-2, produced motor and sensorial adverse effects at the tested dose.

Conclusions:

I.t. N-type VGCC blockers produced a marked antinociceptive effect in mice models of HIV-related pain.
Background and Aims:

Painful Diabetic Polyneuropathy (PDP) is one of the most common long-term complications of diabetes. The fact that effectiveness of conventional pharmacological drugs is often limited creates an urgent need for novel intervention strategies. Dorsal Root Ganglion-stimulation (DRG-stim) has recently emerged as a new neuromodulation modality in pain treatment. The goal of this study was to develop the technique of experimental DRG-stim and gain first insights into the effect of DRG-stim in experimental PDP.

Methods:

Diabetes was induced in female Sprague-Dawley rats by intraperitoneal injection of streptozotocin. Animals were tested for mechanical hypersensitivity using Von Frey testing at baseline, and once a week for 4 weeks following injection, for the purpose of selecting animals that developed PDP. Subsequently, animals were implanted with a monopolar DRG-stim electrode at L5 and stimulated for 30 minutes at 3 days following implantation. Immediately before stimulation, 15 and 30 minutes during stimulation, and 15 and 30 minutes after stimulation, animals were again tested on Von Frey.
First data indicate successful implantation and successful reduction in mechanical hypersensitivity upon L5 DRG-stim in an experimental model for PDP (Figure 1). 

Conclusions:
First data indicate successful implantation and successful reduction in mechanical hypersensitivity upon DRG-stim in PDP rats. Further analysis in more animals is needed to substantiate the pain relieving effect of DRG-stim in experimental PDP and compare its effectiveness with conventional dorsal column spinal cord stimulation.

Acknowledgements:
We would like to thank Prof. Dr. Quinn H. Hogan for his training and advice with regard to the DRG-stim electrode implantation procedure.
Background and Aims:

While is well established that chronic pain (CP) is associated with heightened anxiety, depression as well as deficits in cognitive functioning, its impact on social behavior has received considerably less attention.

Methods:

We therefore studied social interactions between familiar and unfamiliar conspecifics, 1 and 2 months after spared nerve injury (SNI) installation in the left- (SNI-L) or in the right-hind paws (SNI-R). After 6 hours of isolation, social (nose-to-body, nose-to-nose, nose-to-genitals, above and following) and non-social (stand alone and walk alone) interactions were quantified in a neutral arena in 15 minutes sessions.

Results:

We observed that: 1) SNI-L and SNI-R presented no differences in mechanical allodynia; 2) SNI animals present a hyposociability phenotype when compared with Sham-operated animals; 3) no sex-related differences were observed; 4) mechanical allodynia is not affected by social interaction; 4) SNI-R males and females interact less than SNI-L animals, demonstrated a lateralized effect of SNI in social behavior.

Conclusions:

Collectively, our results demonstrated that SNI neuropathic pain model promotes lateralized deficits in social behavior and underlying neuronal activation in rodents.
Background and Aims:

Peroxynitrite is involved in the development of pain of several etiologies via post-translational nitration and modification of various proteins. Pharmacologic scavenging of peroxynitrite inhibit inflammatory and neuropathic pain by blocking nitration of key proteins involved in the regulation of synaptic glutamate homeostasis. Sirtuin 3 is the major mitochondrial protein deacetylase. A recent proteomic survey reported that approximately 20% of mitochondrial proteins are acetylated, implicating SIRT3 as a key regulator of mitochondrial proteome function.

In the present study we evaluated the role of epigenetic modification by class III HDAC in a model of chronic constriction injury (CCI) of the sciatic nerve.

Methods:

Animals were exposed to CCI of the sciatic nerve in the presence or absence of antioxidants. To demonstrate the involvement of SIRT3 modulation by free radicals products during CCI we detected the level of acetylation and activity of SIRT3 of mitochondrial compartment in spinal cord, and we demonstrated the post-translational modulation on cysteine residues of SIRT3 by HNE.

Results:

Our studies revealed that CCI leads to the development of hyperalgesia and allodynia. We reported that neuropathic pain induced by CCI is associated to SIRT3 inactivation in the spinal cord of CCI treated rats and this event seems to be related to mitochondrial protein hyperacetylation.

Conclusions:

These findings demonstrate for the first time that deactivation of sirtuins is involved in hyperalgesia and allodinia and that activation of SIRT3 by antioxidants is beneficial during oxidative stress induced by neuropathic pain.

This work has been supported by funds from PON03PE_00078_2, PON03PE_00078_1/F1, PON03PE_00078_2/F1.
Background and Aims:

Chronic pain can arise from interactions between innate mechanisms and environmental factors, such as stressful events. Since environmental enrichment (EE) provides welfare to animals, we aimed to evaluate the role of different EE types on anxiety and sensibility of rats with neuropathic pain.

Methods:

Male Wistar rats were used. Animals were assigned to two types of EE: (A) After weaning, animals were housed in groups of five in standard size cages (49x34x16cm) and given one type of object (surgical cap, paper roll or tunnels) exchange one for another every week. (B) Animals were already born in an enriched environment and, after 5 weeks of life, housed in larger cages (60x50x22cm) and given three different objects at once (Ping-Pong balls, tunnels, huts, retreats, or surgical cap – exercise wheel was not added), being one of them weekly exchanged. Control group remained in standard cages and didn’t receive objects. Within 7 weeks of life, anxiety and thermal sensibility were evaluated using elevated plus maze and tail flick tests, respectively. Mechanical hyperalgesia was analyzed in the presence of neuropathy (chronic constriction injury) by paw pressure test.

Results:

Both EE protocols tested were effective in diminish anxiety but they didn’t alter thermal sensibility. On the other hand, the simplified EE protocol failed to modulate chronic mechanical hyperalgesia, whereas the second protocol completely abolished such pain behavior.

Conclusions:

Our results suggest that variations in EE protocols provoke alterations in limbic and somatosensory areas that reduce anxiety but differently modulate pain sensibility upon chronic noxious stimulus.

Support: CNPq-141161/2013-2, FAPESP-2013/20795-8
Background and Aims:

Environmental enrichment (EE) can alter the perception of nociceptive stimuli and the analgesic response induced by opioids. The aim of this work was to evaluate the role of animal welfare in pain sensitivity of rats against chronic noxious stimuli and the participation of opioid signaling in this effect.

Methods:

Male Wistar rats were used. Enriched group was already born in an enriched environment. Within 7 weeks of life, under EE condition, chronic constriction injury (CCI) of the sciatic nerve was surgically performed. EE effects in mechanical and thermal hypernociception or tactile allodynia were analyzed before and 7 and 14 days after surgery, using rat paw pressure, Hargreaves and von Frey hair tests, respectively. Naloxone was used to evaluate the involvement of endogenous opioids. After 14 days, animals were euthanized and serum was obtained to quantify endogenous opioid levels using EIA kit. Opioid receptors expression was evaluated by Western Blotting.

Results:

EE completely abolished allodynia, and mechanical and thermal hyperalgesia, after 14 days of CCI. Naloxone treatment reversed analgesic effect of EE. Beta-endorphin and met-enkephalin serum levels were augmented in enriched animals only in the presence of CCI, without changing opioid receptors expression in spinal cord, PAG and DRG.

Conclusions:

EE, without exercise wheel, abolish chronic pain behavior by endogenous opioid pathway activation, demonstrating that welfare per se is able to control chronic pain behavior. This work contributes for the understanding of endogenous mechanisms involved in pain control and in the diversity of responses to different treatments used for patients.

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THE ZEBRAFISH AS INNOVATIVE ANIMAL MODEL TO STUDY NEUROPATHIC-LIKE PAIN.

V. Malafoglia¹, S. Ilari², F. Lauro², L.A. Giancotti³, G. Muscoli³, G. Bellipanni⁴, A. Giordano⁴, W. Raffaeli⁷

¹ISAL Foundation, pain therapy, Rimini, Italy
²San Raffaele Roma S.r.l., pharmacology, Roccioletta di Borgia- Catanzaro, Italy
³Institute of research for food safety and health IRC_FSH- University of Magna Grecia, Health sciences, Catanzaro, Italy
⁴Center for Biotechnology- Sbarro Institute for Research and Molecular Medicine, Biology, Philadelphia, USA

Background and Aims:

Canonical animal model studies have been showed that disruption of tissues and degeneration of axons projecting through muscle and skin are the most common consequences of burn damage. This condition causes neuropathic-like pain due to both inflammation and axonal degeneration. To contrast burns symptoms, destroyed tissues have to be rebuild and pain has to be alleviated. While tissue regeneration techniques have been developed, less is known on the treatment of the induced pain. Thus, appropriate animal models are necessary for the development of the best treatment for pain induced in burned tissues. Here we propose the zebrafish as innovative animal model to study neuropathic-like pain.

Methods:

We present an extreme thermal noxious stimulation test using zebrafish larvae, in order to study axon innervating degeneration, by using a gene trap line expressing Gal4 and uas:egfp under the control of the endogenous nsf promoter gene. Moreover we analyze pain marker genes over-expression (c-fos, c-jun, bdnf, pacap1b) through molecular biology approaches, at specific time points after the stimulus.

Results:

We demonstrated that degeneration of axons innervating the affected tissues and over-expression of specific genes in sensory tissues are conserved mechanisms from zebrafish to mammals. Thus we showed that zebrafish could be a new model to study burn-induced inflammation and neuropathic-like pain.

Conclusions:

We have established a new assay of nociception in zebrafish larvae that induce effects similar to post-burn neuropathic pain. We are now looking at the possible heat receptor that could mediate such sensation, focusing on transient receptor potential (TRP) family.

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QUANTITATIVE SENSORY TESTING IN ANIMAL MODELS OF CHRONIC PAIN: A PILOT STUDY
B. Monteiro¹, M. Moreau¹, C. Otis¹, L.P. De Lorimier², J.P. Pelletier³, E. Troncy¹
¹Université de Montréal, Biomedical Sciences, St. Hyacinthe, Canada
²Centre Vétérinaire Rive-Sud, Oncology, Brossard, Canada
³Research Center of the Université de Montréal Hospital Centre, Osteoarthritis Research Unit, Montreal, Canada

Background and Aims:
Quantitative sensory testing (QST) evaluates the patient’s somatosensory profile. This pilot study aimed to compare the sensory sensitivity of healthy and affected dogs with chronic pain.

Methods:
Static and dynamic QST included punctate tactile (electronic von Frey) and mechanical (Wagner™ algometer) thresholds, and conditioned pain modulation (CPM) index, respectively.

Healthy client-owned dogs (n=7) were evaluated twice in a day 4 hours apart. Data from healthy dogs were compared to laboratory dogs before and after (9 weeks) surgically-induced osteoarthritis (OA) (n=6) and client-owned dogs with natural osteosarcoma (OSA) (n=8). Inferential statistics were done at a level of 5% (data presented as mean±SD).

Results:
In healthy dogs, intra-class correlation coefficients for tactile and mechanical thresholds at the primary site were 0.96 and 0.36, respectively; and 0.89 for mechanical threshold at a distal site. A CPM effect was observed in healthy dogs: 8.89±0.58 N pre- vs. 9.5±0.38 N post-conditioning stimulus (P=0.032).

Primary tactile allodynia was observed in OA-induced dogs (P=0.014), and mechanical threshold was decreased in OA-induced (2.72±0.74 N) and OSA dogs (6.69±2.35 N); (P<0.05 for both painful conditions). Secondary mechanical allodynia was noted in OA-induced dogs (P=0.013). No CPM effect was observed in OSA dogs (suggesting deficient descending inhibitory control). The mechanical threshold of laboratory dogs at baseline was high (9.44±1.1 N) and no CPM effect was observed, suggesting a situation of stress-induced analgesia.

Conclusions:
The proposed QST protocol seems a reliable and sensitive method to evaluate somatosensory changes associated with canine chronic pain.
ROLE OF CAPSAICIN-SENSITIVE PRIMARY AFFERENTS IN A MOUSE MODEL OF NITROGLYCERIN-INDUCED PERIPHERAL HYPERSENSITIVITIES

S.J. Kim¹, J.H. Yeo¹, S.Y. Yoon², S.G. Kwon¹, J.H. Lee³, A.J. Beitz⁴, D.H. Roh¹
¹Kyung Hee University, School of Dentistry, SEOUL, Republic of Korea
²Seoul National University, School of Dentistry, SEOUL, Republic of Korea
³Seoul National University, College of Veterinary Medicine, SEOUL, Republic of Korea
⁴University of Minnesota, College of Veterinary Medicine, St Paul, USA

Background and Aims:

Despite the relatively high prevalence of migraine, the underlying mechanisms, especially in relation to chronic migraine are unknown. In this study, we tried to investigate the role of capsaicin-sensitive primary afferents (CSPAs) in the development of peripheral hypersensitivities in a mouse model of nitroglycerin (NTG)-induced chronic migraine.

Methods:

Nitroglycerin (10 mg/kg) was repetitively administrated to the mice every alternate day for nine days. To verify whether resiniferatoxin (RTX)-induced destruction of CSPAs altered peripheral hypersensitivity, RTX (0.02 mg/kg) was injected five days prior to the first NTG administration.

Results:

Two hours post-injection, NTG produced acute mechanical and heat hypersensitivity in the hind paws and facial cold alldynia. Mechanical and heat hypersensitivities in the hind paws, and facial cold allodynia, were progressive and long-lasting. Resiniferatoxin did not affect NTG-induced mechanical allodynia in the hind paws or facial cold alldynia, but did inhibit the development of thermal hyperalgesia in the hind paws of NTG-treated mice. Nitroglycerin produced significant thermal hyperalgesia in transient receptor potential type V1 (TRPV1) wild-type mice, but not in TRPV1 knockout mice.

Conclusions:

Nitroglycerin-induced mechanical hypersensitivity in the hind paws, and facial cold alldynia, are not mediated by the activation of CSPAs. This suggests that peripheral hypersensitivity in migraine patients could be differentially expressed, depending on the body site, modality tested, and the primary afferent fiber types innervating the particular body region.

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EVALUATING IMMUNOMODULATORY AND ANTINOCICEPTIVE EFFECTS OF CROTOXIN CONJUGATED TO NANOSTRUCTURED SILICA SBA-15 IN A MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

M.B.M. Sant'Anna¹, A.C. Giardini¹, N.B. Teixeira¹, F.S.R. Lopes¹, L.F. Kimura², S.C. Sampaio³, O.A. Sant'Anna⁴, Y. Cury¹, G. Picolo¹
¹Butantan Institute, Special laboratory of pain and signaling, São Paulo, Brazil
²Butantan Institute- Institute of Biomedical Sciences, Special laboratory of pain and signaling- Department of Pharmacology, São Paulo, Brazil
³Butantan Institute, Laboratory of Pathophysiology, São Paulo, Brazil
⁴Butantan Institute, Laboratory of Immunology, São Paulo, Brazil

Background and Aims:

Multiple sclerosis is a chronic inflammatory demyelinating disease that induces pain in 50% to 80% of patients. Crotoxin (Ctx), the main neurotoxin of Crotalus durissus terrificus snake venom has prolonged anti-inflammatory, immunomodulatory and antinociceptive activities, making it potential drug for the treatment of pain. However, toxicity is a limiting factor for its use. SBA-15 silica when used as an adjuvant may reduce the toxicity and potentiate immune response of various compounds. We aimed to assess whether CTX, when conjugated to the SBA-15 has its toxic effect reduced and its antinociceptive effect enhanced, and to assess its interference on the immune and inflammatory responses in EAE model.

Methods:

To determine the LD₅₀, five doses of CTX or CTX:SBA-15 were used. EAE was induced by immunization of female C57BL/6 mice with MOG₃₅₋₅₅ peptide and CFA, followed by pertussis toxin injection. Animals were treated with CTX or CTX:SBA-15. Mechanical nociceptive threshold was evaluated using electronic von Frey. Clinical signs were assessed according to scores from 0 to 5. Glial cells activation and myelin loss were evaluated by Western Blotting.

Results:

Results showed that CTX:SBA-15 induced an increase of LD₅₀ when compared to CTX. A single dose of CTX:SBA-15 was effective in modulating both mechanical hypersensitivity and clinical signs in animals with EAE. Also, treatment with CTX:SBA-15 reduced glial cells activation and prevented demyelination.

Conclusions:

These results suggest that SBA-15 decreases the toxic effect of CTX, allowing the increase of its dose and its use for pain management and clinical signs control in the EAE model.

Support: FAPESP-2014/19397-0
Background and Aims:

Crotoxin (CTX), the main neurotoxin of *Crotalus durissus terrificus* snake venom, has prolonged anti-inflammatory, immunomodulatory, antitumoral and antinociceptive activities, making it potential drug for the treatment of pain. It is also known that the SBA-15 silica when used as an adjuvant may reduce toxicity and potentiate immune responses of various compounds. We aimed to investigate the effect of CTX when coupled to SBA-15 silica (CTX:SBA-15) on modulation of acute and chronic phase of neuropathic pain development.

Methods:

C57Bl/6 mice were used. To determine the LD$_{50}$, five doses of CTX or CTX:SBA-15 were used. Neuropathy was induced by the partial sciatic nerve ligation (PSNL model). Animals were treated with CTX, CTX:SBA-15 or vehicle. To assess nociceptive threshold von Frey hairs were used. Glial cell activation was verified by Western blotting.

Results:

The results showed that CTX:SBA-15 induced an increase of LD$_{50}$ when compared to CTX in 35%. Treatment with CTX: SBA-15 in the acute phase, in a single or repetitive (five) doses induced antinociception that lasted up to 6 days after the last dose administration. At the 14$^{th}$ day after surgery, CTX: SBA-15 induced analgesic effect for at least 5 days after the last dose administered. The activation of glia cells observed in these models is reduced by the treatment with complex CTX:SBA-15.

Conclusions:

These results suggest that SBA-15 decreases the toxic effect of CTX, allowing the increase of its dose. The complex CTX:SBA-15 induces long lasting analgesic effect, confirming its potential use for pain modulation even in chronic states.

Background and Aims:

Opioids (e.g. morphine) provide eminent analgesia in acute pain. However, their efficacy is weakened in neuropathic pain: nerve injury causes maladaptive overactivation of endogenous pronociceptive systems, counteracting the antinociceptive opioid effect. Therefore, the idea of simultaneous activation of the opioid system and modulation of a chosen pronociceptive system has been proposed in search for better analgesic effect in neuropathic pain.

Methods:

Two types of bifunctional drugs (hybrids) were designed: 1) acting as antagonists of neurokinin 1 receptors (NK1R), involved in pain transmission 2) binding to nociceptin receptors (NOP) that contribute both to pain sensation and analgesia. All hybrids tested contain opioid receptors (OP) agonist pharmacophores. We compared the analgesic efficacy of the compounds administered intrathecally in mice using the tail-flick test in acute pain model. Chronic constriction injury (CCI) was performed on mice to induce neuropathy. Different aspects of analgesia were measured next in the von Frey (mechanical stimulus) and cold plate test (low temperature stimulus).

Results:

Both types of hybrids diminished neuropathic pain. OP-NOP hybrids were efficient at doses as low as 0.005 nmol; their analgesic action lasted up to 180 min. after injection. In contrast, the prepared OP-NK1 hybrids performed robust, but short-lasting analgesic effect only in higher doses starting from 1 nmol.

Conclusions:

Results point to a superior efficacy of OP-NOP hybrids towards improved neuropathic pain treatments. Acknowledgements: Supported by grant NCN MAESTRO 2012/06/A/NZ4/00028; statutory funds IF-PAS; J. S - KNOW scholarship sponsored by MSiHE, Poland. 2Collaboration between the MDEIE, Canada and the Research Foundation – Flanders (FWO Vlaanderen).
Background and Aims:

This study aimed to evaluate the feasibility and repeatability of electrical (E), mechanical (M) and thermal (T) nociceptive testing in dogs.

Methods:

Sixteen client-owned healthy dogs [4.5 (2-9 years); 30 ± 18 kg (six males/10 females)] were included. Nociceptive stimulation was applied to the dorsal aspect of the metacarpus and the plantar aspect of the metatarsus in a randomized order until a behavior response (escape reaction, etc.) was observed or a cut-off reached. For E, transcutaneous electrical stimulation (TENS) was provided using two adhesive electrodes. For M, an increasing pressure was applied with a flat tip of an algometer. For T, a probe was applied at 25°C and decreased gradually to 0°C. Tests were performed twice (60 seconds apart) and by two observers. Retesting was performed five hours later. Sham testing was performed for E and T. Statistical analysis included mixed linear model; inter-observer repeatability was calculated using intra-class correlation coefficient (ICC) (p < 0.05).

Results:

There was no influence of time or limb tested on mean values for E and M (p < 0.05). Only five dogs responded to thermal stimulation and data for T were not tested. Collection of data was feasible (easy) in 99% for E, 93.6% for M and 93.5% for T. Inter-observer repeatability was excellent for E (91.3%) and moderate for M (60.9%). False-positive responses were 15% and 28.6% for E and T, respectively.

Conclusions:

Electrical nociceptive testing was feasible, repeatable and superior to M/T. Data will be used for comparison with dogs with naturally-occurring neuropathic pain.
Aim of investigation: It has been reported that there are non-/low-responders to pregabalin, commonly used for diabetic peripheral neuropathy (DPN), however few studies focused on the pregabalin-refractory DPN. Therefore in this study, we tried to investigate the mechanisms of the pregabalin-refractory DPN using STZ rats, classically used as an animal model of DPN.

Methods and results: STZ rats showed increase of blood glucose, mechanical allodynia and decrease in nerve conduct velocity of their tails, sustainably within 8 weeks after a single injection of STZ (60 mg/kg, i.v.). The anti-allodynic effects of amitriptyline (3-50 mg/kg) and oxycodone (0.3-1mg/kg) were observed but not by diclofenac (10mg/kg) in both 2 weeks and 8 weeks after STZ-injection. Pregabalin (10-30mg/kg) showed significant anti-allodynic efficacies in 2 weeks, but not in 8 weeks after STZ-injection, even taking into account of the decrease of its plasma concentration. The changes of expression of alpha-2-delta-1 in dorsal root ganglion were not observed after STZ-injection. Intrathecal pregabalin (3-30ug) did not significant anti-allodynic effects and the efficacies of systemic pregabalin (10-30mg/kg) were significantly attenuated by the intrathecal yohimbine in 2 weeks after STZ-injection.

Conclusions: The STZ rats in 8 weeks could be a good model for the pregabalin-refractory DPN. The efficacy of pregabalin in DPN might be mediated by the supra-spinal noradrenergic descending pain modulatory pathways, not by the spinal alpha-2-delta-1, and the dysfunction of the pathways might involve in the pregabalin-refractory DPN.
BACKGROUND AND AIMS:

Following nerve injury sensory neurons develop ectopic activity thought to be critical for the induction and maintenance of peripheral neuropathic pain. Local anaesthetics and anti-epileptic drugs can suppress such hyper-excitability however these drugs are complicated by motor, CNS and cardiac dysfunction. Here we show that a glutamate-gated chloride channel (GluCl) modified to be activated by the non-toxic drug Ivermectin, but not glutamate, is highly effective in silencing sensory neurons and treating neuropathic pain related hypersensitivity.

METHODS:

Using sensory neuron cultures we performed detailed in vitro analysis of GluCl efficacy and translated these findings in vivo by delivering AAV-GluCl specifically to mouse dorsal root ganglion. This allowed us to use sensory withdrawal assays to assess GluCl silencing capability in vivo, in both naïve and nerve-injured animals.

RESULTS:

GluCl activation potently inhibited the ability of human and rodent sensory neurons to respond to electrical and algogenic stimuli, in vitro. Intrathecal delivery of AAV9-GluCl generated high levels of sensory neuron transduction (L4 DRG, 66.1 ± 9.6%) that was still evident 7 months later. This enabled reproducible and reversible increases of thermal (+48.4 ± 15.6%, P=0.007) and mechanical (+63.0 ± 20.9%, P=0.015) pain thresholds by Ivermectin, with no motor deficits. Established mechanical pain related hypersensitivity secondary to traumatic nerve injury was reversed by Ivermectin; mirrored at the cellular level with a cessation of ectopic activity.

CONCLUSIONS:

These findings emphasise the importance of aberrant afferent input in the maintenance of neuropathic pain and the potential for targeted chemogenetic silencing as a new treatment modality for neuropathic pain.
Background and Aims:

Traumatic injury to the spinal cord results in acute necrosis and delayed secondary expansion of neurological damage, and leads to a lifetime of paralysis, sensory dysfunction, and chronic pain. Excessive excitation is a primary source of neural injury evoked by various insults, causing neuronal cytotoxicity, and slowly expanding lesions. The proposed studies attempt to reduce persistent hyperexcitability of neurons during acute phases of spinal cord injury (SCI), thereby preserving neurological function that would be lost during the chronic stage. KCNQ channels are abundant in spinal neurons and axons, controlling their excitability. Retigabine, a specific KCNQ channels opener could be a plausible treatment to reduce SCI-induced pathology.

Methods:

We produced contusive SCI at T10 in adult, male rats, which then received 10 consecutive days' treatment with retigabine or vehicle 3 hours or 3 days after contusion. Two different concentrations and two different delivery methods were used.

Results:

With pumps (250 mg/kg/hr) delivery 3 hours after contusion, retigabine promoted recovery of locomotor function, not intraperitoneal delivery. Remarkably, retigabine delivery in both method significantly attenuated the development of mechanical stimuli-induced hyperreflexia (hindlimb and torso) although there were no significant difference in thermal threshold. Retigabine delivered by pump 3 days after contusion only significantly attenuated the development of mechanical hypersensitivity, no effect on locomotor function. Finally, we found early application of retigabine protect gray matter, not the white matter after SCI.

Conclusions:

Our result indicates that very early opening of KCNQ channels promotes locomotor function recovery and preemptively mitigates development of neuropathic pain following SCI.
Background and Aims:

Chemotherapy-induced neuropathic pain (CINP) is one of serious dose-limiting side effect. Sinomenine, an alkaloid originally isolated from Sinomenium acutum, is used as an alternative medicine for various diseases such as rheumatoid arthritis. The present study was designed to examine whether sinomenine could produce analgesic effect and enhance lower-dose gabapentin-induced analgesic effects in a mouse model of oxaliplatin-induced neuropathic pain.

Methods:

CINP was induced by single intraperitoneal (i.p.) administration of oxaliplatin (10mg/kg). Sinomenine (10, 20, 30 or 40mg/kg, i.p.) or/and gabapentin (10, 30, 100, 200 or 300mg/kg, i.p.) was injected at day 14 after oxaliplatin injection. Gabapentin-induced motor impairment/sedation was measured by the rota-rod test.

Results:

Sinomenine administration dose-dependently reduced oxaliplatin-induced mechanical allodynia. Gabapentin also dose-dependently reduced CINP. However, high dose of gabapentin (200 and 300 mg/kg) induced severe sedative effect. Interestingly, sub-dose of sinomenine (20mg/kg) in combination with a low dose of gabapentin produced an analgesic effect similar to that of the high dose of gabapentin, but without significant sedative effects.

Conclusions:

These data showed that sinomenine could suppress CINP and significantly enhance gabapentin-induced analgesia, implying that a combination of low dose gabapentin with sinomenine might be a novel strategy for CINP management that could eliminate gabapentin’s side effects.

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NEUP7-0317
INVOLVEMENT OF DOPAMINE RECEPTORS WITHIN THE DORSAL HIPPOCAMPUS IN SUPPRESSION OF THE FORMALIN-INDUCED OROFACIAL PAIN.
L. Zarepour

Iran university of medical science, Medical physiology, Tehran, Iran

Background and Aims:

It is widely established that the dopaminergic system has profound effects on pain modulation in different regions of the brain including the hippocampus, the salient area for brain functions. The orofacial region is one of the most densely innervated areas of the body susceptible to acute and chronic pains.

Methods:

we tried to examine the effects of dopamine receptors located in the dorsal hippocampus (CA1) region upon the modulation of orofacial pain induced by the formalin test. To induce orofacial pain in male Wistar rats, 50μl of 1% formalin was subcutaneously injected into the upper lip. In control and experimental groups, two guide cannulae were stereotaxically implanted in the CA1, and SKF-38393 (0.25, 0.5, 1 and 2μg/0.5μl saline) as a D1-like receptor agonist, SCH-23390 (1μg/0.5μl saline) as a D1-like receptor antagonist, Quinpirole (0.5, 1, 2 and 4μg/0.5μl saline) as a D2-like receptor agonist and Sulpiride(3μg/0.5μl DMSO) as a D2-like receptor antagonist or vehicles were microinjected.

Results:

Results indicated that SKF-38393 at the dose of 1 and 2μg significantly reduced pain during the first and second phases of observed pain while SCH-23390 reversed such analgesic effect. Moreover, there is a significant difference between groups in which animals received 2 and 4μg quinpirole or vehicle in the first phase (early phase) of pain. The three high doses of this compound (1, 2 and 4μg) appeared to have an analgesic effect during the second (late) phase.

Conclusions:

Current findings suggest that the dorsal hippocampal dopamine receptors exert an analgesic effect during the orofacial pain test.
METHYLGLYOXAL, AN ADVANCED GLYcation END PRODUCT, INDUCES THE INTEGRATED STRESS RESPONSE (ISR) IN NOCICEPTORS TO PROMOTE DIABETIC NEUROPATHIC PAIN

P. Barragan-Iglesias¹, J. Kuhn¹, T. J. Price¹
¹University of Texas at Dallas, School of Behavioral and Brain Sciences, Dallas, USA

Background and Aims:

Methylglyoxal has been suggested to play a causal role in the pathogenesis of diabetic peripheral neuropathic pain. However, the mechanisms underlying Methylglyoxal-induced pain over the longer term are not known. Recently the endoplasmic reticulum (ER) stress pathway was proposed to regulate DRG excitability. We hypothesized that MGO induces ER stress in DRG leading to a sensitization of these neurons.

Methods:

Primary DRG cultures
Western blot
Calcium Imaging
SUnSET assay
Mechanical hypersensitivity testing

Results:

Methylglyoxal (1 micromolar, 3-48 h) up-regulates the ER stress markers BiP, p-PERK and p-eIF2 alpha but not p-ERK, p-RS6 or p-eIF4E in DRG cultures. ISRIB (200 nanomolar) attenuates the eIF2alpha phosphorylation. Methylglyoxal decreases nascent protein synthesis in DRG neurons. In addition, methylglyoxal enhances the excitability of primary DRG cultures. Methylglyoxal (0.01-0.1 nmol, i.pl.) causes a transient nocifensive response and a short-term mechanical hypersensitivity. However, methylglyoxal (10 nmol) causes a transient nocifensive response and a long-lasting mechanical hypersensitivity. Pain behavior produced by methylglyoxal (0.01-0.1 nmol) is not blocked by the TRPA1 antagonist A967079 (30 micrograms, i.pl.) but ISRIB blocks methylglyoxal-induced mechanical hypersensitivity (6.64-22.15 nmol, i.pl. or 2.5 mg/kg, i.p.) suggesting an ISR-dependent mechanism. Moreover, 4-PBA blocks mechanical hypersensitivity (10-100 mg/kg). 4-PBA (10-100 mg/kg, i.p.) and ISRIB (2.5 mg/kg, i.p.) also reversed established long-lasting mechanical hypersensitivity. In addition, the AMPK activators A769662 (20 nmol, i.pl.) and metformin (200 mg/kg, p.o., daily from day 5 to day 15) inhibit and reverse MGO induced long-lasting mechanical hypersensitivity.

Conclusions:

Experiments are ongoing in our lab to determine what links MGO to the ISR and cellular excitability.
Background and Aims:

A decrement of AMP-activated protein kinase-eNOS bioavailability is critical for the pathogenesis of diabetic peripheral neuropathy (DPN). We evaluated the neuro-protective effect of cinacalcet on DPN by activating the AMPK-eNOS pathway using db/db mice.

Methods:

The db/db mice and db/m controls were divided to receive either a regular diet or a diet containing cinacalcet. Mice were evaluated sciatic motor nerve conduction and tactile response. We performed Mason’s trichrome staining and immunohistochemistry for type IV collagen and 8-hydroxy-deoxyguanosine, an oxidative DNA damage marker. For immunofluorescence double staining, apoptosis was detected by ApopTag Fluorescein In Situ Apoptosis Detection Kit. The sciatic nerve specimens were double stained and we measured unmyleinated fiber areas and axonal areas using NIH Image J under a transmission electron microscope. Western blot assay was performed total protein of sciatic nerve with specific antibodies for CaSR, CaMKK, total LKB1, phospho-Ser428 LKB1, total AMPK, phospho-Thr172 AMPK, total eNOS, phospho-Ser1177 eNOS, PGC-1.

Results:

The db/db mice showed sensorimotor impairment, nerve fibrosis and inflammation, apoptosis, disorganized myelin with axonal shrinkage and degeneration, and fewer unmyleinated fibers in the sciatic nerve (Fig 1) compared to db/m mice. Cinacalcet administration, without causing any changes in blood glucose and Ca++ concentrations, significantly increased the paw withdrawal mechanical threshold and decreased the motor conduction latency, and ameliorated the deterioration of sciatic nerve pathology (Fig 2), accompanied by increases in the expressions of calcium-sensing receptor-CaM KK and phosphorylation of AMPK-eNOS (Fig 3) in diabetic mice.

Conclusions:

The Cinacalcet may play an important role in the prevention and amelioration of DPN by amplifying AMPK signaling.
Slack potassium channels are expressed throughout the nervous system where they are important for the generation of sodium-activated potassium ($K_{\text{Na}}$) currents and the excitability of neurons. Using different mouse models of pain in Slack knockout (Slack$^{-/-}$) mice, we found that Slack modulates mechanical hypersensitivity after peripheral nerve injury. However, the detailed function of Slack channels in pain processing is unknown. Here we focused on the functional roles of Slack channels in peripheral neuropathic pain.

Methods:

Slack$^{-/-}$ mice; patch-clamp recordings; neuropathic pain models; immunostaining; western blot

Results:

In the present study, genetic and pharmacological approaches were used to further investigate the roles of Slack channels in neuronal physiology and pain. In isolated dorsal root ganglion neurons, we found that deletion of Slack channels reduced $K_{\text{Na}}$ currents and also changed the pattern of action potential firing. In vivo, pharmacological Slack activation reduced neuropathic pain behavior.

Conclusions:

Our data suggest that Slack channels control the sensory input in neuropathic pain states and could be a potential drug target for peripheral neuropathic pain.
NEUP7-0225
NITROUS OXIDE (N2O) EXPOSURE REDUCED NEUROPATHIC PAIN AND ASSOCIATED SPINAL NEURO-INFLAMMATION IN RAT
C. Mallet¹, E. Chapuy¹, Y. Aissouni¹, J. Barbier¹, A. Milet², G. Farjot², B. Bessière², A. Eschalier¹
¹Clermont Auvergne University, Neuro-Dol INSERM 1107 Pharmacology of Pain, Clermont-Ferrand, France
²Air Liquide Santé International, Centre de Recherche Paris-Saclay, Jouy-en-Josas, France

Background and Aims:
A pre-clinical study in a rat neuropathic model showed that a single nitrous oxide (N₂O) exposure induced a delayed analgesic effect days after exposure. The aim of this study is: (1) to confirm the delayed analgesic effect of N₂O and (2) to study the impact of gas exposure on spinal neuro-inflammation.

Methods:
Thermal sensitivity was evaluated before and 14 days after ligature of the sciatic nerve (chronic constriction injury model, CCI) measuring the withdrawal latency of the ipsilateral paw after immersion in a warm (46°C) or cold (10°C) bath. After, animals were exposed to a N₂O-O₂ (50/50%) gas mix or a N₂-O₂ (50-50%) gas control mix during 1h15. Thermal sensitivity of animals was evaluated during 3 days. From other animals submitted to the same procedure, dorsal spinal cord has been sampled the 2nd day post-exposure in order to study neuro-inflammation.

Results:
Compared to controls, a reduction of thermal sensitivity induced by CCI was observed at 1st and 2nd days after 50% N₂O exposure. This was accompanied by (1) a reduction of microglial activation, (2) a decrease of IL-1β, TNFα, BDNF levels; (3) a decrease of the p65 subunit of the NFκB, and (4) a reduction of the phosphorylation of the NR2B subunit of the NMDA receptor.

Conclusions:
These results suggest that a single N₂O exposure in a neuropathic animal model leads to a reduction of the spinal neuro-inflammation that could contribute to the maintained analgesic effect of this gas. Thus, this study highlights a new therapeutic use of N₂O to relieve neuropathic pain.
Background and Aims:

Trigeminal Neuralgia (TN) remains one of the most painful conditions known to man. Vascular compression of the trigeminal nerve is the primary cause of TN. A subpopulation of patients responds to the anti-seizure, voltage-gated Na+ channel (VGSC)-blocking drug carbamazepine. However, carbamazepine was largely ineffective in clinical trials on other neuropathic pain conditions, including peripheral nerve compression injuries. Based on these clinical observations, and data on the role of VGSCs in neuropathic pain and the afferent subpopulation specific response to injury, we hypothesized the therapeutic selectivity of carbamazepine reflects the unique combination of changes in VGSCs in the trigeminal nerve.

Methods:

We employed rat models of somatic (sciatic nerve, SN-CCI) and trigeminal (infraorbital nerve, ION-CCI) nerve compression, and assessed the sensitivity of spontaneous (conditioned place preference test) and evoked (von Frey and orofacial pain tests) pain behavior to carbamazepine. PCR analysis of trigeminal (TG) and dorsal root ganglia (DRG) was used to assess the potential contribution of changes in VGSCs expression to behavioral differences.

Results:

Carbamazepine (50 mg/Kg), but not vehicle, diminished spontaneous and evoked pain behavior in ION-CCI rats, but had no effect in SN-CCI rats. Preliminary PCR analysis suggested elevated NaV1.9 expression in the TG of ION-CCI rats, but not in the DRG of SN-CCI rats.

Conclusions:

These results agree with our initial hypothesis and suggest possible novel strategies to treat TN by targeting the selective pattern of changes in VGSCs.

The work was supported by generous donations from TN patients and family members as well as NIH grant R01NS064988.
Background and Aims:

Neuropathic pain imposes substantial challenges to clinical practice. In a quest for novel treatment options recent research efforts are focused on elucidating the molecular underpinnings of neuropathic pain conditions. Despite enormous progress, a global view of entire protein networks and a quantitative comparison of their changes during neuropathic pain is still missing. Yet, the benefits of such knowledge would be immense. This is exemplified by evolving strategies of network medicine which postulate that critical hubs of dysfunctional disease-related protein networks may serve as novel therapeutic targets.

Methods:

Here we present a comprehensive proteome analysis of 6 regions along the pain axis (from the periphery to several regions of the CNS) in a mouse model of neuropathic pain. To this end we employed emerging data-independent acquisition mass spectrometry (DIA-MS). DIA-MS is uniquely suited to perform proteome profiling with high sensitivity and reproducibility – prerequisites for faithfully interrogating protein network dynamics.

Results:

We reproducibly quantified the same set of several thousands of proteins in each analyzed region of the pain axis. Our dataset reveals (i) region-specific and (ii) pain-associated proteome changes including dozens of proteins that have not been described in the context of pain before.

Conclusions:

Our study yields unprecedented insights into the dynamics of protein networks during neuropathic pain and opens new avenues for ongoing efforts to define a molecular signature of neuropathic pain.
Background and Aims:

Recent studies have shown that HCN channel dysfunction contributes to the hyperexcitability observed in the medial prefrontal cortex (mPFC) after peripheral nerve injury. However, the molecular mechanisms underlying neuropathy-associated changes in HCN channel activity remain elusive. Since chronic pain and anxiety can increase noradrenergic transduction in the mPFC, our primary aim was to investigate whether the ascending noradrenergic system plays a role in HCN channel regulation in neuropathic conditions.

Methods:

Whole-cell patch-clamp recordings in acute brain slices were performed on superficial prefrontal neurons from sham and spared nerve injured (SNI) rats to investigate HCN channel function. Immunofluorescence experiments were performed on tissue extracted from PFA-perfused sham and SNI rats to determine changes in noradrenergic fiber density in the mPFC. Von Frey and acetone tests were used to assess behavioral effect of clonidine in SNI rats.

Results:

We discovered a significant increase in noradrenergic innervation within the mPFC of nerve-injured animals compared to controls. Moreover, we demonstrated that adrenergic receptors, particularly α2 adrenoceptors, can modulate the voltage-dependent activation of HCN channels and the abnormal prefrontal excitability following nerve injury. Microinfusions of the α2 adrenoceptor agonist clonidine in the mPFC of SNI rats caused modality-specific analgesic effects, suggesting a behavioral significance for these receptors in neuropathic pain conditions.

Conclusions:

Our findings provide insights into the role of catecholaminergic neuromodulation in neuropathic pain and suggest that altered noradrenergic transduction may play an important role in the HCN channel dysfunction and pyramidal hyperexcitability observed in the neuropathic mPFC.
Background and Aims:

Clinically, tongue cancer patients do not occasionally complain of obvious tongue pain in the early stage of tongue carcinogenesis. However, the exact mechanism of nociceptive modulation in cancerous tongue is not still known. In this study, we examined the involvement of endothelin signaling in modulation of tongue pain in the early stage of tongue carcinogenesis in rats.

Methods:

Cancer cells (SCC-158) were inoculated into the tongue under the deep anesthesia. Changes in tongue mechanical sensitivity, a mount of Endothelin-1 and β-endorphin in cancerous tongue, were examined following cancer cells inoculation. Moreover, Effect of endothelin-A or μ-opioid receptor antagonism on the tongue mechanical sensitivity were also examined.

Results:

Tongue mechanical sensitivity did not change in the early stage of tongue carcinogenesis, was significantly reduced from day 11 onward. Endothelin-A receptor was expressed in cancer cells. A mount of Endothelin-1 was significantly increased, and Endothelin-A receptor antagonist administration into cancerous tongue induced the mechanical hypersensitivity in the early stage of tongue carcinogenesis. μ-opioid receptor was expressed in trigeminal ganglion neurons innervating to the cancerous tongue, m-opioid receptor antagonist administration to the tongue significantly enhanced the mechanical hypersensitivity, and amount of β-endorphin was significantly increased in the early stage of tongue carcinogenesis.

Conclusions:

These findings suggest that β-endorphin released from the cancer cells via endothelin-1 signaling is involved in modulation of tongue pain in the early stage of tongue carcinogenesis.
NEUP7-0242
PN6047; A NOVEL AND POTENTIALLY FIRST-IN-CLASS TREATMENT FOR CHRONIC PAIN.

B. von Mentzer¹, I. Starke², G. Sunden³, D. Kendall⁴
¹PharmNovo AB, Medicine, ÖCKERÖ, Sweden
²PharmNovo AB, Chemistry, ÖCKERÖ, Sweden
³PharmNovo AB, Patents, ÖCKERÖ, Sweden
⁴PharmNovo AB, Pharmacology, ÖCKERÖ, Sweden

Background and Aims:

The delta opioid receptor (DOR) has been suggested as a target in a variety of disease states, particularly in chronic pain. PN6047 is a potent and very selective DOR agonist with a uniquely G protein biased signalling profile, which enables full receptor activation with very limited agonist-induced receptor desensitization.

Methods:

PN6047 has been tested in rodent models of neuropathic pain (sciatic nerve ligation) and knee osteoarthritis (intra-articular monoiodoacetate) using von Frey measurements of mechanoreception.

Results:

PN6047 has no appreciable activity at any other neurotransmitter receptors, ion channels or enzymes apart from the DOR. It potently reduces mechanical hypersensitivity in rodent chronic pain models (half maximal effect, 1 mg/kg oral) with no overt signs of analgesic tolerance, unwanted side effects, tissue pathology or toxicity following multiple dosing. Prediction of human ADME/PK-properties is favorable with moderate oral bioavailability and half-life. PN6047 has an estimated therapeutic safety margin of >50 times its effective analgesic dose.

Conclusions:

Alternatives are needed to the only partially effective tricyclic antidepressants and anti-epileptic drugs which presently dominate chronic pain therapy. Replacements are also needed to address the world-wide epidemic of addiction to standard morphine-like opioids which are massively and inappropriately prescribed for chronic pain sufferers. PN6047 could be one such replacement. PN6047 has the potential to be a novel first-in-class treatment for chronic pain and, with its very promising pre-clinical profile, PN6047 has a very good chance of reaching the clinic and becoming a widely prescribed chronic pain medicine.
Background and Aims:

Following peripheral nerve injury a plethora of central changes occur within the dorsal horn of the spinal cord, including putative changes to the physiological and anatomical connectivity of central and primary afferent neurons (West et al. 2015). However, the structural changes to connectivity within the dorsal horn that underlie peripheral nerve injury are still relatively unknown.

Methods:

Here we developed a methodology to study connectivity by labeling synapses within the dorsal horn. A novel antigen retrieval method was employed which consisted of application of heat, proteinase K and pepsin to the tissue in sequence, and robustly unveiled synaptic puncta throughout the tissue section. To assess the puncta in an automated fashion, an in-house ImageJ plugin suite was developed. This suite first deconvolves confocal Z-stacks and thresholds them to produce robust representations of synaptic puncta, and assesses synaptic puncta in user-defined ROIs in 3D using a stereologically-consistent approach leading to an unbiased sample of objects representative of the population they are drawn from. This method assesses both synapse number as well as size and shape characteristics, and can assess large numbers of synaptic puncta in reasonable timeframes.

Results:

This method reveals a distinct distribution pattern of pre- and post- synaptic markers synaptophysin and PSD95 in dorsal horn, and demonstrates a significant loss of PSD95+ puncta in lamina 2i of dorsal horn at 21+ days post peripheral nerve injury (SNI; p<0.01, One-Way ANOVA).

Conclusions:

This method successfully quantifies synaptic puncta throughout the dorsal horn, and provides a useful tool for assessing changes in connectivity.
CHEMOCETIC DISSECTION OF THE CENTRAL NORADRENERGIC SYSTEM: DISSOCIATION OF ANALGESIC AND AVERSIVE CIRCUITS

S. Hirschberg¹, Y. Li¹, A. Randall², E.J. Kremer³, A.E. Pickering¹
¹University of Bristol, Physiology- Pharmacology and Neuroscience, Bristol, United Kingdom
²University of Exeter, Medical School, Exeter, United Kingdom
³Institut de Génétique Moléculaire de Montpellier, Centre Nationnal de la Recherche Scientifique, Montpellier, France

Background and Aims:

The Locus coeruleus (LC) is the principle noradrenergic nucleus in the CNS. Anatomical studies suggest that the LC has distinct output modules, providing selective innervation of some target areas. For example, there are discrete populations that innervate the prefrontal cortex (LC-PFC) and the spinal cord (LC-SC).

We hypothesise that stress-like adverse effects and analgesic effects that are caused by tonically increased noradrenergic activity are mediated by these distinct subpopulations of LC neurons and their postsynaptic target cells.

Methods:

Retrogradely transported CAV2 based vectors were developed to express a genetically “engineered” excitatory ionophore (PSAM) specifically activated by the selective agonist (PSEM308) under a catecholaminergic neuron specific promoter. Chemogenetic activation of LC neurons was electrophysiologicaly verified and subsequently employed to activate LC-PFC and LC-SC neurons in naïve rats and rats that underwent tibial nerve transection (TNT).

Results:

Activation of LC-SC neurons (10mg/kg PSEM308) increased thermal withdrawal latency but had no effect in conditioned place preference (CPP) and open field experiments (N=9). In contrast, activation of LC-PFC neurons was associated with aversive and anxiety-like behaviour (N=7) in CPP and open field test without any analgesic effect.

In the TNT model, activating LC-SC neurons significantly reduced mechanical and cold evoked hypersensitivity and improved incapacitance between the injured and uninjured hind leg (N=7). The animals also exhibited conditioned place preference for the PSEM308 paired environment.

Conclusions:

These findings demonstrate that by using a selective therapeutic strategy to activate pontospinal noradrenergic neurons it is possible to dissociate analgesic from aversive noradrenergic circuits and this is effective in a neuropathic pain model.
A QUANTITATIVE ANALYSIS OF THE INHIBITORY TERMINALS IN THE MOUSE DORSAL HORN IN THE SPARE NERVE INJURY MODEL OF NEUROPATHIC PAIN

K. Werynska¹, S.J. West¹, D. Bennett¹
¹University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom

Background and Aims:

Neuropathic pain is a growing concern worldwide. Lesions to the nervous system start a cascade of functional and structural changes sustaining the pain. Unfortunately, their nature remains largely unknown which is reflected in the lack of satisfying treatments. One of the ongoing challenges is understanding reason behind the loss of inhibitory tone in the dorsal horn after nerve injury. Furthermore, the quantitative distribution of inhibitory terminals in that region remains unclear. The aim was to quantify the distribution patterns of the inhibitory terminals in the dorsal horn, in a naïve state and at 3- and 6-week timepoints after the peripheral nerve injury.

Methods:

Here, a quantitative analysis of VGAT- and Gephyrin-labeled inhibitory terminals brings into focus the laminal distribution of the pre- and postsynaptic components. IB4 labelling was used for laminar delineation. Furthermore, a new technology has been utilized to assess large numbers of synapses in an unbiased manner (see “A Method to assess synaptic puncta within the Dorsal Horn: A Tool for assessing shifts in connectivity”).

Results:

A robust increase in number of both inhibitory terminals was observed between laminae I, II and III in the naïve tissue (p<0.05). Interestingly, there was no difference in the presynaptic or the postsynaptic puncta after the peripheral nerve injury (p>0.05).

Conclusions:

The results indicate that the overall number of the inhibitory terminals in the dorsal horn is not affected after the peripheral injury. The study presents patterns of the VGAT- and Gephyrin-labeled synaptic puncta and suggests the effect of injury on circuit-level processing in dorsal horn.
Background and Aims:

Tissue injury leads to the release of proalgesic molecules that promote pain by sensitizing pain-sensing neurons (nociceptors) to heat and mechanical stimuli, whereas they have no effect on cold sensitivity. Nonetheless, cold allodynia is prevalent in many neuropathies, with little known of the cellular and molecular mechanisms promoting cold pain. The transient receptor potential melastatin-8 (TRPM8) has been shown to be involved in cold allodynia, yet the molecular mechanisms that lead to TRPM8-dependent cold hypersensitivity are unknown. Here we show that the glial cell line-derived neurotrophic factor family ligand (GFL) artemin and its receptor GFRα3, along with TRPM8, are critical components of a molecular pathway that underlies cold allodynia.

Methods:

We used a microarray screen for genes enriched in TRPM8 neurons to identify GFRα3 and behavioral assays of mice lacking TRPM8 or GFRα3 to examine the role of GFRα3 and TRPM8 in the development of cold allodynia.

Results:

Here, we show that artemin, and to a lesser extent nerve growth factor (NGF), induces TRPM8-dependent cold hypersensitivity. The effects of both are dependent on GFRα3 expression, and cold allodynia induced by nerve injury or chemotherapeutics is absent in GFRα3-null mice. Furthermore, cold allodynia is blocked in animals treated with neutralizing antibodies against artemin whereas, in all cases, heat and mechanical hyperalgesia are unchanged.

Conclusions:

In striking contrast to the redundant mechanisms sensitizing other modalities after injury, these data demonstrate that induction of cold allostodynia absolutely requires GFRα3, suggesting that artemin-GFRα3 signaling via TRPM8 can be targeted to selectively treat cold pain.
Background and Aims:

Chemotherapy-induced neuropathy (CIPN) is a dose limiting side effect of many cancer treatments. Independently from the mechanism of action chemotherapeutics alter neural and glial function inducing mitotoxicity, cytokines release and enhancing nociceptive input. We described a role of the chemokine prokineticin (PK) and its receptors (PKRs) in the development of neuropathic pain of different origin. Here we assess the role of PK system in the development of CIPN consequent to the treatment with Bortezomib (BTZ) and Vincristine (VCR) evaluating a potential therapeutic effect of a prokineticin antagonist.

Methods:

CIPN was induced in mice by repeated injections of BTZ or VCR. Painful neuropathy was assessed evaluating the presence of hyperalgesia and allodynia. The PK antagonist PC1 was daily administrated in presence of a well established neuropathy and its treatment continued until the end of the chemotherapeutic schedule. At different times PK2, PKR1, PKR2 and cytokines were evaluated as mRNA (Real time PCR) and protein (multiplex ELISA) in DRG, nerves and spinal cord.

Results:

In BTZ and VCR protocol the presence of CIPN well correlated with an up regulation of PK members and pro-inflammatory cytokines and in the peripheral nervous system. The PK antagonist was able to counteract the development of thermal hyperalgesia and allodynia allowing the continuation of the chemotherapeutic treatment. The effect may be due to its capacity to contrast neuroinflammation normalizing the levels of proinflammatory cytokines simultaneously to a reduction of PK2 and PKR1 in DRG and peripheral nerves.

Conclusions:

PK antagonism may represent a new pharmacological strategy to contrast experimental CIPN.
Background and Aims:

With its 96 electrodes designed for delivery of a defined electric field to cell cultures in microplates, the Cellaxess® Elektra platform can be used for in-situ electroporation and transfection. In addition, it can also be used for electric field stimulation (EFS) to induce membrane depolarization of excitable cells. Functional EFS data can be automatically aligned with high content analysis (HCA) data to analyze excitability exclusively from a sub-population of neurons.

By combining these capabilities, the aim was to specifically investigate the modulating effects of siRNA or compounds on excitability in the pain-signaling population of DRG neurons.

Methods:

Adult rat DRG neurons were transfected with siRNAs silencing the pain-relevant targets NaV1.7 and TrkA or exposed to pain relevant compounds, followed by analysis of the EFS response in RIIβ-positive, nociceptive neurons.

Results:

By using TTX, the analysis was confirmed to be specific to nociceptive neurons. By selectively analyzing the RIIβ-positive population, the potency of the pan-Trk inhibitor K252 was increased whereas no potency change was observed for the peripheral μ-opioid receptor antagonist loperamide. NaV1.7 or TrkA siRNA decreased the number of EFS responding RIIβ-positive neurons by 60% and 47%, respectively.

Conclusions:

The Cellaxess® Elektra platform provides a powerful methodology for efficient, large scale RNAi and small molecule screening. Moreover, by combining the efficiency of the Cellaxess® Elektra with HCA, we have developed an assay capable of detecting siRNA or compound effects in a specific neuronal sub-population, being of significant value for target identification and target deconvolution in physiologically relevant culture systems.
Background and Aims:

Significant research has implicated spinal microglia as a critical mediator of mechanical hypersensitivity associated with peripheral nerve injury (PNI). After injury, microglia signalling produces downregulation of the potassium chloride cotransporter KCC2, resulting in disinhibition of lamina 1 neurons and development of hypersensitivity. However, we recently showed that microglia do not contribute to pain hypersensitivity in female mice. It remains to be determined whether KCC2 downregulation is involved in pain processing in females despite a sex difference in microglial involvement. Thus, we interrogated the role of KCC2 in neuropathic pain in both sexes.

Methods:

Neuropathic pain was induced in rodents using spared nerve injury or spinal nerve ligation. Investigation of chloride-mediated disinhibition in the spinal cord was conducted using in-vivo electrophysiology. KCC2 expression was interrogated using immunohistochemical techniques. Mechanical sensitivity was assessed with von Frey fibers. Drugs were applied via intrathecal injections.

Results:

First, we found that KCC2 blockade produces disinhibition in both sexes. Second, we demonstrated that nerve injury results in chloride dysregulation in males and females. Third, we determined that there was no sex difference in KCC2 downregulation after nerve injury in the dorsal horn of the spinal cord. Finally, we found that pain hypersensitivity associated with PNI was alleviated by intrathecal application of a chloride extrusion enhancer in both sexes.

Conclusions:

Our experiments indicate that KCC2 downregulation is critical in the mediation of pain hypersensitivity in females, despite a sex difference in upstream signalling.
THE ANTI-DIABETIC DRUG METFORMIN CAN REGULATE VOLTAGE-GATED SODIUM CHANNEL NAV1.7 VIA THE UBIQUITIN-LIGASE NEDD4-2
L. Gillet\textsuperscript{1}, C. Laedermann\textsuperscript{1}, M. Pertin\textsuperscript{1}, I. Décosterd\textsuperscript{1}
\textsuperscript{1}CHUV - UNIL, Pain Center - Department of Fundamental Neurosciences, Lausanne, Switzerland

Background and Aims:
Voltage-gated sodium channels (NaVs) expression/function in DRG neurons is often found to be dysregulated in neuropathic pain. The ubiquitin-ligase NEDD4-2 is a potent post-translational regulator of NaVs, and is downregulated in the spared nerve injury model (SNI) of neuropathic pain, leading to hyperexcitability of DRG neurons. The anti-diabetic drug metformin, an AMPK-activator, was shown to decrease sensory neurons excitability and allodynia after SNI. Since AMPK can inhibit ion channels activity by Nedd4-2 activation, we investigated whether the effect of metformin can occur through post-translational modification of NaVs.

Methods:
Sodium currents (I_{Na}) were recorded in voltage-clamp, from HEK293 cells co-transfected with NaV1.7 and NEDD4-2, and from isolated mouse DRG neurons, after 12-hours of incubation with metformin 20 mM. Neurons excitability was studied in current-clamp. Cell-surface expression of NaV1.7 was studied using cell-surface biotinylation and western blotting.

Results:
Metformin significantly reduced I_{Na} by 42±6\% in HEK293 cells co-transfected with NEDD4-2 and NaV1.7. When NEDD4-2 was not co-transfected, metformin had no effect on I_{Na}. Cell-surface biotinylation showed a decreased NaV1.7 expression after metformin treatment (19±7\%), only when NEDD4-2 was co-transfected. Incubation of isolated DRG neurons with metformin significantly reduced I_{Na} (46±11\%) in control neurons but not in neurons lacking NEDD4-2. Finally, metformin decreased DRG neurons excitability partially in a Nedd4-2-dependent way.

Conclusions:
Our results suggest that metformin effect on DRG neurons excitability is related to post-translational modulation of NaVs by their regulator NEDD4-2. Comprehension of mechanisms of action of metformin opens new alternatives for the diminution of hyperexcitability related to neuropathic pain.
Background and Aims:

Disruption of the blood-nerve barrier (BNB) is pivotal in the development of local neuroinflammation, peripheral sensitization and neuropathic pain following peripheral nerve injury (PNI). Among the key events of such disruption, activation of innate-immunity Toll-Like Receptor 4 (TLR4) and inactivation of Sonic Hedgehog (SHH) signaling pathways within endoneurial endothelial cells are essential. However, preemptive inactivation of TLR4 signaling or sustained activation of SHH signaling do not prevent the local alterations observed following PNI, suggesting the implication of another signaling pathway.

Methods:

Using the infra-orbital nerve chronic constriction injury model (IoN-CCI), we investigated the implication of Wnt/β-catenin pathway and its role in the phenotypical transition between neuritis and neuropathy, using an ad-hoc neuritis model, the simple nerve stretch (SNS). In PNI models (CCI and SNS) vs controls, mRNA expression levels and/or immunochemical detection of major readouts (Fzd-7, VE-cadherin, Patched-1, Gli-1) and/or Tight Junction (TJ) proteins (Claudin 1, Claudin 5, Occludin) were investigated and vascular permeability assessed by sodium fluorescein extravasation.

Results:

IoN-CCI induced early and transient alterations in the VE-cadherin/β-catenin/ Frizzled-7 AJ complex, shown to mediate local BNB disruption via β-catenin-dependent TJ protein downregulation. Furthermore, Wnt pathway also mediated the endoneurial TLR4/SHH crosstalk. Finally, differences in both Wnt/β-catenin signaling involvement and endoneurial vascular permeability were observed between SNS and CCI that might participate in the transition between neuritis and neuropathy.

Conclusions:

An endoneurial endothelial crosstalk between Wnt/β-catenin- and SHH-mediated signaling pathways mediates the chronic disruption of the BNB following PNI, resulting in increased irreversible endoneurial vascular permeability and eventually neuropathic pain.
NEUP7-0151
EVIDENCE FOR A ROLE OF CONTACTIN ASSOCIATED PROTEIN-2 (CASPR2) IN NEUROPATHIC PAIN
J. Dawes¹, H. Kuehn¹, G. Weir¹, D. Bennett¹
¹University of Oxford, NDCN, Oxford, United Kingdom

Background and Aims:

CASPR2 is a neurexin-like protein which interacts with shaker type potassium channels (STKCs) such as Kv1.1 and 1.2. Auto-antibodies against CASPR2 are associated with neuropathic-like pain in patients. The aim of this project was therefore to assess whether CASPR2 has a role in neuropathic pain.

Methods:

Tissue was taken from mice subjected to a spared nerve injury and analysed using QPCR, western blot, in situ hybridisation and immunohistochemistry. Patch clamp analysis was used to assess CASPR2 overexpression in cultured DRG neurons.

Results:

Initially we assessed the expression of CASPR2 using QPCR in whole DRG and found a significant down-regulation following nerve injury. Using in situ hybridisation coupled with immunohistochemistry the loss of CASPR2 was assessed in both injured and uninjured neurons. In comparison to sham, there was a significant reduction in CASPR2 mRNA signal 7 days post-surgery in uninjured neurons (19.4±0.8 a.u. vs 14.3±1.4 a.u., respectively). Interestingly this loss was far greater in ATF3 positive neurons (3.0±1.1 a.u.). A similar reduction was also seen at day 21. The overexpression of CASPR2 was able to reduce DRG neuronal excitability in vitro and we have now started to assess pain-like behaviour in mice overexpressing CASPR2 using the neuron-specific promoter Thy1.

Conclusions:

Following nerve injury there is a significant reduction in CASPR2 expression in DRG neurons, particularly those expressing the injury marker ATF3. The overexpression of CASPR2 was able to modulate DRG neuronal excitability. This protein interacts with STKCs and a loss of their function is associated with neuropathic pain.
Background and Aims:

Lactate (LA) is known to accumulate in ischemic tissue, in particular during excessive muscle work and in pain states of myocardial infarction. Sensory neurons are equipped with numerous nociceptors, of which ASIC3 and TRPA1 have been demonstrated to be sensitized and activated by LA. However, it is unclear how LA acts on TRPV1, a main receptor for acidosis in sensory neurons.

Methods:

Our study focused on patch clamp, calcium imaging and CGRP release experiments investigating the effect of LA on TRPV1 expressed in HEK293T cells and in native DRG neurons.

Results:

Membrane currents of TRPV1 evoked by capsaicin, protons, heat and pro-algesic agents were clearly inhibited by physiological and pathophysiological concentrations of LA. Additionally, LA reduced the TRPV1-dependent Ca\(^{2+}\) influx, and inhibited CGRP release from sciatic nerves containing TRPV1. LA shifts the open probability of TRPV1 towards more positive voltages and forces the channel to its closed state at physiological potentials. Mutation of I680A constitutively opened the lower gate of TRPV1 and simultaneously abolished LA inhibition. Approaches on excised patches and cell-attached recordings revealed that LA inhibits TRPV1 from the extracellular side and independently of intracellular
Conclusions:

LA modulates as a potent endogenous inhibitor gating of TRPV1 channels and seems to prevent the multimodal nerve terminal from further depolarization under ischemic conditions. We here show for the first time that TRPV1 distinguishes between acidosis and lactic acidosis what might be important for the regulation of pain perception during ischemic states.
BASIC SCIENCE (ANIMALS): CELLULAR/ MOLECULAR ASPECTS - PART 2

NEUP7-0029
PEPTIDE BASED INHIBITOR OF THE SCAFFOLDING PROTEIN PICK1 UNDERLYING MALADAPTIVE SYNAPTIC PLASTICITY
M. De Luca¹, N. Riis Christensen¹, M. Richner², C. Bjergaard Vægter², A. Bach³, K. Strømgaard³, U. Gether¹, K. Lindegaard Madsen¹
¹University of Copenhagen, Neuroscience and Pharmacology, Copenhagen, Denmark
²University of Aarhus, Biomedicine, Aarhus, Denmark
³University of Copenhagen, Drug Design and Pharmacology, Copenhagen, Denmark

Background and Aims:

Ionotropic glutamate receptors are responsible for the majority of the excitatory neurotransmission in both the central and peripheral nervous system. Different functional modalities of the glutamatergic synapses are encoded in a complex multiprotein machinery - including many scaffolding proteins, receptors, etc. - termed the Post Synaptic Density (PSD), that allows individual synapses to generate highly specific biological responses to differential external stimuli. Among the scaffolding ensemble involved in the synaptic plasticity of glutamatergic synapses, PICK1 (protein interacting with C Kinase 1) is a promising pharmacological target.

The pharmacological targeting of specific intracellular components of PSD enables a selective modulation of discrete responses rather than affecting general excitatory neurotransmission. In this way, serious side effects might be reduced opening new ways of treating diseases involving glutamatergic dysfunctions, such as neuropathic pain.

Methods:

Mechanical Allodynia has been evaluated by von Frey filaments to determine the paw withdrawal threshold. Furthermore repeated administrations and dose dependence experiments have been performed.

Results:

We have developed a PICK1 peptide inhibitor showing a target affinity in the low nanomolar range. Intrathecal injection of the peptide in mice, hypersensitized by the spared nerve injury model of persistent peripheral neuropathic pain, reversed the mechanical allodynia for an extended period of time in a dose dependent manner.

Conclusions: The pharmacology of ionotropic glutamate receptors remains strongly underdeveloped. Although numerous diseases, including neuropathic pain, involve over-activation or sensitization of the glutamate system. We have developed a new lead compound with a great potential as a new treatment for neuropathic pain.
NEUP7-0055
ANALGESIC EFFICACY AND MODE OF ACTION OF EMA300, A SMALL MOLECULE ANGIOTENSIN II TYPE 2 (AT2) RECEPTOR ANTAGONIST FOR NEUROPATHIC PAIN
N. Khan¹, A. Muralidharan¹, M. Smith¹
¹The Centre for Integrated Preclinical Drug Development CIPDD, University of Queensland, Brisbane, Australia

Background and Aims:

Recently, EMA401, a highly selective, orally bioavailable, small molecule angiotensin-II type 2 (AT₂) receptor antagonist showed promising efficacy in patients with postherpetic neuralgia in a randomised, double-blind, placebo-controlled Phase 2 clinical trial. Hence, this study was designed to further investigate the mode of action of AT₂ receptor antagonists, in a rat model of peripheral neuropathic pain.

Methods:

Male Sprague-Dawley rats (200-225g) were used for inducing unilateral chronic constriction injury (CCI) model. On day 14-15 (post-CCI surgery) rats received single bolus doses (i.p.) of either EMA300 at 10mg/kg (n=10) or vehicle (n=12) and their ipsilateral (L4-L6) dorsal root ganglia were collected to investigate the analgesic mode of action of EMA300. GraphPad-Prism™v7 was used for statistical analyses and the statistical significance criterion was \( P<0.05 \).

Results:

The single bolus dose of EMA300 at 10 mg/kg (i.p) but not vehicle produced significant relief of mechanical allodynia \( (P<0.05) \) developed in CCI-rats. IHC and western-blotting data suggest that EMA300 has potentially produced analgesic effect in CCI rats by attenuating the augmented AngII/AT2R signaling in CCI-rats. Interestingly, herein we showed for the first time that EMA300 normalised NGF levels sparing changes in its receptor, TrkA suggesting that AngII/AT2 pathway has a possible direct impact on NGF expression. Additionally, EMA300 significantly decreased the up-regulated expression levels of phospho-ERK (pERK) and -p38 (pp38) in DRG tissues thereby mitigating mechanical allodynia developed in CCI-rats.

Conclusions:

Augmented AngII/AT2R signaling in the ipsilateral lumbar DRGs of CCI-rats was attenuated by a single bolus dose of EMA300 to normalise the NGF-isoforms matching their levels to sham-rats.
K+ CURRENTS IN NEURONS FROM RAT DORSAL ROOT GANGLIA REVEAL DIFFERENTIAL EXPRESSION IN INFLAMMATORY AND NEUROPATHIC CHRONIC PAIN

P. Lima¹,², B. Szwarc², J. Serrão², M. Sousa², M.A. Roberto³
¹Universidade Nova de Lisboa, NOVA Medical School / Faculdade de Ciências Médicas, Lisboa, Portugal
²Sea4Us, Biotecnologia e Recursos Marinhos- Lda, Sagres, Portugal
³Centro Hospitalar de Lisboa Central EPE-, Hospital S. José, Lisboa, Portugal

Background and Aims:

With chronic pain (CP) nociceptive fibers involved in pain transmission are hiper-excitabile. This may be caused by abnormal firing patterns modulated by voltage-gated potassium (Kv) channels among others. Our goal is to elucidate the shared and specific mechanisms of neuropathic chronic pain in rat neuropatic and inflammatory pain rat-models by studying the biophysics of Kv currents/channels in small diameter DRG neurons.

Methods:

Neuropathic (chronic constriction injury of sciatic nerve- CCI) and inflammatory (knee injection with CFA) chronic pain models were established and mechanical sensibility was scored with von Frey monofilament. Animal care and experimental studies with Wistar rats were in accordance with Directive 2013/63/EU. After 4 weeks (CCI) and 3 weeks (CFA) of injury, rats were euthanized by decapitation after anesthesia with pentobarbital. L4-L6 DRGs were removed, isolated and used within 8 hours. Whole-cell voltage-clamp technique was used to record Kv currents.

Results:

Recordings revealed two K+ current components: one fast-inactivating (Ifast) and a second slowly-inactivating (Islow). Data revealed that Ifast is dramatically decreased in both CCI- and-CFA derived DRG neurons when compared to Naïve. Surprisingly, Islow was augmented in CCI but not in CFA. Details regarding voltage-sensitivity of activation and inactivation are given. Results are discussed in context of the predicted effects on excitability. We also found that Kv1.3 is over expressed in CCI-derived DRG neurons, suggesting it as Kv underlying the augmented Islow in CCI.

Conclusions:

Data demonstrate that neuropathic-and-inflammatory pain differentially alter the biophysical and expression properties of Kv currents/channels.
Background and Aims:

Impairment in Glycine (GlyR) mediated inhibitory neurotransmission is thought to play a critical role in the disinhibition that account for the development and maintenance of central pain hypersensitivity. We evaluated the expression of GlyR beta subunit in neuropathic (Chronic Constriction Injury, CCI) and inflammatory (Zymosan injected) models of chronic pain.

Methods:

We evaluated the expression of GlyR auxiliary beta subunit after 3 days of Chronic Constriction Injury and after 4 hours of zymosan A injection by RT-qPCR and Western Blot analysis of spinal cord samples.

Results:

RT-qPCR analysis of spinal cord samples showed increased beta subunit gene expression after 3 days of CCI surgery, and similar results were obtained by Western blot analysis. Inflammatory pain model showed increased level of beta subunit gene expression after 4 hours of zymosan A injection. Thus, remarking the beta subunit regulation as a common component in both pain models. These results were associated with increased expression of interleukin 6 (IL-6), which is reported to reduce glycine-induced currents, and therefore promoting spinal neural hyper-excitability, characteristic of chronic pain status.

Conclusions:

These results suggest that auxiliary GlyR beta subunit may play a substantial role in establishing and maintaining GlyR-mediated pain sensitization during neuropathic and inflammatory injury. Taking together, these findings suggest an important, albeit underappreciated, role of auxiliary beta subunit in the allosteric modulation of glycine receptors in chronic pain development.
Painful tissue alterations are accompanied by changes in the extracellular matrix (ECM). However, knowledge about the influence of ECM on pain sensitization signaling in nociceptive neurons is sparse. Thus, we aimed to identify ECM components which alter sensitization signaling in nociceptive neurons, and to analyze their subgroup specificity and cellular mechanism.

We analyzed ~1 million cultured primary sensory neurons from rat DRGs for an influence of 17 ECMs on basal and NGF-, 5-HT-, and OSM-induced pErk1/2 and/or pRlI-PKA signaling at 7 time points (510 conditions), using immunocytochemistry and a High-Content-Screening (HCS) microscopy approach. Single-cell data was analyzed for mechanistic changes by applying a computational model using ordinary differential equation constrained mixture modelling (ODE-MM). Further subgroup and mechanistic analysis was pursued via HCS microscopy.

We identified the class of collagens to modulate NGF-induced pErk1/2 but not pRlI-PKA kinetics. In contrast, none of the other ECMs altered basal and induced pErk1/2/pRlI-PKA levels. Neuronal subgroup comparability was validated by subgroup markers and ODE-MM. Collagens did not modulate GDNF-induced pErk1/2 kinetics indicating TrkA subgroup specificity. Mechanistic analysis identified NGF-mediated effects on TrkA receptor level to be the reason for differential pErk1/2 signaling. Furthermore, we could show that collagens induce long-lasting elevations of pErk1/2 levels and downstream CGRP expression. Collagen content and composition is strongly modified in painful tissue alterations such as wounds or tumors. Our results suggest that collagens augment nociceptor sensitivity long-lastingly in such pathologies. The observed elevated CGRP expression proposes a further increased pain signaling by para- and autocrine CGRP activity.
Background and Aims:

TRPM8 is the main molecular entity responsible for detection of cold temperatures in the somatosensory system. This calcium-permeable cation channel is activated by cold, cooling compounds such as menthol and voltage. It has been suggested that TRPM8 function could be regulated by several kinases, in both recombinant systems and cold thermoreceptor neurons. Among these kinases, the role of protein kinase C (PKC) could be relevant in inflammatory and painful conditions. In order to explore the mechanism underlying PKC regulation, we evaluated its effect in TRPM8 function and its contribution to the phosphorylation levels of the channel.

Methods:

We analyzed the phosphorylation state of immunoprecipitated TRPM8 channels, and assessed channel function using calcium imaging in both HEK293 cells and in trigeminal neurons in culture, and using extracellular recording of corneal cold thermoreceptors.

Results:

We found that TRPM8 is a phosphoprotein, and the inhibition of the basal kinase activity using staurosporine enhances TRPM8 cold and menthol responses. Furthermore, the activation of PKC, using either phorbol esters or activating a G-protein signaling cascade, reduces the responses of TRPM8 to both agonists, and causes a shift of 2ºC in its temperature threshold to warmer temperatures. In corneal cold thermoreceptors, PMA produces a reduction of the ongoing activity and maximal response to cold.

Conclusions:

Altogether, these results indicate that kinase activity, and specifically PKC, acts as a negative modulator of TRPM8 function, suggesting a relevant role of this regulation in cold thermoreceptor neurons.

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Background and Aims:

Neuropathic pain is a severe clinical problem, often appearing as a result of damage to the nervous system and as a co-symptom of many diseases. Neuropathic pain appears to be less opioid responsive than nociceptive pain. The molecular basis for the lower opioid efficacy is not clear. Therefore, the opioid gene expression and opioid specific receptor signaling was examined at the spinal and supraspinal levels.

Methods:

Albino Swiss mice were subjected to sciatic nerve ligation (CCI). The development of neuropathic pain symptoms and the changes of mRNA levels of genes encoding opioid receptors and prohormones (qRT-PCR) and the opioid GTPgammaS binding were measured in the contra and ipsilateral lumbar spinal cord and thalamus 14 days after injury.

Results:

A decrease in the opioid receptor mRNA levels as well as GTPgammaS binding of opioid receptor ligands at the ipsilateral site of the spinal cord and in the contralateral thalamus was observed 2 weeks after injury. No significant changes were present in the opioid prohormone gene expression in the spinal cord and in the thalamus.

Conclusions:

The decrease of opioid receptor expression and GTPgS binding in specific spinal cord and thalamic pain pathways may contribute to suppression of opioid analgesic effects under neuropathic pain.

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Background and Aims:

The blood-nerve barrier (BNB) consisting of the perineurium and endoneurial vessels is sealed by tight junction proteins. We have previously shown that recombinant tissue plasminogen activator (rtPA) injected close to sciatic nerve opens the BNB via claudin-1 downregulation for delivery of analgesics. Here, we examined tPA in the pathophysiology of peripheral neuropathy and its effect on the cytoplasmatic anchoring protein zona occludens-1 (ZO-1) and the adherens molecule JAM-C in the BNB.

Methods:

Wistar rats underwent chronic constriction injury (CCI) or were treated by a single perisciatic application of 10 µg rtPA (all approved by the local animal care committee) and analysed subsequently.

Results:

TPA mRNA was profoundly upregulated in the sciatic nerve 7 d after CCI. CCI elicited mechanical allodynia, BNB leakage for large molecule tracers, downregulation of ZO-1 and JAM-C mRNA/protein in the cytosol or membrane fraction as well as a loss of ZO-1 immunoreactivity in perineurium and endoneurial cells, but no change of JAM-C immunoreactivity. Similarly, after perisciatic rtPA injection, ZO-1 and JAM-C mRNA, cytosolic/membrane protein and ZO-1 immunoreactivity were downregulated in the sciatic nerve and the BNB was opened. Neither mechanical hyperalgesia/allodynia nor any nerve degeneration or macrophage infiltrations were observed after rtPA in contrast to CCI. Five days after rtPA, ZO-1 mRNA returned to baseline. Mechanistically, microRNA-155, which is known to destabilize ZO-1 via Akt activation was also upregulated in CCI or after rtPA application.

Conclusions:

RTPA transiently opens the BNB. This could contribute to the generation of neuropathic pain as observed in CCI via endogenous TPA.
Background and Aims:

In this study we used a mouse model of diabetes to evaluate the effect of mesenchymal stromal cells from human adipose tissue (hASC) and of their secretome (conditioned medium CM) on neuropathic pain, neuroinflammation and peripheral immune activation.

Methods:

Diabetes was induced in male mice with low STZ doses. When allodynia was established (14 days after STZ), mice were treated by intravenous injection with either $1 \times 10^6$ hASC or with CM from $2 \times 10^6$ serum-free cultured cells. Mechanical and thermal allosthy of IL-1 and of IL-10 were measured (ELISA) in nerve, dorsal root ganglia and spinal cord. The release of Th1 and Th2 cytokines by splenocytes was also evaluated.

Results:

Both hASC and secretome reversed neuropathic hypersensitivity. The effects of hASC and CM was very rapid, 3 hours after treatments, and long lasting, since it was maintained 14 weeks after a single hASC or CM injection. Both hASC and CM treatments restored a correct IL-1/IL-10 cytokine balance in nerve, DRG and spinal cord that was altered in diabetes. In the periphery, a Th1 polarization was present and hASC and CM re-established a correct Th1/Thelper2 balance. No effect on blood glucose was evident but diabetic animals regained weight after hASC or CM treatment.

Conclusions:

HASC treatment may be a promising approach for diabetic complications such as neuropathic pain and we suggest that since their effect is likely to be mediated by their secreted products, cells may eventually be substituted with CM.
CONTRIBUTION OF CENTRAL TRPV1 CHANNELS IN THE 5-HT-DEPENDENT DESCENDING FACILITATION UNDERLYING THE MAINTENANCE OF NEUROPATHIC PAIN

F. Wei

University of Maryland- Baltimore, Neural and Pain Sciences, Baltimore- MD, USA

Background and Aims:

Our previous study has demonstrated the expression of TRPV1 in 5-HT-containing neurons in the rat rostral ventromedial medulla (RVM) and its involvement in mechanisms of descending facilitation underlying the development of persistent pain. In the present study, we further examined cellular and molecular mechanisms in which how central TRPV1 works.

Methods:

Trigeminal neuropathic pain model was used. Combination of pain behaviors, patch-clamp recordings in rat brainstem slices, immunostaining and Western Blot analysis were performed.

Results:

Functional blockade or knockdown of RVM TRPV1 significantly suppressed rIL-1β or rTNF-α-induced increase of both NMDA-induced inward currents and evoked EPSCs (eEPSC) in RVM neurons, and decreased rIL-1β-induced enhancement of both pTRPV1 and pNR1 in RVM tissue and behavioral hypersensitivity. Neutralizing endogenous IL-1β in the RVM significantly prevented enhancement of pTRPV1 induced by CCI. Next, we examined possible linkage between TRPV1 and NMDAR. Bath application of capsaicin obviously amplified eEPSCs. Intra-RVM microinjection of capsaicin increased local pNR1 expression. Finally, we identified intracellular signaling mediating interaction between TRPV1 and NMDAR. Capsaicin-enhanced eEPSCs were blocked by quenching intracellular Ca²⁺ using BAPTA in an intracellular recording solution, and pretreatment of selective inhibitors for Src, PKC or PKM. In addition, CCI-, intra-RVM rIL-1β-induced increase of pPKMζ and pNR1 in RVM tissue could be attenuated by functional blockade or knockdown of local TRPV1.

Conclusions:

Together, these data indicate that central TRPV1 channels in the RVM may contribute to the maintenance of 5-HT-dependent descending facilitation and behavioral hypersensitivity after nerve injury by bridging interaction between local glial reactivation and neuronal hyperexcitability.
Background and Aims:

Parkin controls neuronal cell function and associated with pathophysiology of neurodegenerative diseases. p53 is a transcription factor negatively regulating parkin expression. We investigated the function of parkin/p53 signaling in the DRG neurons for the development of the diabetic pain hypersensitivity.

Methods:

Male C57BL/6 (20-25g) mice were used. Diabetes was induced by the intraperitoneal injection of streptozotocin (STZ, 200mg/kg). Control mice received saline injection. Nociceptive thresholds against mechanical threshold by von Frey filaments was determined up to 3 weeks after the treatment. Parkin and p53 expression in L4 and L5 DRG was assessed by the immunohistochemistry and RT-PCR. Separately, selective p53 inhibitor pifithrin-α (2mg/kg) was administrated intraperitoneally prior to the STZ treatment. Same dose of pifithrin-α was repeatedly injected every 3 days up to 3 weeks.

Results:

Nociceptive threshold decreased 1-3 weeks after the STZ treatment. Immunohistochemistry revealed that most of DRG neurons were positive for parkin in control. Parkin expression decreased 3 weeks after the STZ treatment. RT-PCR demonstrated parkin mRNA decreased significantly after the STZ treatment. In contrast, p53 mRNA increased significantly after the STZ treatment. Pifithrin-α inhibited reduction of the mechanical threshold and parkin mRNA after the STZ-treatment.

Conclusions:

DRG neurons express parkin in naive condition. Expression of parkin decreased in diabetic mice. Inhibition of p53 prevented down-regulation of parkin and development of pain hypersensitivity in diabetic mice.
BACKGROUND AND AIDS:

C-Low Threshold MechanoReceptors (C-LTMRs) are a unique subset of non-nociceptive C-fibers that innervate exclusively hairy skin. At steady-state, C-LTMRs have been shown to convey low intensity mechanical stimuli (caress and gentle touch) and to detect cooling temperatures.

Recent data unravelled an important modulatory role for C-LTMRs in pain processing following tissue injury, both of inflammatory and nerve lesion origin. Yet, our knowledge regarding the molecular and cellular modes of action of C-LTMRs is still in its infancy.

To provide a better understanding of this aspect of sensory neuroscience, our team has recently published RNA-seq based data allowing us to considerably broaden the molecular repertoire of C-LTMRs. Besides Tyrosine Hydroxylase, V-GluT3 and Tafa4, already shown to be restricted to C-LTMRs in DRG, we found nine other genes highly enriched in this subset. One of them encodes for the basic Helix-Loop-Helix (bHLH) family transcription factor bHLHa9.

METHODS:

To understand the role of bHLHa9 in somatic sensory biology and in C-LTMRs in particular, I have performed a large panel of behavioural tests on mice lacking its expression, from mechanical to thermal stimulation under acute conditions.

RESULTS:

Interestingly, bHLHa9 knock-out (KO) mice exhibited a broad defect in temperature sensation. We are currently investigating the pain behaviour of these mice following tissue injury.

CONCLUSIONS:

This is the first time the KO of a gene highly enriched in C-LTMRs has been shown to induce temperature sensation defects and potentially the first time C-LTMRs are shown as directly involved in temperature sensation in a live animal.
Background and Aims:

Background and aims: Next-generation sequencing technologies have revolutionarily advanced sequence-based research with the advantages of high-throughput. RNA-seq is now being used to uncover multiple facets of transcriptome enabling to identify numerous genes which could represent potential therapeutic targets for the treatment of chronic pain.

Methods:

Methods: We coupled high-throughput RNA sequencing with the translating ribosome affinity purification (TRAP) to evaluate translation efficiencies within the trigeminal (TG) and dorsal root ganglia (DRG) in different conditions. We used a reporter transgene that encodes an EGFP-tagged ribosomal protein L10a (Rpl10a) driven by the Nav1.8 promoter.

Results:

Results: First, the predicted EGFP-Rpl10a expression patterns in trigeminal and dorsal root ganglia of the Nav1.8 reporter line was confirmed by immunofluorescence. As expected, The Nav1.8 promoter-driven EGFP-RPI10a was specifically expressed in nociceptors as indicated by the co-staining with Prph and CGRP while only a small portion of the large diameter neurons expresses EGFP. GFP immunoprecipitation of extracts from mouse DRG and TG successfully recovered polyribosome-associated mRNAs from the EGFP-RpI10a reporter mouse, with very little background RNA from mice without the GFP-containing transgene. mRNAs purified by EGFP immunoprecipitation from sensory neurons remained intact and were highly enriched for nociceptors-specific genes, including Nav1.8 and Calca(CGRP).

Conclusions:

Conclusion: This genetically targeted TRAP methodology is a generalizable method useful for the identification of molecular changes in any cell type in response to disease, or pharmacological manipulations. This approach will provide us a better understanding of which mRNAs contribute to in vivo long-term sensitization of nociceptors after chronic pain.
BACKGROUND AND AIDS:

Previously using male mice we demonstrated that CCI-induced neuropathic pain induces hippocampal pathology which includes an increase in TNF, a decrease in myelin and synaptic proteins, and depression-like symptoms. Using genetic approaches we further determined that CCI induced pain and subsequent hippocampal neuropathology are dependent on TNF/TNFR1 signaling. Because chronic pain tends to occur more frequently and severely in females, we investigated the therapeutic effects of inhibiting TNFR1 signaling using XPro1595, a specific inhibitor of solTNF, in male and female mice.

METHODS:

The CCI model of neuropathic pain was used in male and female mice. One week following CCI surgeries, neuropathic mice received subcutaneous delivery of either saline or XPro1595 (10 mg/kg) every 2nd day and were blindly tested for pain weekly.

RESULTS:

In male mice, XPro1595 accelerated recovery from neuropathic pain which began after 2 weeks of drug delivery; however, in female mice there was no effect of XPro1595 on pain. Next, western blotting was used to investigate changes in levels of a glutamate transporter (GLT-1) and glutamate receptor subunit (NMDAR1) in brains of these animals since neuropathic pain has been shown to invoke an increase in glutamergic signaling. In male mice, inhibiting solTNF reduces glutameric signaling in the hippocampus and cortex while in female mice inhibiting solTNF increased NMDA mediated glutameric signaling.

CONCLUSIONS:

Together, these results suggest that solTNF signaling plays a role in pain induction following CCI in males but not females. This could be due to the sexually dimorphic effects of solTNF inhibition on glutamate signaling components following CCI.
Background and Aims:

The incidence of neuropathic pain in the elderly is significantly higher than adults and adversely impacts quality of life. Previous studies report exaggerated inflammatory and behavioral responses, as well as differential nociceptor activity after insult in aged animals. Overall, very little is known about how aging alters the development of neuropathic pain after surgery. Therefore we sought to determine the behavioral and inflammatory response following spared-nerve injury (SNI) in aged versus adult animals.

Methods:

Young adult (3-6 month) and aged (24-26 month) male FBN rats underwent SNI and the development of neuropathic pain was assessed using mechanical and cold hypersensitivity measures. Behavioral data was collected 3, 5, 7, 10, 14, and every 7 days for 56 days post-surgery. Spinal cords and DRGs were collected either 5 or 60 days post-surgery to assess gene expression and immunohistochemistry (IHC) was also performed.

Results:

Surprisingly, aged animals took significantly longer to develop signs of neuropathic pain than young adult rats as measured by both mechanical hypersensitivity and cold allodynia (21 days aged vs. 5 days young). We assessed DRG and spinal cord tissue ipsilateral to the injury for inflammatory and ER stress genes and observed an age-and surgery-related upregulation of ATF4, IL-6, and SK2 5 days post-surgery. However, there was no difference in behavior or gene expression 60-days post surgery.

Conclusions:

These experiments demonstrate a connection between age, inflammation, ER stress, and pain plasticity. The altered kinetics in the onset of neuropathic pain after injury in the aged hints at potential therapeutic targets in resolution pathways.
Background and Aims:

The purpose of the present study is to evaluate the mechanisms underlying ectopic tooth-pulp pain associated with acute pulpitis.

Methods:

Complete Freund’s adjuvant (CFA) or saline was applied to the upper first molar tooth pulp (M1) in rats under the 3-mix-anesthesia, and capsaicin was applied to the upper second molar tooth pulp (M2) on day 3. Fluorogold (FG) neuronal tracing study was conducted to assess if there are any trigeminal ganglion (TG) neurons innervating multiple tooth pulps. Digastric muscle (Dig) EMG activity was also analyzed to evaluate if M2 capsaicin administration enhances jaw reflex.

Results:

Dig EMG activity was significantly larger in M1 CFA-applied rats compared with M1 saline-applied rats. The number of FG-labeled neurons encircled with glial fibrillary acidic protein (GFAP) and Connexin43 (Cx43)-IR cells in TG was significantly larger in M1 CFA-applied rats compared with M1 saline-applied rats. Application of Cx43 cell inhibitor (Gap26) into TG caused significant reduction of capsaicin-induced Dig activity, and satellite cell and Cx43 activation.

Conclusions:

These findings suggest that M1 pulpitis causes spreading of satellite cell activation within TG under the mechanisms of Cx43 activation, resulting in M2 pulp hypersensitivity.
Background and Aims:

Botulinum neurotoxin serotype A (BoNT/A) shows antinociceptive properties and has a large clinical applications in pain therapy. The goal of our in vitro/in vivo study was to examine whether BoNT/A affects the protein levels of glial activation markers and nociceptive factors.

Methods:

We tested the effect of the single intraplantarly (i.pl.) injection of BoNT/A (300pg/paw) on analgesia in neuropathy using behavioral tests in animal chronic constriction injury (CCI) model and on nociceptive factors level by using biochemical study (Western blott) and after lipopolysaccharide (LPS) stimulation.

Results:

Our results show that i.pl. BoNT/A injection diminished pain-related behaviors in neuropathy. Western blotting analyses demonstrated the upregulation of the pronociceptive proteins in the spinal cord and DRG, but no changes in the antinociceptive proteins levels in neuropathy. BoNT/A injection suppressed the CCI-induced upregulation of IL-18 and IL-1β in the spinal cord and/or DRG, increased the levels of IL-10 and IL-1RA in the DRG. Moreover, BoNT/A diminished LPS-induced microglial pro-inflammatory (IL-1β, IL-18, IL-6) and anti-inflammatory (IL-10) factors.

Conclusions:

Our results suggest that BoNT/A significantly attenuates pain-related behavior and microglial activation and restores the neuroimmune balance in a CCI model by decreasing the levels of pronociceptive factors (IL-1β and IL-18) and increasing the levels of antinociceptive factors (IL-10 and IL-1RA) in the spinal cord and DRG. Acknowledgements Supported by National Science Centre Poland grant Harmonia 5-2013/10/M/NZ4/00261 and statutory funds. Anna Piotrowska - holder of KNOW scholarship sponsored by Ministry of Science and Higher Education, Poland.
Background and Aims:

Chemotherapy-induced painful neuropathy (CIPN) is a severe side-effect of chemotherapeutic agents, frequently resulting in premature termination of therapy. Current treatments for CIPN have limited efficacy and undesirable side effects. Better understanding of the mechanisms underlying CIPN and the development of novel therapies is therefore needed. Recently, preclinical data has indicated that chemokine signalling is a crucial mediator of neuron/non-neuronal communication. Indeed, in a murine model of Vincristine-induced neuropathic pain, we observed that inhibition of chemokine signalling in monocytes, which are recruited from the circulation in the sciatic nerve, is a promising therapeutic strategy for CIPN. Specifically, inhibition of endothelial CX3CL1/monocyte CX3CR1 signalling shows potential for the prophylactic treatment of CIPN, with CX3CR1 knock-out mice displaying significantly delayed vincristine-induced pain. CX3CR1 is expressed by both patrolling and inflammatory monocytes, however, only inflammatory monocytes express the chemokine receptor CCR2.

Methods:

Here we elucidate the role of CCR2+ monocytes in vincristine-induced pain, using a transgenic reporter line and pharmacological inhibition of peripheral CCR2.

Results:

We find that CCR2+ monocytes have a more prominent role in later stages of vincristine-induced pain as opposed to pain induction.

Conclusions:

The delayed onset of pain observed in CX3CR1 knock-out mice could therefore be orchestrated by patrolling monocytes, whilst later stages of pain are regulated by CCR2+ inflammatory monocytes. Our results indicate that whilst CX3CR1 inhibition is a promising prophylactic treatment for CIPN, CCR2 inhibition could be more effective for the treatment of established CIPN and that tailored therapies targeting chemokines in non-neuronal cells are a promising alternative to current analgesics.
Background and Aims:

Microglia are known to be involved in spinal cord injury-induced central neuropathic pain (SCI-CNP), which affects about 41% of SCI patients and lacks effective treatments. We previously showed that the omega-3 polyunsaturated docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) modulated microglial activation in vivo. Here, we examined whether these compounds could attenuate SCI-CNP in adult male rats.

Methods:

Primary microglial cultures were prepared from rat pups at postnatal days 3-5. After 4 days in culture, cells were treated for 4 hours with DHA or EPA at different concentrations when simultaneously exposed to lipopolysaccharide (LPS). Some cells were fixed and processed for immunocytochemistry, and others were harvested for measuring TNF-α and IL-1β mRNA levels using qPCR. To assess the in vivo effects on SCI-CNP, a contusion model of SCI was used (MASCIS). DHA (250nmol/kg) or EPA (1000nmol/kg) was administered intravenously 30 minutes after injury and then every 3 days up to 6 weeks. Complex pain-related behaviours were examined.

Results:

DHA (0.8µM) and EPA (32µM) significantly reduced the number of iNOS-positive microglia in vitro when compared to LPS only. TNF-α and IL-1β mRNA levels were also significantly reduced. In contusion animals, DHA and EPA treatments significantly attenuated brainstem and cerebral responses to at-level mechanical stimuli, and pain-related burrowing and thigmotaxis behaviours.

Conclusions:

Our in vitro and in vivo data suggest that DHA and EPA are efficacious in preventing SCI-CNP development. Future studies will explore their potential in treating already established SCI-CNP.

Supported by Development Trust of Aberdeen University
Background and Aims:

Targeting chemokine signaling pathways is crucial in neuropathy development. The aim of our study was to investigate the influence of maraviroc (MVC; CCR5 antagonist) on neuropathic pain symptoms, opioid effectiveness, glial markers and nociceptive factors in rat model. Additionally, we exam MVC influence on primary glial cultures after lipopolysaccharide stimulation.

Methods:

Intrathecal (i.t.) catheters were implanted in Wistar rats and chronic constriction injury (CCI) of sciatic nerve was established. MVC was administered preemptively i.t. and then once daily for 7 days. The last day rats received a single i.t. injection of morphine or buprenorphine. Then von Frey and cold plate tests were conducted. Primary glial cultures were treated with MVC 30 min before LPS stimulation. The biochemical changes were examined by qRT-PCR and Western blot.

Results:

Our results demonstrated MVC not only diminished the development of neuropathic pain but also intensified morphine and buprenorphine analgesia. Additionally, we indicated that MVC downregulated CCR5 and its ligands, CCL3, 4, 5 and intracellular pathways in the spinal cord. Furthermore, MVC diminished "classical" activation markers: IL-1β, IL-18, IL-6, NOS2 and upregulated "alternative" antinociceptive activation markers: IL-1RA, IL-18BP and IL-10 in the spinal cord. In parallel, similar changes were observed after MVC in LPS-induced microglial and astroglial cell cultures.

Conclusions:

Our results suggest the modulation of CCR5 by MVC as a novel therapeutic approach for neuropathy, by modulating intracellular pathways, enhancing opioids effectiveness and restoring the balance between pro- and antinociceptive factors.

Acknowledgments: Supported by National Science Centre, Poland grant Harmonia 5-2013/10/M/NZ4/00261; APM,KK,AMJ- KNOW scholarship
Background and Aims:

Infiltrating macrophages contribute to the mechanisms underlying neuropathic pain at the site of nerve damage in the periphery and in the dorsal root ganglia (DRG) by releasing mediators which sensitize neurons.

Using flow cytometry analysis of leukocytes (CD45+ cells) isolated from 7 day-neuropathic mice, we observed a significant infiltration of macrophages (F4/80+CD11b+ cells) in ipsilateral DRG and especially CD206+CD11c+ cells (M1 macrophages) whilst CD206+CD11c- cell (M2 macrophages) numbers were comparable in sham and injured DRG. Then, we explored whether macrophages release exosomes containing microRNAs (miRs) as a possible mechanisms to regulate sensory neuron activity in the DRG.

Methods:

Exosomes are small vesicles containing (micro) RNA and protein cargos that are secreted by all cells types, including immune cells.

Results:

In our study, cultured peritoneal macrophages demonstrated presence of miR 21-5p, Let 7b-5p, miR 155-5p and miR 134-5p. Incubation with LPS resulted in significant release of exosomal markers TSG101 and CD81 in the culture medium and intracellular increase of miR-155 and miR-21, but not miR-let7b and miR-134. Overexpression of miR-21-5p in peritoneal macrophages induced up- regulation of pro-inflammatory markers nuclear factor-κB (NF-κB) and inducible nitric oxide synthase (iNOS) and down-regulation of anti-inflammatory markers IL-10 and arginase-1. Flow cytometry analysis of miR 21-5p transfected macrophages confirmed in these cells a significant reduction of M2 phenotype in favor of M1.

Conclusions:

Future studies will assess whether the release of exosome containing miRs from macrophages in the DRG contributes to the ongoing nociceptive neuron activity under neuropathic pain states.
Background and Aims:

Neuropathic pain that develops in a majority of individuals with spinal cord injury (SCI) is refractory to treatment. SCI-induced pain is associated with robust inflammation in the spinal cord, however, the immune response in the DRG is not well characterized. Rehabilitative exercise is a non-invasive clinical therapy used predominantly to improve locomotor function after SCI, but since exercise is immunomodulatory, we hypothesized that post-SCI exercise would reduce neuropathic pain by modulating the immune environment of the injured system with a focus on the DRG.

Methods:

Adult, female, Sprague-Dawley rats received a unilateral C5 spinal cord contusion and were separated into SCI and SCI+Exercise groups. At 5 days post injury, SCI+Exercise group was exercised on automated running wheels for 20 minutes/day, 5 days/week for 4 weeks. Pain was assessed via von Frey and mechanical conflict avoidance operant testing. Macrophage recruitment and inflammation in the DRG were assessed using ELISA, immunocytochemistry and qPCR.

Results:

Macrophage chemoattractant, CCL2, was transiently elevated at 24 hrs post-SCI in the DRG. Macrophage infiltration was seen by 24 hrs post-SCI and persisted chronically. Post-injury exercise reduced the incidence of pain by 83% in conjunction with reduced number of ED-1+ macrophages in the DRG. We are currently using qPCR to determine whether post-injury exercise modulates acute and/or chronic pro-inflammatory cytokine production.

Conclusions:

By examining the inflammatory response in the DRG we will better understand possible mechanisms for the onset of neuropathic pain and for the efficacy of exercise. Supported by: Commonwealth Universal Research Enhancement (CURE) Program Grants (MRD), NIH #NS055976 (JDH).
Background and Aims:

The role of kynurenine pathway in neuropathic pain requires further extensive research. Several studies suggest a role of this system in the pathophysiology of neurodegenerative diseases (i.e., Huntington's, Alzheimer's, and Parkinson's diseases, migraines, multiple sclerosis), brain disorders and autoimmune disorders. The aim of our studies was to examine the role of kynurenine 3-monooxygenase (Kmo) inhibitors on neuropathic pain symptoms.

Methods:

Chronic constriction injury (CCI) of the sciatic nerve was performed according to Bennett and Xie (1988). Behavioral studies consisted of the allodynia/hyperalgesia measurements, biochemical studies comprised the RT-PCR and/or Western blot analysis in the tissue (spinal cord, DRG) and primary glia cultures. The experiments were carried out according to IASP rules (Zimmermann, 1983).

Results:

Our findings establish KMO inhibition reduce allodynia and hyperalgesia 7 day after CCI. Minocycline decreased pain and in parallel spinal and DRG Kmo expression, as well as improved the lipopolysaccharide (LPS)-induced upregulation of Kmo mRNA expression in microglial cell cultures. Kmo inhibitors reduced the mRNA expression of CD40, IL-1beta, IL-6 and NOS2 in the spinal cord and/or the DRG. Furthermore, we demonstrated that Ro61-6048 decreased the protein levels of IBA-1, IL-6, IL-1beta and NOS2 in the spinal cord and/or in the DRG.

Conclusions:

These results provide further evidence for the involvement of the Kmo inhibitors in neuropathic pain and highlight a potential role of these enzyme as novel therapeutic targets to evaluate for the treatment neuropathy.

Acknowledgments: Supported by National Science Centre grant- Sonata2015/17/D/NZ4/02284, Harmonia5 2013/10/M/NZ4/00261 and by Institute of Pharmacology statutory funds.
Background and Aims:

EMA300 is a selective, small molecule, angiotensin II type 2 (AT\textsubscript{2}) receptor antagonist that evokes dose-dependent anti-allodynia in rodents with a chronic constriction injury (CCI) of the sciatic nerve, with anti-allodynia abolished in AT\textsubscript{2} receptor knockout CCI-mice\textsuperscript{1}. The present aim was to assess a role for infiltrating angiotensin II (Ang II)-expressing CD3\textsuperscript{+} T-cells as a source for augmented Ang II expression levels in the ipsilateral lumbar dorsal root ganglia (DRGs) of CCI-rats\textsuperscript{1}.

Methods:

Ethics approval was from The University of Queensland. Briefly, a unilateral CCI (or sham-procedure) was induced in rats. A blinded tester measured von Frey hindpaw withdrawal thresholds (PWTs) pre-surgery and at 2-weeks post-surgery. CCI-rats with ipsilateral PWTs $\leq$6g, received intraperitoneal EMA300 at 10mg/kg, vehicle, or no treatment; PWTs were assessed at 1 h post-dose. Rats were euthanised and perfusion-fixed with 4% paraformaldehyde. Lumbar DRGs were collected and analysed using immunofluorescent antibodies for Ang II expression by CD3\textsuperscript{+} T-cells. Between-group comparisons were by one-way ANOVA.

Results:

In CCI-rat ipsilateral lumbar DRG sections, augmented Ang II expression was co-localised in part with a subset of CD3\textsuperscript{+} T-cells with an ~9.8-fold increase in number relative to that for sham-rats. At the time of peak EMA300 anti-allodynia, the number of Ang II-expressing CD3\textsuperscript{+} T-cells was reduced ($P$≤0.05) to match the number in the corresponding sections from sham-rats.

Conclusions:

Our findings suggest a role for infiltrating Ang II-expressing CD3\textsuperscript{+} T-cells as a source for augmented Ang II expression levels in the ipsilateral lumbar DRGs of CCI-rats.

\textsuperscript{1}Smith et al., Pain Medicine (2013)
Background and Aims:

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Pain represents an extremely common and difficult to treat symptom in MS, and is widely believed to have a central neuroinflammatory basis. We aimed to test the effects of intrathecal injection of recombinant interleukin (IL)-35, a novel anti-inflammatory cytokine, on pain behaviours and neuroinflammation in mice with experimental autoimmune encephalomyelitis (EAE).

Methods:

We used MOG$_{35-55}$-induced EAE in female C57BL/6 mice to model pain in MS. Intrathecal IL-35 therapy was commenced following the onset of clinical disease on day 11 post-EAE induction. Mechanical allodynia was assessed using von Frey filaments applied to the whisker pad and spontaneous pain was measured using the mouse grimace scale. Neuroinflammation was analysed using a combination of flow cytometry and immunohistochemistry.

Results:

IL-35 therapy reduced facial allodynia on days 12, 14 and 16, as well as facial grimacing on day 19 post-EAE induction. Flow cytometric analysis on day 16 revealed that this was associated with an increase in IL-10-producing T cells in the spinal cord and brainstem. Immunohistochemistry analysing the spinal and principle trigeminal nuclei on day 20 showed a decrease in IBA-1$^+$ monocytes/microglia, no change in P2ry12$^+$ homeostatic microglia, and a decrease in 4c12$^+$ monocytes in mice receiving IL-35 therapy.

Conclusions:

Overall, these findings suggest that intrathecal IL-35 therapy reduces pain behaviours in EAE, and appears associated with an upregulation of IL-10-producing T cells and a reduction in the numbers of infiltrating monocytes in the CNS.
Background and Aims:

Multiple sclerosis (MS) is an inflammatory condition of the CNS. Often, sensory disturbances arise which have been widely attributed to an inflammatory environment, including the development of neuropathic pain. Regulatory T-cells (Treg-cells), potent immunosuppressor cells within the adaptive immune system, may counteract a pro-inflammatory nociceptive environment. We aimed to characterise the phenotype of Treg-cells across stages of experimental autoimmune encephalomyelitis (EAE), an animal model of MS, and to assess the effects of adoptive transfer of Treg-cells on pain behaviours and neuroinflammation in EAE-affected mice.

Methods:

EAE was induced in female C57BL/6 and transgenic DEREG (depletion of regulatory T-cell) mice via immunisation with MOG35-55. Neuroinflammation and pain behaviours were assessed at distinct stages of EAE. Treg-cells were isolated from EAE-affected DEREG mice and adoptively transferred via intrathecal injection into EAE-affected C57BL/6 mice. Mechanical allodynia was assessed using von Frey filaments applied to the whisker pad. Neuroinflammation was assessed using flow cytometry and immunohistochemistry.

Results:

Chronic-stage EAE elicited a desirable Treg-cell phenotype, with anti-inflammatory cytokine production most elevated. Following intrathecal adoptive transfer, Treg-cells were found to accumulate in the brain of recipient animals. These cells also transiently ameliorated mechanical allodynia at the clinical-peak of EAE when injected intrathecally at disease onset. Further, decreased astrogliosis was seen in the trigeminal nuclei of Treg-injected compared to saline-injected EAE animals.

Conclusions:

These findings suggest that Treg-cells may modulate neuroinflammation and neuropathic pain following adoptive transfer in EAE. Spinal delivery of Treg-cells may thus provide a novel therapeutic intervention for neuropathic pain in MS.
ATTENUATION OF MECHANICAL PAIN HYPERSENSITIVITY BY TREATMENT WITH PEPTIDE5, A CONNEXIN-43 MIMETIC PEPTIDE, INVOLVES THE NLRP3 INFLAMMASOME IN NERVE-INJURED MICE

R. Tonkin¹, C. Perera¹, S. Duffy¹, P. Makker¹, J. Lees¹, S. O'Carroll², L. Nicholson², C. Green³, C. Gorrie⁴, G. Moalem-Taylor¹

¹The University of New South Wales, Translational Neuroscience Facility - School of Medical Sciences, Sydney, Australia
²University of Auckland, Department of Anatomy and Medical Imaging and the Centre for Brain Research - Faculty of Medical and Health Sciences, Auckland, New Zealand
³University of Auckland, Department of Ophthalmology - Faculty of Medical and Health Sciences, Auckland, New Zealand
⁴University of Technology Sydney, School of Life Sciences - Faculty of Science, Sydney, Australia

Background and Aims:

Accumulating evidence points to a key role for spinal astrocytes in the pathogenesis of neuropathic pain. Astrogliosis is associated with the opening of undocked connexin43 (Cx43) hemichannels and efflux of small excitatory molecules, such as ATP. An increased extracellular concentration of ATP causes assembly of the NLRP3 inflammasome, which activates caspase-1 triggering the release of key pro-inflammatory cytokines.

Methods:

Mice were subjected to a chronic constriction injury (CCI) of the sciatic nerve and an intrathecal injection of Peptide5 (a Cx43 mimetic peptide) ten days after CCI, followed by pain tests using von Frey filaments at 8 and 24 hours. The ipsilateral spinal cord was then removed and analysed by Western blot for Cx43 and inflammasome components. In addition, in vitro work was performed on purified astrocytes isolated and cultured from mixed cortical cells of mouse pups.

Results:

In vitro studies showed that primary astrocytes pre-treated with Peptide5 for 1 hour had significantly reduced ATP release in response to extracellular calcium depletion, as compared to controls. In vivo studies in nerve-injured mice showed that intrathecal injection of Peptide5 significantly improved mechanical pain hypersensitivity 8 hours following injection. Spinal cord analysis showed that Peptide5 reduced CCI-induced increases of Cx43 and the NLRP3 inflammasome proteins to naïve levels.

Conclusions:

Our findings demonstrate that spinal delivery of Peptide5, following nerve injury, attenuates mechanical pain hypersensitivity by the specific closure of Cx43 hemichannels. This reduction in neuropathic pain behaviour is associated with decreased NLRP3 complex, directly linking Cx43 hemichannel opening to inflammasome activation in the spinal cord.
Background and Aims:

Sigma-1 receptors (σ₁Rs) are a neuromodulatory intracellular chaperone able to migrate to the plasma membrane under cellular stress to interact with several receptors and channels. σ₁R knockout (σ₁R-KO) mice show a reduced neuropathic pain phenotype, although the underlying mechanisms are not well known. We investigated σ₁Rs cellular distribution in dorsal root ganglia (DRG) and macrophage infiltration into DRG in the sciatic spared nerve injury (SNI) model of neuropathic pain.

Methods:

We used female CD-1 wild-type (WT) and σ₁R-KO mice. Immunohistochemistry was performed 7 days after SNI to study the expression of σ₁Rs, NeuN (neurons), ATF3 (cellular stress) and Iba1 (macrophages) in the L4 DRG.

Results:

σ₁Rs are exclusively expressed in DRG neurons (NeuN positive cells) of WT mice, and they were homogeneously distributed in the cytoplasm of most neurons under normal conditions. After SNI, the subcellular distribution of σ₁Rs changed, as it was concentrated in the plasma membrane of many injured (ATF-3 positive) large DRG neurons, but was still restricted to neurons only. We also detected a massive infiltration of peripheral macrophages in the injured WT DRG, and this was strongly attenuated in σ₁R-KO mice.

Conclusions:

σ₁Rs migrate to neuronal plasma membrane and modulate macrophage infiltration into the injured DRGs after SNI. These results might suggest a role for σ₁Rs in the cross-talk between the injured DRG neurons and macrophages.
NEUP7-0298
DISTINCT MOLECULAR SIGNATURE OF DORSAL HORN ASTROCYTES IN A MOUSE MODEL OF NEUROPATHIC PAIN
J. Kuhn¹, J. Braz¹, K. Hamel¹, A. Basbaum¹
¹University of California San Francisco, Anatomy, San Francisco, USA

Background and Aims:
Glial cells contribute to many neurological diseases, including the mechanical hypersensitivity associated with neuropathic pain. In mouse models of nerve injury-induced pain, activation of microglia precedes the activation of astrocytes. However, little is known about the molecular signature of the reactive astrocytes or of the mechanisms through which activated microglia influence astrocyte reactivity.

Methods:
Here we used the sciatic nerve transection model and intrathecal injections of the microglia-activating chemokine colony stimulating factor 1 (CSF1) together with behavioral, anatomical and gene expression analyses to identify reactive astrocytes in a mouse model of neuropathic pain.

Results:
Surprisingly, Aldh111-reporter mice show only sparse expression in the superficial dorsal horn, indicating a distinct molecular signature of dorsal horn astrocytes. In addition to the commonly used astrocyte marker GFAP, nerve injury induces upregulation of the astrocyte gene Sperinf1, which is usually coexpressed with GFAP and Aldh111 and associated with proinflammatory gene expression.

Intrathecal injection of CSF1, which we have shown induces spinal cord microglia activation, resulted in sustained mechanical hypersensitivity accompanied by induction of GFAP-expressing astrocytes in the superficial dorsal horn. Based on the finding that microglia activation is sufficient for astrocyte reactivity, we are now testing the hypothesis that microglia are necessary for nerve injury-induced astrocyte activation. Our studies use a mouse model in which Cre-mediated deletion of CSF1 from sensory neurons prevents microglia activation and injury-induced hypersensitivity. Conclusions:

Dorsal horn astrocytes show a distinct molecular signature in response to nerve injury that likely arises after microglia activation.
Background and Aims:

Inflammation in the orofacial region results in pain and is associated with many pathological states, including migraine, neuralgias and temporomandibular disorder. Although extensively studied, the mechanisms responsible for these conditions are not known and effective treatments are lacking. We reported earlier that the proinflammatory TNF plays an important role in regulation of trigeminal ganglion (TG) neuron function in vitro. In the present study we investigated the role of TNF in mechanical hypersensitivity in mice. The aim of the present study was to analyse behavioural differences and changes in transient receptor potential vanilloid subfamily member 1 (TRPV1) gene and protein expression in TG following facial inflammation and or administration of novel TNF biologic - XPro 1595 in mice.

Methods:

We employed a Complete Freund’s Adjuvant (CFA)-induced model of orofacial pain and evaluated the effect of peripheral blocking of TNF activity by administration of XPro1595.

Results:

We show that CFA administration into the lower lip causes hyperalgesia and an increase in both expression of (TRPV1) mRNA and in the mean TRPV1 protein content in TG neurons. We also show that i.p. administration of XPro1595 prevents both CFA-induced mechanical hypersensitivity and up-regulation of TRPV1 protein in TG neurons.

Conclusions:

We conclude that one of the possible regulatory mechanisms of TNF in pain involves up-regulation of TRPV1, and that blocking of TNF can prevent hyperalgesia caused by inflammation in the orofacial region.

Acknowledgments This work has been supported by the Polish National Science Center, based on the Decision No. DEC- 2012/05/B/NZ4/02385.
Background and Aims:

Systematic review and meta-analysis are powerful approaches to summarising research, but publication of new material means they rapidly become outdated. For our systematic review of the neuropathic pain literature we had identified 33,814 publications; after dual screening 6,506 relevant publications will contribute to a family of systematic reviews categorised by the pain model used, including chemotherapy-induced peripheral neuropathy (CIPN). This magnitude of data is not sustainable with conventional systematic review methods and we therefore explored the utility of machine-learning approaches to facilitate the process.

Methods:

We used the initial set of 33,814 publications as a training set for 5 independent machine-learning approaches; 13 classifiers were created and applied to an updated search performed in November 2015. A 10% random sample of these publications were checked for inclusion/exclusion by two independent investigators.

Results:

This updated search identified 11,880 unique publications and machine-learning approaches identified 3,461 for inclusion. In our random 10% sample, the approach that fitted best had a sensitivity and specificity of 95% and 84%, respectively. Using text mining we identified 359 CIPN studies in the updated search, compared with 181 in the original search, giving a rate of publication of 9 papers per month. Automated extraction of study meta-data and risks of bias is ongoing.

Conclusions:

We show the application of machine-learning to facilitate living systematic reviews in the field of neuropathic pain, where investigators only have to screen a fraction of the search results; and publication rate, even in the narrow area of CIPN, which underlines the importance of this approach.
Background and Aims:

The voltage-gated sodium channel (Nav) contributes to generation and propagation of the action potentials (APs) in neurons. Recent studies in human and animals suggest that Nav1.7 and Nav1.8 subtypes are key contributors to the nociceptive processing in both physiological and pathological states; dysfunction or decreased expression of these channels may provide relief of pain. Therefore, the blockage of Nav1.7 and Nav1.8 may be a promising pharmacotherapy for pathological pain. Here we describe the in vitro property of a novel Nav blocker DSP-2230.

Methods:

Electrophysiological experiments were performed by the whole-cell patch-clamp recording. Na+ currents were recorded from recombinant human Nav1.7-, Nav1.8- or Nav1.5-expressing cells. The dorsal root ganglion (DRG) neurons were isolated from newborn rats, and then Na+ currents and APs were recorded.

Results:

We revealed that DSP-2230 inhibited Nav1.7-, Nav1.8- and Nav1.5-derived Na+ current with IC50 values of 9.3, 5.5 and 151 μM, respectively, suggesting a good selectivity for avoiding cardiovascular side effects. As for the modulatory effects of DSP-2230 on channel gating properties of human Nav1.7, DSP-2230 decreased the peak of Na+ current at the resting state, depolarized the activation curve and decelerated the kinetics of activation and inactivation. In the experiments using isolated rat DRG neurons, DSP-2230 inhibited Na+ currents and decreased the frequency of evoked APs.

Conclusions:

In conclusion, a Nav1.7/1.8 blocker DSP-2230 showed unique profiles in selectivity and modes of action, which are clearly different from other Nav blockers.
Background and Aims:

DSP-2230 is a novel compound that has been shown to selectively inhibit voltage gated sodium channel currents at human Nav1.7 and Nav1.8 subtypes that are expressed primarily on peripheral nerves. DSP-2230 demonstrated significant antiallodynic effects in multiple animal models of pain, including neuropathic pain models that have been shown to be predictive of efficacy in humans, as well as significant efficacy in an inflammatory pain model. Here we describe the safety and pharmacokinetics profiles of DSP-2230, which is ready for the phase II clinical trial.

Methods:

The effects of DSP-2230 on locomotor activity and motor coordination were evaluated in rats. Potential effects on cardiac repolarization were evaluated in an in vitro hERG study and effects on ECG parameters were evaluated in anesthetized dogs and conscious monkeys.

Results:

At single oral doses of up to 300 mg/kg, DSP-2230 had no effects on locomotor activity and motor coordination. In CV safety assessments, administration of DSP-2230 to dogs and monkeys did not result in any effects on the QT interval and other CV parameters. In order to evaluate potential “off-target” effects of DSP-2230, the ability of DSP-2230 to displace specific binding at 68 receptors was evaluated at a concentration of 10 µM. Greater than 50% inhibition was seen in only 2 receptors (sigma 1 receptor and sodium channel).

Conclusions:

These findings suggest that DSP-2230 has positive potential as a drug for the treatment of neuropathic pain without CV or CNS side-effects, which are present with current drugs such as non-selective sodium channel blockers and anti-epileptics.
PROPHYLACTIC ADMINISTRATION OF ZERUMBONE ATTENUATES ALLODYNIA AND HYPERALGESIA THROUGH THE SUPPRESSION OF INFLAMMATORY MEDIATORS IN CHRONIC CONSTRICTION INJURY-INDUCED NEUROPATHIC PAIN

E.K. Perimal1, B. Gopalsamy1, A.A. Farouk1, A.S. Mohamad1, M.R. Sulaiman1
1Faculty of Medicine and Health Sciences - Universiti Putra Malaysia, Biomedical Science, Serdang, Malaysia

Background and Aims:

Neuropathic pain affects approximately 6% to 10% of the worldwide population. It remains a clinically challenging condition. Existing drugs do not provide optimal pain relieve in many of the patients. Our previous studies using Zerumbone (Zer), a sesquiterpene compound isolated from the rhizomes of a Southeast Asian ginger plant, Zingiber Zerumbet showed to possess antinociceptive and anti-inflammatory properties when tested on models of nociception and inflammation. This study investigated the effects of prophylactic administration of Zer on allodynia and hyperalgesia in a mouse model of chronic constriction injury (CCI)-induced neuropathic pain.

Methods:

Intraperitoneal administrations of Zer (5, 10 or 50 mg/kg) from day one post-surgery were carried out to identify the onset and progression of pain conditions. Responses towards mechanical and cold allodynia and mechanical and thermal hyperalgesia were assessed on day 3, 5, 7, 9, 11 and 14. Blood plasma and spinal cord levels of IL-1β, IL-6, TNF-α and IL-10 were screened using ELISA on day 14.

Results:

Zer (10 and 50mg/kg) significantly attenuated mechanical and cold allodynia as well as mechanical and thermal hyperalgesia on all days of behavioral testing without showing any signs of motor impairment using the rota rod test. Blood plasma and spinal levels of IL-1β, IL-6 and TNF-α and not IL-10 was significantly (p<0.05) suppressed by the Zer treatment.

Conclusions:

Zer exhibits its antiallodynic and antihyperalgesic properties via reduced sensitization at nociceptor neurons possibly through the suppression of the inflammatory mediators. Zer may prove to be a novel and beneficial alternative for the management of neuropathic pain.
NEUP7-0101
DSP-2230, A NOVEL SODIUM CHANNEL BLOCKER, SUPPRESSES NOCICEPTIVE BEHAVIORS IN PRECLINICAL PAIN MODELS
Y. Takada¹, T. Kamei¹, F. Ishibashi¹, A. Ono¹, K. Ikeda¹, Y. Oyamada¹
¹Sumitomo Dainippon Pharma Co.- Ltd., Drug Research Division, Suita, Japan

Background and Aims:

The voltage-gated sodium channels Nav1.7 and Nav1.8 are located peripheral neuron and play a key role in action potential production and pain sensation. Recent genetic studies have identified Nav1.7 dysfunction in different human pain disorders, and previous studies have demonstrated that decreased expression of Nav1.8 attenuates the development and maintenance of neuropathic pain. Thus, the Nav1.7 and Nav1.8 sodium channels may provide a unique target for the pharmacotherapy of pain in humans. Here, we report that DSP-2230, which is a novel compound that inhibits Nav1.7 and Nav1.8 sodium channels, has demonstrated antiallodynic effects in multiple rat models of pain.

Methods:

Experimental neuropathic pain models were produced by rat L5 spinal nerve ligation (SNL) or chronic constriction injury (CCI). Streptozotocin-induced mechanical hypersensitivity was used as a diabetic neuropathy model. Formalin or capsaicin-induced nociceptive behaviors were used as inflammatory pain models.

Results:

Oral administration of DSP-2230 attenuated mechanical hypersensitivity in SNL and CCI models with minimum efficacious doses (MEDs) of 10 and 30 mg/kg, respectively. DSP-2230 also significantly attenuated mechanical hypersensitivity in streptozotocin-induced rat with a MED of 5 mg/kg. In addition, suppressive effect of DSP-2230 on formalin-induced nociceptive behaviors selectively in the second phase was observed with a MED of 30 mg/kg, which was comparable to the effect of diclofenac, non-steroidal anti-inflammatory drug. Finally, DSP-2230 inhibited the development of mechanical hypersensitivity induced by intraplantar injection of capsaicin with a MED of 10 mg/kg.

Conclusions:

These data demonstrate that novel sodium channel blocker DSP-2230 is a promising candidate for multiple pain conditions in human.
NEUP7-0159
LOW-LEVEL LASER THERAPY (904 NM) REDUCES HYPERALGESIA THROUGH DAILY IRRADIATION AT DORSAL ROOT GANGLIA IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC NEUROPATHIC RATS

W. Vieira1, S. Magalhães1, J. Schiavuzzo1, C. Parada1
1State University of Campinas UNICAMP,
Department of Structural and Functional Biology - Institute of Biology, Campinas, Brazil

Background and Aims:

Diabetic neuropathy develops as a complication of diabetes and just a few of the used therapies have been successful. Efficacy of low-level laser therapy (LLLT) in painful clinical conditions has been established by several recent studies. The aim of this study was verify the pain relief potential of LLLT in Streptozotocin (STZ)-induced diabetic neuropathic rats.

Methods:

All experiments were approved by the UNICAMP Ethic’s Committee (CEUA #3902-1). Male Lewis rats (LEW/HsdUnib) (200-250 g; 6-8-weeks-old) received a STZ-low dose (25 mg/kg) once a day during five consecutive days. Animals were considered diabetic when reach a 250 mg/dL blood glucose level and were submitted to eletronic von Frey test at 0, 7, 14, 21, 24 and 28 days after STZ injections. 0.1 M sodium citrate buffer was used as control. After being considered neuropathic, rats were submitted to daily LLLT (after the 21st day) (GaAs 904 nm; 2.03 Joules; 70 mW; 0.001 cm² output; 29 seconds). LLLT was performed transcutaneously at the dorsal region between L4/L5 DRG (right and left) while the animals were kept under anesthesia (2% isoflurane).

Results:

LLLT was able to reduce the intensity of hyperalgesia (Δ withdrawal threshold, g) on the 24th and 28th days after STZ injections when compared to diabetic neuropathic non-irradiated rats (p<0.001; Two-Way ANOVA followed by Bonferroni post hoc test).

Conclusions:

The data of this study, although preliminary, suggest that LLLT could be a promising treatment to alleviates pain during diabetic neuropathy. The probable action mechanism of this therapy is under investigation in our laboratory.
Background and Aims:

Cell therapy represents a promising step in the treatment of spinal cord injury (SCI), but the ideal type of stem cell remains open. Here we evaluated the ability of fetal neural stem cells (fNSC) from different regions to reverse chronic pain, as well as to promote motor recovery after SCI.

Methods:

Wistar rats were submitted to traumatic SCI using the NYU Impactor. After 10 days, the spinal cord was re-exposed and the animals received a transplant of culture medium (sham group) or fNSC extracted from periventricular substance (PV group, GABAergic precursors) or ventral pons/medulla region (VPM group, serotonergic/ noradrenergic precursors) from embryos with 14 days of intrauterine life. Behavioral assessments were performed during 8 weeks.

Results:

Thermal hyperalgesia assessment, by hot plate test, showed that PV group improved ≈47.73% in relation to sham (p<0.05) at the 7th and 8th week after transplantation and MPV group improved 45.88% (p<0.05) at the 7th week and 59.31% (p<0.01) at the 8th week compared to sham. Mechanical allodynia assessment, by von Frey filaments, showed that PV group improved 52.08% (p<0.05) and the MPV group improved 49.77% (p<0.01) in relation to sham at the 8th week of evaluation. BBB test showed a slight improvement (19%) of the MPV group compared to sham, however with no statistical difference. No difference between the groups was observed in the inclined plane test.
Conclusions:

Inhibitory neuronal precursors transplantation are able to attenuate the painful sensation but not motor recovery in animals submitted to SCI.

Acknowledgement: FAPESP/CNPq
Background and Aims:

Previously, we showed that Telocinobufagin (TCB, 3β,5β-Dihydroxy-5β,14β-bufa-20,22-dienolide), a cardiotonic steroid identified in amphibians and after in humans, has an important antinociceptive effect (not affected by naloxone) on animal models of pain (Patents: US 8,541,395B2; EP 2440211B1). More recently, we have shown that compounds derived from TCB also presented strong analgesic activity. However, toxicological screening of these analgesics may be important for the extension of the therapeutic potential. So, the aim of this work is to study the safety acute testing of compounds derived from TCB.

Methods:

The toxicological screening was carried out in mice and rat through the oral route, with doses of 5, 10, 20 and 40 mg/Kg. The first assay was used for selection of the maximum tolerated dose. After this, the second assay was the acute toxicity testing by the up-and-down method and the animals were observed for 14 days, including detailed clinical signs.

Results:

After the assay for maximum tolerated dose of ten derived analgesics from TCB, two of these compounds, sTCB3 and sTCB4, were selected for the next phase, since the animals tolerated dose of 40 mg/Kg for both treatments. Next, the acute toxicity testing by the up-and-down method shows that sTCB4 presented the higher experimental therapeutic index, three times higher than that of morphine.

Conclusions:

Preclinical safety acute testing shows that sTCB4 presented an experimental TI three times higher than that of morphine, suggesting that it be a template for development of new non-opioid analgesics for treatment of the human NP.

GRANTS: FINEP, CNPq, TECNOVA/SECITECE, INOVAFIT/FUNCAP
Background and Aims:

Multiple sclerosis (MS) is an inflammatory, autoimmune, demyelinating disease of the central nervous system. Pain is a common symptom reported by almost 50% of people suffering from MS. Crotalphine, a peptide synthesized from the sequence of a purified analgesic compound from the C.d. terrificus snake venom, induces potent and long-lasting analgesic activity. This effect is mediated by peripheric type 2 cannabinoid receptor, followed by endogenous opioid peptide release. Our objective was to assess the effect of Crotalphine in the EAE model, evaluating its effect in the pain and progression of EAE.

Methods:

EAE was induced by immunization of C57BL/6 female mice with MOG35-55 peptide and complete Freund’s adjuvant, followed by pertussis toxin injection. Pain threshold was determined using an electronic pressure-meter test. Clinical signs were assessed according to scores from 0 to 5. Crotalphine (50μg/kg) was administered in a single dose on the 5th day after immunization, or in 5 intercalated doses (1 dose every 3 days).

Results:

Our results showed that EAE decreased the pain threshold, starting at day 4 and lasting for 10 days. Motor accompaniment was started on days 10-11. Crotalphine caused partial reversion of EAE-induced hyperalgesia. This effect was maintained up to the 10th day. The Crotalphine decreased the severity of the clinical signs of EAE when compared to saline-treated animals.

Conclusions:

These results suggest that crotalphine could be useful in the control of EAE. These data also point to the fact that substances derived from animal toxins may have therapeutic potential for treating MS.

Support: CNPq-467211/2014-0, FAPESP-2011/17974-2, CAPES
NEUP7-0322
EPA AND DHA ENRICHED FISH OIL TREATS AND PREVENTS NEUROPATHIC PAIN BEHAVIOR IN MICE AFTER PERIPHERAL NERVE INJURY
R.V. Silva¹, C.K.F. Lima¹, F.C. Dias¹, B.L.R. Santos¹, J.T. Oliveira², A.L.P. Miranda¹
¹Universidade Federal do Rio de Janeiro - UFRJ, Faculty of Pharmacy, Rio de Janeiro, Brazil
²Universidade Federal do Rio de Janeiro - UFRJ, Faculty of Medicine, Rio de Janeiro, Brazil

Background and Aims:

Neuropathic pain (NP) is a multifactorial condition arising from injury or malfunction of somatosensory system, leading to peripheral and central sensitization, in which neuroinflammation is a crucial process. Omega 3 PUFAs, EPA and DHA, are well known for their immunomodulatory activity by generating potent lipid mediators. Aim: To investigate the therapeutic potential of EPA and DHA enriched fish oil (EFO) in a model of NP.

Methods:

NP was induced by partial sciatic nerve ligation (PSNL). Thermal and mechanical hypersensitivity was assessed on 5th, 7th and 9th day after nerve injury. Animals received daily oral treatment for 5 days beginning 5 days after surgery (therapeutic protocol) or for 10 days beginning on the surgery day (preventive protocol) with vehicle arabic gum 5%, EFO (2.3 or 4.6 g/kg) or gabapentin (100 mg/kg). On 9th day animals were euthanized and dorsal root ganglia (DRG), spinal cord (SC) and sciatic nerve (SN) were collected for further analysis (ATF-3 expression, TNF production, MPO activity). Additionally, locomotor activity and electrophysiology were performed. Animal ethics committee: CEUA-UFRJ 011/16.

Results:

EFO (4.6 g/kg) reversed mechanical allodynia and thermal hypernociception (therapeutic protocol). EFO (2.3 g/kg) prevented NP decreasing allodynia, hypernociception, TNF in SC and DRG ATF-3 expression, suggesting reduced microglial and neuronal activation. MPO activity was reduced on SN in both protocols.

Conclusions:

EFO reverses and prevents mechanical and thermal hypersensitivity after injury. The molecular mechanisms involved seem to be related to a decreased neuroinflammation, neuronal activation and fibers regeneration. EFO arises as a safe and efficacious therapeutic alternative for NP treatment.
BASIC SCIENCE (ANIMALS): OTHER - PART 2

NEUP7-0264
DISCOVERY OF AN HETEROCYCLIC-BASED H-PHE-PHE-NH₂ ANALOGUE WITH ANTI-ALLODYNIC EFFECT IN MICE AND WITHOUT NEUROTOXIC LIABILITY
A. Skogh¹, A. Lesniak², F.Z. Gaugaz³, A. Jonsson², R. Svensson³, R. Fransson¹, F. Nyberg², M. Hallberg², A. Sandström¹
¹Uppsala University, Medicinal Chemistry, Uppsala, Sweden
²Uppsala University, Pharmaceutical Science, Uppsala, Sweden
³Uppsala University, Pharmacy, Uppsala, Sweden

Background and Aims:

H-Phe-Phe-NH₂, a derivative of endomorphin-2 (EM-2, H-Tyr-Pro-Phe-NH₂) has previously been identified as a high affinity ligand for the substance P 1-7 (SP₁₋₇, H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-OH) binding site. The dipeptide exerts profound antiallodynic and antihyperalgesic effects when delivered intrathecally to diabetic mice. In the current study, properties and effects of a novel heterocyclic-based H-Phe-Phe-NH₂ analogue, AS097 in a spared nerve injury model of neuropathic pain after systemic administration was investigated.

Methods:

Human and mouse plasma stability tests were performed with LC-MS/MS analysis. Cytotoxicity was evaluated in rat primary neuronal cultures with the lactate dehydrogenase and MTT cell proliferation assays. Neuropathic pain was induced in male NMRI mice by tightly ligating the tibial and common peroneal nerves while leaving the sural nerve intact. The antiallodynic activity was assessed in the von Frey test.

Results:

AS097 did not show any effect on primary cortical cell culture survival. The half-life of AS097 exceeded 180 min in both human and mouse plasma. When AS097 was administrated systemically into neuropathic mice, a dose-dependent increase in mechanical thresholds in the von Frey test was observed. Additionally, AS097 was equipotent to the full-length SP₁₋₇ amide but showed greater potency than gabapentin and morphine in attenuating mechanical allodynia and its effect was reversed by D-SP₁₋₇ (H-Arg- D-Pro-Lys-Pro-Gln-Gln- D-Phe-OH) – a SP₁₋₇ binding site antagonist.

Conclusions:

The heterocyclic-based analogue of H-Phe-Phe-NH₂ is stable in plasma and might serve as a lead for further development of analgesics useful against neuropathic pain.
NEUP7-0015
USE OF THE NON-MEDICATION COMPLEX IN THE TREATMENT OF NEUROPATHIC DISCOGENIC LOW BACK PAIN
O. Tondiy¹
¹, Kharkiv, Ukraine

Background and Aims:

The effect of the combination of the physiotherapy (low-frequent variable magnetic field, electrical stimulation) and of the acupuncture on the patients having neuropathic discogenic low back pain was investigated.

Methods:

110 patients aged from 20 to 50 (49 females and 61 males) having neuropathic low back pain (osteochondrosis, osteoarthritis, spondyloarthritis) for 24 – 41 days were observed. The pain was examined and measured according to the visual analogue scale. The patients were divided into two groups. The first group (72 patients) received in addition acupuncture (individual points) and physiotherapeutic complex with low-frequent variable magnetic field and electrical stimulation treatment on the projection of pain. Every procedure exposure was 12 – 15 min. The complete course was 10 – 12 procedures. The second group (control, 38 patients), received only the basic medication (non-steroid anti-inflammations and anticonvulsants).

Results:

The pain intensity of the patients in the first group was reduced after 10 – 12 days of treatment (65.3% patients) compared to the control group, where pain reduction after 20 – 26 days of treatment (31.6% patients); p<0.01.

Conclusions:

The addition of the non-medication therapy (combination of acupuncture, low-frequent variable magnetic field and electrical stimulation) to the treatment of acute discogenic pain resulted in earlier remission.
Background and Aims:

Analgesic effect of STR-324, a stable natural analog of the natural human dual enkephalinase inhibitor opiorphin, in a rat model of peripheral neuropathic pain, and assessment of c-Fos expression at the spinal level.

Methods:

Male Sprague Dawley rats underwent a left L5-L6 spinal nerves ligation. An Alzet pump was set to deliver intravenously postoperatively during 7 days either a saline solution or 10 µg/h, 50 µg/h, or 250 µg/h of STR-324. Mechanical allodynia, thermal hyperalgesia, and tonic pain were assessed each day from D0 before surgery to D+7 after surgery by von Frey filaments, Hargreaves method, and spontaneous pain related behavior, respectively. C-Fos expression was assessed at D+3.

Results:

There was a significant inhibition of mechanical alldynia from Day4 to Day7 in the 10 µg/h and 50 µg/h STR-324 groups (P < 0.01 and P < 0.05 respectively), of thermal hyperalgesia from Day2 to Day6 in the 10 µg/h STR-324 group (P < 0.05), and of tonic pain from Day1 to Day6 in the 50 µg/h STR-324 group (P < 0.05). C-Fos expression was significantly reduced.
in the STR-324 group.

Conclusions:

In this model, STR 324 reduced mechanical allodynia, thermal hyperalgesia and tonic pain, and may be therefore of interest for peripheral neuropathic pain management. Reduction of c-Fos expression demonstrates that STR-324 acts through inhibition of endogenous nociceptive pathways.
Background and Aims:

We investigated the effect of botulinum neurotoxin type A (BoNT-A) on mechanical allodynia and hyperalgesia associated with infraorbital nerve constriction (ION-CCI) in rats.

Methods:

ION-CCI rats received a subcutaneous BoNT-A injection into the whisker pad area on day 7 postoperatively and underwent pain assessment on days 14 and 21 postoperatively. Rats were assigned to one of four treatment groups (n=5 each): ION-CCI+BoNT-A 20pg (low-dose group), ION-CCI+BoNT-A 200pg (high-dose group), ION-CCI+saline, and Sham. Mechanical allodynia and hyperalgesia were evaluated preoperatively (baseline) and on days 7, 14, and 21 postoperatively. After noxious mechanical stimulation of whisker pad skin, the number and distribution pattern of the phosphorylated extracellular signal-regulated kinase (pERK)-immunoreactive (IR) neurons were analyzed in the trigeminal spinal subnucleus caudalis (Vc) and upper cervical spinal cord (C1-C2).

Results:

On day 21, nocifensive behavior was attenuated by high-dose but not low-dose BoNT-A administration. In addition, after noxious mechanical stimulation of whisker pad skin, the numbers of pERK-IR cells in the superficial laminae of Vc and C1-C2 were significantly lower in the high-dose BoNT-A group than in the ION-CCI+saline group.

Conclusions:

The present findings suggest that, by suppressing Vc neuronal activity, high-dose intradermal injection of BoNT-A at the site of ION innervation alleviates mechanical facial allodynia and hyperalgesia associated with ION-CCI.
Background and Aims:

Stimulation of the secondary somatosensory cortex (S2) has attenuated pain in humans and inflammatory nociception in animals. Here we studied S2 stimulation-induced antinociception and its underlying mechanisms in an experimental animal model of neuropathy induced by spinal nerve ligation (SNL).

Methods:

Effect of S2 stimulation on heat-evoked limb withdrawal latency was assessed in lightly anesthetized rats that were divided into three groups based on prior surgery and monofilament testing before induction of anesthesia: i) Sham operated group, ii-iii) (mechanically) hypersensitive and non-hypersensitive SNL groups. In a group of hypersensitive SNL animals, a 5-HT₁A receptor agonist was microinjected into the rostroventromedial medulla (RVM) to assess whether autoinhibition of serotonergic cell bodies blocks antinociception. Additionally, electrophysiological single unit recordings were performed to assess the effect of S2 stimulation on nociceptive responses of wide-dynamic range (WDR) neurons in the spinal dorsal horn of anesthetized hypersensitive SNL animals.

Results:

Stimulation of S2, but not that of an adjacent cortical area, induced bilaterally antinociception in hypersensitive but not in non-hypersensitive SNL or sham-operated animals. Antinociception was prevented by a 5-HT₁A receptor agonist in the RVM. In spinal WDR neurons, heat-evoked discharge was delayed by S2 stimulation, and this antinociceptive effect was prevented by blocking spinal 5-HT₁A receptors.

Conclusions:

The results indicate that S2 stimulation suppresses nociception in SNL animals if SNL is associated with tactile alldynia-like hypersensitivity. In hypersensitive SNL animals, S2 stimulation-induced spinal antinociception is mediated by descending serotonergic pathways acting on the spinal 5-HT₁A receptor.
Background and Aims:

The orexin has been known as a peptide regulating wakefulness and appetite. In addition, recent studies have reported the suppressive effect on nociception. The insular cortex (IC) is an important region to process nociception, receives dense orexinergic inputs, and expresses both orexinergic receptor 1 (OX1) and 2 (OX2). Therefore, orexin possibly regulates IC activities, which may contribute to suppress nociception. However, it remains unknown how cortical orexinergic receptors modulate synaptic transmission.

Methods:

In the present study, we performed paired whole-cell patch-clamp recordings from fast-spiking cells (FS) and pyramidal cells (Pyr) in the rat, and examined the effects of orexinergic ligands on unitary inhibitory postsynaptic currents (uIPSCs).

Results:

Application of 100 nM orexin A or orexin B enhanced the amplitude of uIPSCs in FS to Pyr connections. The enhancement of uIPSCs by orexin A/B was not accompanied by a change in paired-pulse ratio. OX1 has more preference to binding to orexin A rather than to orexin B, whereas OX2 comparably binds to orexin A and orexin B. Therefore, next, we examined the effects of OX1 and OX2 selective antagonists on the orexin-induced facilitation of uIPSCs to find which orexinergic receptors play a major role in facilitating uIPSCs. Pre-application of SB334867, an OX1 antagonist, or TCS-OX2-29, an OX2 antagonist, diminished the orexin-induced facilitation of uIPSCs, suggesting that both receptors contribute to facilitate uIPSCs.

Conclusions:

This orexinergic facilitation of inhibitory transmission in IC may reduce IC excitation, and as a result, nociception is suppressed.
Background and Aims:

Our previous studies have demonstrated that electrical stimulation of the periodontal ligament (PDL) elicited excitation in the somatosensory and insular cortices. However, a profile of cortical excitation responding to mechanical stimulation of the PDL has been unknown. In this study, we performed optical imaging to identify the cortical regions responding to mechanical and electrical stimulation of the maxillary first molar PDL, and elucidated differences in cortical responses between mechanical and electrical stimulation.

Methods:

We performed in vivo optical imaging to identify the cortical responses evoked by mechanical stimulation of the maxillary first molar in the rat. Furthermore, morphine was systemically applied (2.5mg/kg; s.c.) to explore how a potent nociception blocker changes cortical excitation responding to the PDL stimulation.

Results:

The initial response to electrical stimulation of the maxillary molar PDL induced excitation in the secondary somatosensory cortex (S2) and the insular oral region (IOR), whereas mechanical stimulation elicited excitation in the caudal part of the primary somatosensory cortex (S1). On the other hand, the maximum response to both the mechanical and electrical stimulation induced excitation in S1 and S2/IOR. Systemic application of morphine reduced the response in S2/IOR to electrical stimulation of the PDL.

Conclusions:

These findings suggest that electrical and mechanical stimulation principally elicit S2/IOR and S1, respectively. In addition, nociception of the PDL is likely to be processed in S2/IOR.
Background and Aims:

The experience of pain arises from the integration of somatosensory and emotional nociceptive information, which guides the selection of motivationally protective behaviors. However, the computational processing of nociception within emotional neuronal ensemble networks, such as the basolateral amygdala (BLA), is not understood. Furthermore, injury-induced neuroplasticity in the BLA may lead to miscoding of sensory information concomitant with the development of chronic pain. Here, we aim to determine the causal link between neural ensemble activities and their emergent behavioral consequences, along with the evolving co-dynamics due to nerve injury.

Methods:

We utilized recurrent in vivo calcium (Ca\(^{2+}\)) imaging, chemogenetic manipulation, and trans-synaptic circuit tracing of BLA nociceptive neuronal ensembles prior to and throughout the development of neuropathic pain.

Results:

Multidimensional and population vector analyses of Ca\(^{2+}\) spike activity patterns revealed that acute, multimodal nociception (i.e. noxious heat vs. cold vs. mechanical) is innately encoded in a shared ensemble involving hundreds of BLA principal neurons, distinct from general salience and appetitive valence codes. Chemogenetic silencing of this nociceptive ensemble selectively mitigated pain affective-motivational behaviors, with no effect on anxiety, reward, or nociceptive reflex behavior. During a neuropathic state, the representation of normally innocuous stimuli overlaid the nociceptive ensemble, which can be targeted for alleviation of chronic pain affect.

Conclusions:

We have identified unique ensemble representations of abstracted noxious information in the BLA facilitating the innate, negative qualities of the pain experience. Our findings demonstrate how BLA ensemble miscoding assigns negative valence to non-noxious stimuli, which may ground the psychological and motivational dysfunctions of chronic pain.
Background and Aims:

Preemptive analgesia or early treatment of pain attenuates the progression of pain caused by surgical lesions. However, the underlying mechanisms are unclear. The purpose of present study is to investigate underlying mechanisms of effects of preemptive analgesia on the development of trigeminal neuropathic pain.

Methods:

SD-rats were anesthetized with ketamine (40 mg/kg) and xylazine (4 mg/kg). Under anesthesia, ION-CCI was performed. We examined the withdrawal behavioral responses produced by air-puff pressure (4 sec of duration, 10 sec of intervals). Co-localization analysis of p-p38 and GFAP for immunofluorescence images was examined by a confocal laser scanning microscope.

Results:

Perineural application of 2 % of QX-314 reduced neuropathic mechanical allodynia. Double injection of QX-314 significantly attenuated development of mechanical allodynia compared to the single treatment with QX-314. Application of 2% QX-314 did not affect neuropathic mechanical allodynia on POD7 when pain is already established. Application of QX-314 did not affect extravasated Evans’ blue dye concentration and the number of cell with ATF-3 immunoreactivity produced by ION-CCI. The application of QX-314 reduced the up-regulated GFAP and p-p38 expression produced by ION-CCI in the trigeminal ganglion.

Conclusions:

QX314-induced long lasting preemptive analgesia produces inhibition of development of neuropathic pain through a regulation of the satellite glial cells and neuronal p-p38 expression in the trigeminal ganglion. Importantly, these results provide a potential preemptive therapeutic strategy for the treatment of neuropathic pain following nerve injury.
ROLE OF SIGMA-1 RECEPTOR IN THE SPARED NERVE INJURY MODEL OF NEUROPATHIC PAIN

I. Bravo-Caparrós1,2, F.R. Nieto1,2, G. Perazzoli3, R. González-Cano1,2, J.M. Baeyens1,2, E.J. Cobos1,2
1Departament of Pharmacology, School of Medicine- University of Granada, Granada, Spain
2Institute of Neuroscience- Biomedical Research Center, University of Granada, Armilla- Granada, Spain
3Department of Human Anatomy and Embryology, School of Medicine- University of Granada, Granada, Spain

Background and Aims:

Sigma-1 receptors (σ1Rs) are an emerging target for the treatment of neuropathic pain. The role of σ1Rs in neuropathic pain induced by nerve compression is known, but their participation on peripheral neuropathy induced by nerve transection is unexplored. Therefore, we investigated the effect of pharmacological and genetic blockade of σ1Rs in the neuropathic pain associated to the sciatic nerve spared nerve injury (SNI) model.

Methods:

SNI was performed on female CD1 wild-type (WT) and σ1Rs knockout (σ1Rs-KO) mice. Mechanical allodynia (von Frey test), cold-alldynia (acetone test) and heat hyperalgesia (Hargreave’s test) were tested previously to SNI and during three weeks following injury. The effect of σ1Rs antagonist S1RA (16-64 mg/kg, s.c.) and agonist PRE-084 (32 mg/kg, s.c.) were tested seven days after SNI.

Results:

WT mice developed prominent cold and mechanical allodynia after SNI, whereas σ1Rs-KO did not develop cold alldynia and showed reduced mechanical allodynia. These effects were mimicked by the administration of S1RA to neuropathic WT mice, and the effects of the σ1 antagonist were reversed by PRE-084. σ1Rs-KO and WT mice showed a similar thermal hyperalgesia after SNI. However, S1RA abolished SNI-induced thermal hyperalgesia in WT mice, an effect which was reversed by PRE-084.

Conclusions:

σ1 receptors have a relevant role in SNI-induced neuropathic pain. The pharmacological antagonism of this receptor can be useful to prevent and/or treat neuropathic pain produced by nerve section.
ROLE OF THE TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL A1 (TRPA1) ON THE SECONDARY HYPERALGESIA INDUCED BY CAPSAICIN IN MICE

M. Goncalves¹, R. Nassini², J. Ferreira³, P. Geppetti²
¹University of Florence, Health Science Department, Florence, Italy
²UniFI, Health Sciences, Florence, Italy
³Federal University of Santa Catarina - UFSC, Pharmacology, Florianopolis, Brazil

Background and Aims:

Since, many neuropathic pain patients do not receive appropriate treatment due to reduced effective analgesic drugs, the identification of new targets to indicate novel analgesic drugs is urgent. TRPA1 antagonists produce analgesic-like effect in neuropathic mice. Secondary hyperalgesia-induced by capsaicin in health individuals is a human pain model used to produce proof-of-concept for novel analgesic drugs useful to treat neuropathic pain. Similar to neuropathic pain, subcutaneous capsaicin injection cause secondary hyperalgesia maintained by a spinal cord sensitization of the pain pathways. The aim of the present study was to investigate the role of TRPA1, especially at the spinal cord, on the secondary hyperalgesia caused by capsaicin in mice.

Methods:

We used male and female C57/Bi6, TRPA1 wild type and TRPA1 knockout mice (N=6, 20-25 g). The experiments were approved by the Ethics Committee of UniFi and performed following the ARRIVE guidelines. Capsaicin (20 nmol/paw/subcutaneous) was injected and pain-like behaviors were detected. Von Frey filaments were applied to the paw's distal part. TRPA1 antagonist (HC-030031, 10 ug/site/intrathecally) was injected before and after capsaicin. As positive control, gabapentin (70 mg/Kg) was orally administrated 2 hours after capsaicin injection.

Results:

Capsaicin produced secondary hyperalgesia for 6 hours. Pain-behaviors were similar in wild type and TRPA1 knockout mice but secondary hyperalgesia induced by capsaicin was significantly reduced in TRPA1 knockout mice. Capsaicin-induced secondary hyperalgesia was inhibited by HC-030031.

Conclusions:

TRPA1 activation has relevant role in the secondary hyperalgesia induced by capsaicin in mice, indicating it as a target to treat neuropathic pain.
Background and Aims: Neuropathic pain following peripheral nerve injury is associated with hyperexcitability in damaged myelinated sensory axons, which begins to normalize over time. We have previously shown that following nerve injury axonal Kv1 channels switch the expression of its α-subunits and are re-located invading the paranode. These coincide with a marked reduction in spontaneous activity recorded in the spinal nerves and with reduced pain like behaviors. Blocking Kv1 with αDTX reinstates hyperexcitability (eLife 2016). The mechanisms involved in the changes in expression and re-distribution of Kv1 are unknown, and we are currently studying whether they depend on the myelinating cell enwrapping the axon.

Aim. To determine the differential contribution of CNS and PNS myelinating cells in Kv1 redistribution. We compared changes occurring in the peripheral nerve (enwrapped by Schwann cells) with that of central axons (enwrapped by oligodendrocytes).

Methods: We used the rat neuroma model and injected CTB-Alexa555 into the axotomized nerve to trace injured axons. Using IHC and WB we compared nodal structures and Kv1 expression in injured axons in the PNS (sciatic nerve) and in the CNS (dorsal columns).

Results: Preliminary results show that CTB injected at the sciatic nerve transection is transported up to the dorsal columns and allows the detection of axotomized neurons in the dorsal horn. We are currently investigating the integrity of nodal structures and the pattern of Kv1 expression.

Conclusions:
Following nerve injury Kv1 channels act as a brake in hypersensitivity. We are dissecting the mechanisms behind the changes in expression that lead to the dampening of NeuP.

**BASIC SCIENCE (ANIMALS): PHYSIOLOGY - PART 2**

**NEUP7-0214**

**A MOUSE MODEL OF MOTOR AND SENSORY NERVE EXCITABILITY TO IMPROVE TRANSLATIONAL UTILITY**

*P. Makker¹, G. Moalem-Taylor¹, J. Howells²*

¹University of New South Wales, Medicine, Sydney, Australia
²University of Sydney, Brain and Mind Centre, Sydney, Australia

**Background and Aims:**

Non-invasive nerve excitability measurements have contributed to our understanding of axonal membrane changes in peripheral neuropathies. These techniques have been adapted from humans to *in vivo* rat models. However, nerve excitability studies in mice, which are increasingly used as models in pain research, are lacking. We therefore aimed to develop a model of motor and sensory nerve excitability in the mouse caudal nerve using threshold tracking.

**Methods:**

Male and female C57BL6/J mice, aged between 16-20 weeks, were anesthetised using 2% isofluorane. Electrical stimuli were applied via disposable ring electrodes to the proximal caudal nerve *in vivo* and orthodromic compound muscle action potentials (CMAPs) and antidromic sensory nerve action potentials (SNAPs) were recorded from needle electrodes in the distal tail. Multiple excitability parameters were recorded using QtracS threshold tracking software.

**Results:**

The excitability waveforms and parameters for mouse caudal nerve motor axons are comparable to previously published data. The waveforms for mouse sensory axons are qualitatively similar to those from rat and human sensory axons; however mouse sensory axons have significantly reduced superexcitability and shorter relative refractory period compared to human sensory axons, perhaps due to lower Na⁺ conductance.

**Conclusions:**

In this study, we have developed a novel technique to record the excitability of sensory axons antidromically in the mouse tail *in vivo*, using the same site of stimulation as for motor recordings. This will enable direct comparisons between sensory and motor axons and the use of clinically translatable models of peripheral neuropathies in mice.
THE ROLE OF CASPR2 IN REGULATING PAIN-RELATED HYPERSENSITIVITY AND SENSORY NEURON EXCITABILITY

S. Middleton¹, J. Dawes¹, G. Weir², J. Walcher², J. Kuehnemund², G.R. Lewin², D.L.H. Bennett¹

¹University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom
²Max Delbrück Centre for Molecular Medicine, Department of Neurosciences, Berlin, Germany

Background and Aims:

Contactin associated protein 2 (CASPR2) is known to associate with shaker type potassium channels; Kv1.1 and Kv1.2 which are both known to regulate sensory neuron excitability. Here we aim to elucidate the role of CASPR2 in pain and sensory function by phenotyping CASPR2 KO mice.

Methods:

CASPR2 expression in DRG and juxtaparanodes was confirmed using in situ hybridisation and immunohistochemistry. Pain-related behaviours were assessed using the 50% withdrawal von Frey assay, or by chemical application. Both patch clamp analysis and primary afferent recordings were used to characterise excitability measures in DRG neurons and the tibial skin-nerve preparation respectively.

Results:

In wild type mice CASPR2 expression was confirmed in both DRG neurons and juxtaparanodes. CASPR2 expression was absent in CASPR2 KO mice. Behavioural von Frey assays saw CASPR2 KO mice are hypersensitive to mechanical stimuli (WT 0.62±0.03g vs KO 0.43±0.04g, p<0.01), capsaicin and formalin. CASPR2 KO patch clamp analysis revealed that medium sized DRG neurons have a significantly reduced Reobase (WT 690pA vs KO 320pA, p<0.05) and increased firing frequency to graded current injections. Using the ex vivo skin nerve preparation, which involved detailed analysis of stimulus response measures, AP adaptation and sensory ending spontaneous activity, CASPR2 KO mice displayed hypersensitivity hallmarks.

Conclusions:

Phenotyping CASPR2 KO mice has revealed multiple increases in excitability measures, both in electrophysiology and behavioural assays. Here we have implicated CASPR2 as having a crucial role in pain-related hypersensitivity and sensory neuron excitability likely through the modulation of Kv1.1 and Kv1.2.
Background and Aims:

C-fibers express transient receptor potential (TRP) channels and can be activated by capsaicin. The TRP vanilloid (V) 1 receptor became of interest when its influence on heat pain thresholds was elicited and findings suggested that TRPV1 plays a pivotal role in neuropathic pain syndromes. Previously, altered somatosensory function in patients with neuropathic pain carrying the TRPV1 variant 1911A>G (I585V, rs8068080) compared to healthy controls was demonstrated. However, the study did not confirm TRPV1 as a pain susceptibility gene. Therefore, we hypothesized that in healthy volunteers the TRPV1 variant 1911A>G may influence physiological channel function (e.g. mechanical/thermal sensitivity and superficial skin perfusion) during induction of pain using an experimental pain model.

Methods:

The genomic DNA of 25 healthy volunteers (6 males, 19 females, mean age 23.8 ± 2.0, range 22-29 years) was investigated. Before and after topical application of capsaicin (0.6%), skin perfusion measurements (PeriCam-PSI System) and quantitative sensory testing (QST) were performed in the capsaicin-treated area. Intra-individual changes of measurements (prior vs. after capsaicin) as well as group differences were calculated.

Results:

Genotyping of TRPV1 defined 9 homozygous wild type (WT), 8 heterozygous AG (Het) and 8 homozygous GG (Hom)-carriers.

Comparison of groups upon QST revealed lower changes (1)towards loss of warm-detection in Hom (13.1%) compared to WT (92.6%) (p=0.006) and (2)towards gain of heat-pain sensitivity in Hom (20.3%) compared to WT/Het (29.6%) after adjustment for perfusion measurements (p=0.021).

Conclusions:

The mutant genotype (Hom) demonstrated less hypoesthesia in warm-detection and more sensitivity to heat pain suggesting an altered channel function under painful conditions.
BASIC SCIENCE (HUMAN): GENETICS

NEUP7-0268
A SYSTEMATIC REVIEW OF GENETIC RISK FACTORS FOR NEUROPATHIC PAIN
A. Veluchamy¹, H.L. Hébert¹, W. Meng¹, C.N.A. Palmer², B.H. Smith¹
¹University of Dundee, Division of Population Health Sciences- School of Medicine, Dundee, United Kingdom
²University of Dundee, Division of Molecular & Clinical Medicine- School of Medicine, Dundee, United Kingdom

Background and Aims:

Neuropathic pain (NeuP) is an increasingly common chronic pain state and a major health burden, affecting approximately 7-8% of the general population and 26% of individuals with diabetes in the UK. Genetic, environmental and lifestyle factors are known to contribute to the development of NeuP. The aim of this study was to perform a literature-based systematic review and summarise the genetic studies that investigate the influence of genetic factors for NeuP.

Methods:

We conducted a comprehensive literature search limited to human studies using electronic databases including PubMed, MEDLINE, EMBASE, SCOPUS and Web of Science from January 1996 to July 2016.

Results:

We reviewed twenty-seven full-text articles including candidate gene association (n=21), molecular genetic (n=4), and genome-wide association studies (GWAS) (n=2). The present review provided current knowledge of genetic variants in or near twenty-six candidate genes associated with different NeuP conditions mainly post-surgical pain, and diabetic neuropathic pain. The results suggest that even though association studies have reported several genetic markers involved in the risk of developing NeuP, only very few genetic markers (HLA-A, HLA-B, HLA-DRB1, COMT, IL, and OPRM1) were confirmed either in different NeuP conditions or in the same population. Meta-analysis was not performed due to case heterogeneity. NeuP associated candidate genes are mainly involved in neurotransmission signal, immune response and iron metabolism.

Conclusions:

Our review highlights the need for the GWAS with consensus NeuP case definition, larger sample sizes as well as the replication cohorts to validate reported associations. This will aid in elucidating the genetic architecture underpinning NeuP.
EFFECT OF AUTONOMIC TRAINING ON PAIN ALLEVIATION IN BMS PATIENTS: A PRELIMINARY STUDY

K. Watanabe, N. Noma, M. Shinoda, K. Iwata, Y. Imamura

1 Nihon University School of Dentistry, Department of Oral Diagnostic Sciences, Tokyo, Japan
2 Nihon University School of Dentistry, Department of Physiology, Tokyo, Japan

Background and Aims:

Imaging studies have reported that pain in burning mouth syndrome (BMS) is associated with impaired pain modulation system in the brain, and some studies have documented that psychological therapies alleviate chronic pain without clear evidence of improvement of depression and anxiety. We conducted a study whether autogenic training (AT) alleviates chronic pain in BMS patients and discussed the mechanism of the pain modulation.

Methods:

13 BMS patients and 9 healthy controls participated in this study. All BMS patients satisfied diagnostic criteria of ICHD-3 beta classification and possible systemic and local conditions that mimic oral manifestations seen in BMS patients were excluded. All BMS patients and controls performed AT under instruction. Before and right after the AT, participants provided answers to inventories including VAS (pain intensity), POMS, STAI and SDS (psychological background) and were collected blood samples to investigate immune and endocrine parameters.

Results:

AT induced a significant reduction in VAS right after the procedure, although no psychological profiles showed significant changes. Immune and endocrine parameters showed a significant decrease in serum levels of adrenaline, noradrenaline and NK activity.

Conclusions:

These results suggest that AT facilitated pain inhibition with suppressing sympatho-adreno-medullary (SAM) axis and innate immune function, but without improving anxiety and depression. Sample size should be fulfilled, although pain modulation in BMS might be associated with a partially different mechanism from that of psychological distress.
BASIC SCIENCE (HUMAN): HUMAN EXPERIMENTAL MODELS - PART 1

NEUP7-0084
WHAT THE “FIELD” IS ALL THE NOCICEPTION ABOUT? IN-VIVO ELECTROPHYSIOLOGICAL RECORDINGS FROM FAST-CONDUCTING LOW- AND HIGH-THRESHOLD MECHANORECEPTORS IN HUMANS
S. Nagi¹, A. Marshall², A. Makdani², E. Jarocka³, W. Jung¹, M. Trulsson⁴, F. O’Neill², F. McGlone², H. Olausson¹
¹Linköping University, Center for Social and Affective Neuroscience, Linköping, Sweden
²Liverpool John Moores University, School of Natural Sciences and Psychology, Liverpool, United Kingdom
³Umeå University, Department of Integrative Medical Biology, Umeå, Sweden
⁴Karolinska Institutet, Department of Dental Medicine, Stockholm, Sweden

Background and Aims:
Recent findings on circumferential endings in mouse hairy skin (Bai et al. 2015, Cell) have shown that Aβ-field-LTMRs, a class of fast-conducting low-threshold mechanoreceptors (LTMRs), “exhibit hallmarks of myelinated nociceptors”. In the current study, we tested whether human Aβ-field-LTMRs display nociceptor-like properties.

Methods:
We performed single-unit axonal recordings (microneurography) from the peroneal nerve of awake healthy participants. We also investigated the sensations evoked by microstimulation of single afferents.

Results:
We recorded from 40 Aβ-field-LTMRs in the dorsal foot region (amongst other LTMR subtypes). We also found a class of A mechano-nociceptors (AMs) with conduction velocities similar to Aβ-field-LTMRs. Contrary to findings in mice, the monofilament thresholds of human field-units were indistinguishable from other LTMR subtypes, and their responsiveness to increasing force indentations was discordant with the psychophysical reports of pain intensity. Using intra-neural micro-stimulation, a painful percept was never reported for field-units (with ‘buzzing’ as the most frequently chosen descriptor). The spike activity of field-units increased with faster brushing, corresponding with psychophysical reports of increasing touch intensity. Conversely, the AMs did not respond to soft brushing (hence not an LTMR), displayed high mechanical threshold and encoded force in the perceptibly noxious range. In a large-fibre deafferented patient with preserved Aδ function, psychophysical testing revealed aberrant processing of noxious punctate stimuli.

Conclusions:
We demonstrate that human Aβ-field-LTMRs likely contribute to the discriminative aspects of touch akin to other Aβ-LTMR subtypes. We also demonstrate, for the first time, a distinct type of fast-conducting (Aβ range) mechano-nociceptors in human skin which likely contribute to pain processing.
Background and Aims:

Sensory input is conveyed to the CNS by DRG primary afferents synapsing onto spinal neurons of the dorsal horn. This first synapse in the somatosensory system is crucial for the processing of both noxious and non-noxious stimuli.

Methods:

Here we describe the differentiation of induced pluripotent stem cells (iPSC) into sensory neurons that are cultured in microfluidic chambers to fluidically isolate the neurite from the soma. Rodent dorsal horn neurons are seeded onto the human neurites which subsequently form synaptic connections. We utilise the sensitive, genetically encoded calcium indicator – GcAMP6, which is transduced into the dorsal horn neurons to record post-synaptic calcium responses in the second order neurons.

Results:

Human sensory neurons extend neurites into the dorsal horn compartment and form synaptic connections with rodent dorsal horn neurons. Upon stimulation of the human sensory neurons with KCl, ATP or acid, we are able to measure robust calcium influxes in the connected dorsal horn neuron. The post-synaptic response can be modulated pharmacologically by applying glutamate receptor antagonists and sodium channel blockers. We demonstrate the ability to image the activity-dependent vesicle cycling in the synaptic terminal using the FM 1-43 membrane probe.

Conclusions:

We will develop this model further by using human iPSC-derived neurons carrying loss and gain of function SCN9A mutations, with the aim to characterise the effect these mutations have on synaptic transmission. This model allows for the first time the analysis of stimulus-evoked signal transmission in human sensory neurons in vitro, and the effect mutations may have on the post-synaptic response.
Background and Aims:

The spatial alternation of innocuous cold and warm stimuli on the skin can paradoxically provoke a hot, burning sensation, known as the thermal grill illusion (TGI). The spinal and supra-spinal mechanisms underpinning this phenomenon are still elusive.

Methods:

To assess whether spinal mechanisms contribute to the TGI, we leveraged anatomical knowledge on the spatial arrangement of dermatomes and spinal segmental projections. Specifically, we stimulated a series of skin locations on the right arm using one cold (~20°C) and one warm thermode (~40°C). The two stimulus locations had identical physical distance on the skin. However, the distance between the cold and heat projection signals in the spinal cord varied across conditions. In two experiments, 32 participants completed a temperature matching task; i.e., they matched the perceived TGI temperature using another thermal probe on the left arm, after each stimulation trial.

Results:

Participants displayed the classic TGI, overestimating the actual cold temperature on the skin. However, this effect was significantly larger when cold and heat stimuli were delivered within the same dermatome (e.g., T1-T1, C5-C5; overestimation = 9.88±5.60°C) or between dermatomes projecting to adjacent spinal segments (e.g., T1-T2; C5-C6; overestimation = 9.48±5.83°C), with respect to when cold and heat stimuli projected to non-adjacent spinal segments (e.g., T1-C5; T2-C6; overestimation = 4.80±3.21°C).

Conclusions:

We demonstrated that the strength of the illusion is modulated by the segmental distance between cold and heat spinal signals. Our findings show that the perceived quality and intensity of thermal stimuli is influenced by low-level spatial summation mechanisms in the spinal cord.
Background and Aims:

A key issue in understanding neuropathic pain is identification of mechanisms involved in hyper-excitability and pain maintenance and to distinguish molecules that modulate the excitability of pain-conducting sensory neurons. Differences in sensory processing between human and model organisms may, however, lead to poor translation between animal studies and the clinical setting. One solution is to use cultured human DRG neurons and manual patch clamp. While the patch clamp technique provides unparalleled insight into the excitability of native tissue, it is extremely low-throughput and costly. Here, we aimed to identify an alternative in vitro approach with similar translational predictability by utilizing human iPSC-derived sensory neurons and a physiological stimulus in a high capacity format to detect changes in neuronal excitability.

Methods:

Using human hiPSC-derived sensory neurons as model system, we applied electrical field stimulation (EFS) in combination with calcium imaging to enable detection of excitability changes in neurons in a plate-based format using the Cellaxess® Elektra platform.

Results:

We analyzed the activity of clinically used drugs (e.g. gabapentin and naproxen) as well as novel sodium channel inhibitors. We found a good agreement between inhibition of excitability as measured by EFS in human iPSC-derived sensory neurons and by conventional methods in human DRG neurons.

Conclusions:

The results strongly suggest that the Cellaxess Elektra platform provides a dramatically higher-throughput means of quantifying the excitability of human iPSC-derived sensory neurons. This enables identification and prioritization of molecules that are likely to also modulate the excitability of native tissue earlier on in the drug development process.
Background and Aims:

Phase-locked local field potentials (LFPs) elicited in the human insula by transient thermonociceptive stimuli have been described extensively (Frot et al., *Hum Brain Mapp*. 2014). However, these LFPs primarily reflect multimodal activity unspecific for pain (Liberati et al., *PLOS Biol* 2016). The present study aims at identifying nociception-related insular activity using sustained periodic activation of heat-sensitive thermonociceptors (Colon et al., *NeuroImage* 2016).

Methods:

We recorded intracerebral electroencephalography (iEEG) in two patients undergoing a presurgical evaluation of intractable epilepsy, from a total of 15 insular electrode contacts (Fig. 1). Very slow (0.2 Hz) and long-lasting (75 s) thermonociceptive (50°C) and non-nociceptive vibrotactile stimuli were delivered in separate blocks, on the hand contralateral to the insular electrode.

Results: Both thermonociceptive and non-nociceptive vibrotactile stimuli elicited a clear 0.2 Hz periodic EEG response (Fig. 2). For both modalities, this response was maximal at contacts in the posterior insula. A Hilbert spectral analysis revealed that both stimuli also elicited a periodic 0.2 Hz modulation of theta, alpha, beta, and gamma activities. In the posterior insula, the theta band modulation induced by thermonociceptive stimulation was greater than the modulation induced by vibrotactile stimulation (Fig. 3).
Conclusions: These preliminary findings suggest that the slow periodic activation of thermonociceptors can be used to reliably identify – at single-subject level – insular activity related to the sustained activation of thermonociceptors, and that some features of this activity may differentiate it from the activity elicited by sustained vibrotactile stimulation.
Background and Aims:

High concentration capsaicin patches for 1 h are extensively used to treat neuropathic pain of peripheral origin. Sustained application (>24 hrs) resulted in complete abolition of heat sensitivity in healthy volunteers with little impact on mechanical pain sensitivity and a complete suppression of the capacity to induce hyperalgesia (Henrich et al. BRAIN 2015). The interaction of hyperalgesia and hypoalgesia following acute 1 h application, however, is hitherto not fully elucidated.

Methods:

In an investigator-initiated trial high concentration capsaicin patches (Qutenza®, 35 x 35 mm) were attached to the skin of the volar forearm in human volunteers for 60 min. A complete somatosensory profile was obtained by quantitative sensory testing in capsaicin-treated (primary, n=36) and adjacent skin (secondary, n=28) immediately after patch removal and followed for 24 and 48 hrs (n=24).

Results:

After 1 hour of application substantial heat hyperalgesia developed with a heat pain threshold of 35.4±1.9°C vs. 43.0±3.5°C in normal skin (p<<0.0001). In contrast, cold pain thresholds dropped from 16.5±9.2°C to 8.5±8.4°C (p<<0.0001). Cold (3.8 vs. 1.4°C, p<<0.001) and warm (2.5 vs. 1.9°C, p<0.005) detection thresholds increased significantly. Pain sensitivity to pinprick doubled, and dynamic mechanical allodynia developed in primary and - to a similar magnitude - in secondary areas. Notably, all somatosensory changes had completely resolved at 24 and 48 hrs.

Conclusions:

In aggregate, primary heat hyperalgesia was accompanied by primary cold hypoalgesia and thermal hyposensitivity. Pinprick hyperalgesia and dynamic mechanical allodynia revealed central sensitization. However, normal somatosensory profiles after 24 hours revealed that all changes were only temporary.
Background and Aims:

Reduced spatial tactile resolution is a typical clinical finding in CRPS patients. In addition, a reduced representation area of the affected hand in S1 is a consistent finding for CRPS. Associations between S1 hand representation size, clinical deficits in CRPS-patients are suspected, but studies using high resolution fMRI are rare.

Methods:

In 13 CRPS-patients and unilateral affection of the upper limb, the thumb (D1) and pinky (D5) representation in BA3b of S1 was determined. The surface distance between D1/D5 was used as a measure for the size of the hand representation. In order to investigate the impact of the hand representation size on behavior measures, associations with pain intensity (VAS: 4.0±2.5), pinch grip performance, and two-point-discrimination (D1) as a measure for the spatial tactile resolution were analyzed.

Results:

The affected and non-affected hand differed in motor function (p=.036) and tactile resolution (p=.021). Patients with higher D1-D5 distance showed better two-point-discrimination (r=-.62). Our optimized evaluation revealed no difference between D1-D5 distances of the affected and non-affected side. Without quality optimization the non-affected hand (20.9mm) was larger than the affected hand (16.2mm) and this difference was highly significant (p=.001). However, the non-optimized distances showed no associations to the behavioral measures.

Conclusions:

Our data on an association of D1-D5 S1-distance with spatial tactile resolution are in good agreement with other studies. The representation size of non-affected and affected hand did not differ significantly in our small sample for the more advanced mapping procedure. Evaluations on a larger number of patients will be presented at the congress.
Background and Aims:

Evidence suggests that the development and maintenance of chronic pain partly relies on alterations within the descending pain modulation network. The objective of the present study was to identify whether patients with painful trigeminal neuropathy (PTN) display altered resting interactions between brainstem regions that are involved in the descending modulation of pain. Furthermore, we assessed the resting interactions between this system and higher pain processing regions within the cerebrum.

Methods:

A resting-state fMRI scan was performed on a group of patients with PTN (n=27, mean age = 46.76 years) and a group of healthy controls (n=50, mean age = 41.53 years). To restrict analysis to the brainstem, each subject’s fMRI image set was resliced using a brainstem-only template within the SUIT toolbox in SPM12. Using REST software, the nucleus raphe magnus was selected as a “seed” region in a functional connectivity analysis between patients and controls (p<0.05). Following this, a significant cluster within the periaqueductal gray (PAG) was selected as a seed region for a further wholebrain functional connectivity analysis (p<0.05).

Results:

Compared to controls, PTN patients display stronger functional connectivity between the nucleus raphe magnus and spinal trigeminal nucleus as well as the midbrain PAG. Furthermore, the PTN patients show increased connectivity between the PAG and insula and bilateral primary somatosensory cortices.

Conclusions:

These data reveal that neuropathic pain is associated with alterations in resting activity within the endogenous modulation network. Such alterations possibly underlie the maintenance of pain in these patients.
Background and Aims:

Using intracerebral electroencephalography, we recently observed that painful heat stimuli, but not non-painful tactile, auditory, and visual stimuli, elicit an enhancement of gamma-band oscillations (GBOs) in the human insula (Liberati et al., under review). This enhancement could be due to different features of the stimuli, such as (i) their painful quality, (ii) the fact that they activate the spinothalamic system, or (iii) the fact that they convey thermal information. To disentangle these different aspects, we examined whether insular GBOs would be elicited by non-painful cool stimuli and non-thermal but painful mechanical pinprick stimuli.

Methods:

Intracerebral EEG was recorded in three patients undergoing a presurgical evaluation of intractable epilepsy (24 insular contacts). Four types of short-lasting stimuli were delivered on the contralateral hand dorsum: (i) painful 62.5°C laser stimuli activating heat-sensitive nociceptors; (ii) mechanical pinprick stimuli activating mechano-sensitive nociceptors; (iii) innocuous cool stimuli activating cool-sensitive free nerve endings; and (iv) innocuous vibrotactile stimuli activating mechanoreceptors of the medial lemniscus system.

Results:

In all patients, all stimuli elicited clear low-frequency phase-locked local field potential (LFPs). In contrast, laser heat stimuli, mechanical pinprick stimuli, and cool stimuli – but not vibrotactile stimuli – elicited an early latency (150-300 ms) enhancement of GBOs (40-90 Hz) similar to what was observed following laser stimulation in our previous study.

Conclusions:

These preliminary findings suggest that stimuli activating the spinothalamic system elicit a consistent enhancement of GBOs in the human insula, regardless of whether they convey thermal sensations or generate pain.
INFRA-SLOW OSCILLATIONS IN THE DEFAULT MODE NETWORK IN DIFFERENT PAIN STATES

Z. Alshelh1, F. Di Pietro1, E. Mills1, R. Vickers1, R. Akhter2, C. Peck2, G. Murray2, L. Henderson1
1University of Sydney, Anatomy and Histology, University of Sydney, Australia
2University of Sydney, Faculty of Dentistry, University of Sydney, Australia

Background and Aims:

The default mode network has been shown to be modulated by the presence of chronic pain conditions however the extent of these changes remain unknown. In our previous investigation we identify infra-slow oscillations (ISO) in neural activity as a marker of chronic neuropathic pain (NP) and we found a decrease in ISOs in areas encompassing the default mode network (DMN). Therefore, the aim of this study was to explore ISOs using functional magnetic resonance imaging (fMRI) in the DMN in NP, chronic orofacial nonNP and acute pain states.

Methods:

Twenty-seven subjects with NP, 13 subjects with nonNP and 58 pain-free controls were recruited. In 15 of the 58 controls, hypertonic saline was placed into the masseter muscle to induce pain. Resting brain activity was measured using fMRI in all subjects. A voxel-by-voxel analysis was used to measure ISOs in all groups, a two-sample t-test was used to determine differences between pain groups and controls and a DMN mask was used to confine the analysis.

Results:

Significant decreases in ISOs occurred in regions of the DMN in subjects with NP and during acute pain. These decreases occurred in the precuneus and prefrontal cortex. In striking contrast, there were no significant changes in ISOs in individuals with nonNP.

Conclusions:

The decreases in ISOs in the default mode network is not limited to NP pain states or chronic pain states and suggests a reorganization of the DMN in NP and acute states possibly causing an increase in difficulty to focus on tasks.
Background and Aims:

Although the pathophysiology of BMS is not clearly understood, central and peripheral neuropathic mechanisms are thought to be involved. We investigated the temporal and spatial responses of the BMS brain to the noxious heat stimuli.

Methods:

16 right-handed women with primary BMS and 15 sex- and age-matched right-handed healthy female controls. A thermal stimulus sequence of 32°C to 40°C to 32°C to 49°C was repeated four times in a cycle. Warm and noxious heat stimuli were delivered with a Peltier thermode placed on the right palm or right lower lip for 32 sec each in a session. Functional magnetic resonance imaging data were obtained by recording echoplanar images with a block design. Statistical Parametric Mapping 8 software was used to analyze the data.

Results:

Comparison of brain activity in the early 16 sec and late 16 sec of the stimulus revealed pronounced temporal summation in BMS patients during lip stimulation. Repetition of noxious heat stimulus on the lower lip but not on the palm induced habituation in brain activity in the cingulate cortex without reduction in pain perception. Multiple regression analysis revealed a correlation between perceived pain intensity and suppression of brain activity in the anterior cingulate cortex when the repeated thermal sequence was applied at the lower lip. Furthermore, the response of the parahippocampal area differed in BMS patients and controls when the same repeated thermal sequence was applied at the palm.

Conclusions:

BMS patients show specific brain responses due to impaired function of both the central and peripheral nervous systems.
Background and Aims:

Patients with chronic whiplash associated disorders (CWAD) are characterized by pain of traumatic origin, cognitive deficits and central sensitization (CS). Previous studies revealed altered grey matter volume (GMV) in mild traumatic brain injury patients and chronic pain conditions also characterized by CS. It can therefore be hypothesized that GMV alterations also play a role in the persistent complaints of CWAD. This study examined regional GMV alterations in CWAD compared to chronic idiopathic neck pain patients (CINP) and healthy controls. Additionally, in both patient groups relations between regional GMV, and measures of cognition, and pain processing were assessed.

Methods:

Ninety-three women (28 controls, 34 CINP, 31 CWAD) were enrolled. First, T1-weighted Magnetic Resonance Images were acquired to examine GMV alterations in brain regions involved in pain and cognitive processing. Next, cognitive performance, pain cognitions, CS symptoms, and hyperalgesia were assessed.

Results:

Regional GMV of the right lateral orbitofrontal cortex, left supramarginal cortex, and left posterior cingulate cortex was decreased in CWAD compared to controls ($p=0.023$; $p=0.012$; $p=0.047$). Additionally, GMV of the right superior parietal cortex and left posterior cingulate cortex was decreased in CWAD compared to CINP ($p=0.008$; $p=0.035$) (Fig. 1).
Decreased regional GMV correlated with worse cognitive performance, higher maladaptive pain cognitions, CS symptoms, and hyperalgesia in CWAD ($r_s = -0.515$ to $-0.657$; $p<0.01$). Furthermore, in CINP, decreased regional GMV only correlated with worse cognitive performance ($r_s = -0.499$ to $-0.619$; $p<0.01$).

**Conclusions:**

These results provide the first evidence for reduced GMV in cortical regions involved in processing of cognition and pain in CWAD patients.
Background and Aims:

Animal models suggest that chemokines are important mediators in the pathophysiology of neuropathic pain. Indeed, these substances have been called “gliotransmitters”, a term that illustrates the postulated close interplay between glial cells and neurons in the context of chronic pain. However, evidence in humans is scarce. Our aim was to apply a new panel measuring 92 inflammation-related proteins to CSF from patients with peripheral neuropathic pain. Our hypothesis was that we would be able to determine a CSF inflammatory profile for peripheral neuropathic pain, and that we thereby would mirror a postulated process of central neuroinflammation.

Methods:

Cerebrospinal fluid was collected from patients with posttraumatic/postsurgical neuropathic pain (n=11) and healthy controls (n=11). Ninety-two proteins were simultaneously analysed with a multiplex proximity extension assay panel (Proseek® Multiplex Inflammation I, Olink Bioscience, Uppsala, Sweden). Linear discriminant analysis with false discovery rate, as well as orthogonal partial least squares – discriminant analysis, were used for statistical analyses.

Results:

Taking the correlation structure of the data set into consideration, we found that chemokines CXCL1, CXCL5, CXCL6, CXCL10, CCL3, CCL8, CCL11, CCL19, CCL23, LAPTGFb1 and LIF-R were significantly associated with neuropathic pain.

Conclusions:

In a small sample of patients and healthy controls, we have determined the CSF inflammatory profile of patients with severe peripheral neuropathic pain. The same panel has recently been used for serum profiling of patients with painful radicular pain (Moen et al 2016), but this is the first time such an extensive inflammatory fingerprint has been described for the CSF of neuropathic pain patients.
Background and Aims:

Clinicians and scientists aiming to treat and understand chronic pain patient pathophysiology would benefit from molecular tools to measure biological processes underlying and accompanying persistent pain conditions. Our study aimed to find proteins associated with two such conditions, neuropathic pain and fibromyalgia, in cerebrospinal fluid (CSF) samples from patients.

Methods:

We have performed protein profiling of 57 proteins in CSF using an affinity proteomics approach. CSF samples were collected from 25 patients with neuropathic pain and 40 patients with fibromyalgia, and were compared to CSF samples from 135 controls without neurological disease.

Results:

We found two proteins with significant increases in CSF levels between patients and controls (p<0.05). One protein was found to be increased in the CSF of neuropathic pain patients compared to controls. Another one was increased in the CSF of fibromyalgia patients compared to all other groups.

Conclusions:

Our results suggest that these two proteins can be used to distinguish CSF from neuropathic pain patients and CSF from fibromyalgia patients from CSF from people without pain disorders. If validation in additional sample cohorts confirms these findings, it supports a role for these two proteins in the pathophysiology of neuropathic pain and in fibromyalgia respectively.
Background and Aims:

Fibromyalgia syndrome (FMS) is a frequent and disabling pain disorder. Patients describe their pain similar to neuropathic pain characteristics. Previous studies point to impaired sympathetic activity and reduced muscle-oxygenation as mechanism at play in this disease. We analyzed the blood flow in muscle and skin of patients and healthy controls (HC) during venous stasis, arterial ischemia and tilt table testing.

Methods:

Using a combination of laser Doppler flowmetry and remission spectroscopy we examined 20 FMS patients and 10 HC. During tilt table testing, venous stasis and arterial ischemia blood flow in skin and muscle, heart rate and blood pressure were measured.

Results:

In the first phase of orthostatic challenge patients demonstrated a significant increase in blood pressure and heart rate in comparison to HC. Blood flow in skin and muscle did not differ significantly during venous stasis, arterial ischemia and orthostatic challenge between patients and HC. Significant differences could be seen in the blood flow during the recovery phase after venous stasis.

Conclusions:

The observed increase in systolic blood pressure and heart rate in patients after orthostatic challenge support the hypothesis of an increased activity of the sympathetic efferent system in FMS patients. No differences in muscular blood flow between patients and HC could be detected during the actual interventions, but the differences in muscular blood flow in the venous recovery phase might point to different regulation patterns in patients and HC.
Background and Aims:

Peripheral neuropathy is the most common complication of diabetes, involving sensorial and motor nerves. Besides pain (alldynia and hyperalgesia), this condition could be associated with a decline in motor compound action potential with alterations in foot positioning and plantar pressure during gait. The aim of this study was identify motor alterations in Streptozotocin (STZ)-induced diabetic neuropathic rats and correlate it with mechanical withdrawal thresholds.

Methods:

All experiments were approved by UNICAMP Ethic’s Commitee (CEUA 3902-1). Male Lewis rats (LEW/HsdUnib) (200-250 g; 6-8-weeks-old) received a STZ-low dose (25 mg/kg) or 0.1 M sodium citrate buffer (SCB, control group) once a day during five days. Diabetic animals (250 mg/dL blood glucose) were submitted to electronic von Frey and CatWalk tests (Noldus Inc., the Netherlands) at 0, 7, 14, 21 and 28 days after STZ injections.

Results:

STZ (25 mg/kg) but not SCB induced diabetes. After the 14th day (STZ)-induced diabetic rats showed a mechanical hyperalgesia and a reduction in the hindlimbs Footprint Intensities, respectively analyzed by eletronic von Frey and CatWalk tests (p<0.05; Two-Way ANOVA followed by Bonferroni posttest). At 28th day the animals showed alterations in Spatial parameters (Maximum Contact Area; Stride Length; Print Area). These parameters were strongly correlated with mechanical withdrawal thresholds, according to Pearson’s Correlation Coefficients (pcc 0.99; p<0.001).

Conclusions:

This is the first work that characterizes with edge technology the motor alterations in STZ-induced diabetic neuropathic rats. Thus, the CatWalk gait parameters can be used as a coadjuvant tool to investigate the development of hyperalgesia in STZ-induced diabetic rats.
NEUP7-0273
INFLUENCE OF HABITUATION ON PAIN INTENSITY AND PAINFUL CUTANEOUS ELECTRICAL STIMULATION IN COMPARISON TO CONDITIONED PAIN MODULATION
L. Eitner, Ö. Özgül, E. Enax-Krumova, J. Vollert, O. Höffken, C. Maier
1Berufsgenossenschaftliches Universitätsklinikum Bergmannsheil GmbH, Department of Pain Medicine, Bochum, Germany
2Berufsgenossenschaftliches Universitätsklinikum Bergmannsheil GmbH, Department of Neurology, Bochum, Germany

Background and Aims:

Painful cutaneous electrical stimulation (PCES) and corresponding evoked potentials can be used to analyze conditioned pain modulation (CPM) [1]. However, it is unknown whether the pain relief during the conditioning test stimulus results from habituation to the long-lasting PCES. We compared the effects of CPM and habituation on relief of PCES-induced pain and changes of PCES-evoked potentials and analyzed whether increased attention by a random change of electric intensities amplifies the habituation effects.

Methods:

According to a previous study using three custom-built concentric surface electrodes, we applied electrical stimuli on the dominant hand, inducing pain of 40-60 on NRS 0-100 [1]. We assessed PCES-induced pain intensity and PCES-evoked potentials over Cz in 29 healthy subjects (f:16, age 20-35). Before CPM, all subjects received 14-minutes of electrical stimulation with consistent intensity followed by 14-minutes of PCES with variable intensities (group A), group B vice versa. For CPM all subjects received PCES as test stimulus and cold water (10°C) as conditioning stimulus contralaterally [2]. Outcome criteria: change of pain ratings, N1-P1-amplitudes, N1-latencies. Statistics: ANOVA, paired/ unpaired t-tests, p<.05.

Results:

Independently from the sequence, pain intensity (12±17%; p<.01) and N1-P1-amplitudes of PCES-evoked potentials (10±16%; p<.05) decreased significantly during PCES. CPM resulted in a significant stronger decline of pain ratings (36±19%; p<.001) and amplitudes (24±34%; p<.001). Latencies remained unchanged.

Conclusions:

Habituation and CPM reduce pain and evoked potentials simultaneously. However, effects of CPM are significantly stronger and cannot be exclusively explained by habituation effects.

THE EFFECTS OF KANGAROO MOTHER CARE ON THE FUSS AND CRYING TIME OF COLICKY INFANTS

Z. farhadikoutenaei1, Z. Akbarian Rad2, M. Haghshenasmojaver2, Y. Zahedpasha2, M. Ahmadpour2

1Babol University of Medical Sciences, Dentistry, Babol, Iran
2Babol University of Medical Sciences, pediatric, Babol, Iran

Background and Aims:

Infantile colic is a common complaint in the first few weeks of the neonate’s life. There is not a specific therapy for this disease. Various therapeutic options are recommended for reducing the pain and restlessness in the affected infants. Skin-to-skin contact via Kangaroo Mother Care (KMC) is known to increase the pain threshold and it seems to be a proper method for the care of these infants. This study aimed to evaluate the effect of KMC on infantile colic.

Methods:

This case-control study was performed from March 2012 to March 2013. Subjects consisted of 55 exclusively breastfed infants ageing between 15-60 days with excessive fuss and crying who were referred to the Children’s Clinic of Ayatollah Rohani Hospital in Babol, North of Iran. The neonates who weighed less than 2500 grams or had been diagnosed with genetic or clinical disorders were excluded from this study. The studied infants were subjected to KMC for at least 2 hours a day. Standard questionnaires were completed through interviews and Barr Scale was also conducted. The collected data were analyzed by SPSS software V.11.5 and T-test and a P-value of less than 0.05 was considered as significant.

Results:

According to the results of this study, the fuss and crying time of the infants before the KMC was 2.21±1.54 hours per day while it reduced to 1.16±1.3 hours per day after the implementation of KMC (p=0.001).

Conclusions:

KMC could be practiced at home as a simple and safe method of diminishing the fussiness and crying time in colicky infants.
Background and Aims:

Incurable diseases cause a lot of pain and suffering. Due to chronic nature, patients have more psychological problems. Therefore, we cannot limit the cancer treatment to the clinical aspects. The aim of the study was to determine the relationship between duration of using of social media and the degree of pain feeling in cancer patients.

Methods:

This cross-sectional study was performed among cancer patients referring to Shiraz University of medical sciences clinics in 2016. One hundred patients were selected with convenience method. Our investigation tools were the questionnaire included sections assessing demographics to describe the population and the social media experience (regarding usage, preference social media activities, the number of hours spent on social media). The instrument used to measure pain was visual Analog scale.

Results:

The most important findings were:

1- Incidence of pain among cancer patients that using social media was 71% (25.7% had mild pain, 30.5% had moderate pain and 14.8% had severe pain).

2- There was a significant relationship between the severity of pain and the use of social media (p<0.05).

3- 15% of patients with high score of pain, spent less time on social media than lower 15%.

Conclusions:

Based on the results of the study, cancer patients suffered from mild pain was the most users of social media and the patients with high scores of pain, spent less time on social media. The use of social media may also recommend to patients by healthcare providers as a low cost and effective modality to decrease the negative effects of pain.
THE EFFECT OF PROBIOTIC LACTOBACILLUS REUTERI ON REDUCING THE PERIOD OF RESTLESSNESS IN INFANTS WITH COLIC

M. HAGHSHENAS MOJAVERI1, Z. Akbarian Rad1, Y. Zahedpash1, M. Ahmadpour1, K. Hajar1, Y. Taghipour1
1Babol University of Medical Sciences, pediatric, Babol, Iran

Background and Aims:

Abdominal colic during infancy is a common complaint of parents within the first three months after the child's birth. As the intestinal microflora in these infants is different from non-colic newborns, we aimed to determine the effects of probiotics on the balance of intestinal microflora and reducing restlessness in infants with colic.

Methods:

This randomized clinical trial was performed on 44 breastfed infants (20-60 days old), with a birth weight of > 2500 g, suffering from colic, based on Wessel's definition. These infants received 1-5 drops of placebo (label 1) per day for five consecutive days. Afterwards, they were administered 2-5 drops of Lactobacillus reuteri (label2) (17938 DSM) per day for 14 consecutive days. The parents were contacted twice on a daily basis and the duration of cramps and restlessness in infants was recorded and evaluated (IRCT=2014012713489).

Results:

Among 44 infants, 36 cases completed the study. The mean period of restlessness after 5 days of receiving placebo and L. reuteri was 275±142.8 and 172±88.3 min, respectively (p<0.001). Also, after 14 days of L. reuteri administration, the mean period of restlessness was 106±53.67 min.

Conclusions:

The results showed that probiotic L. reuteri reduced crying and restlessness duration in infants with colic.
BEST POSTER SESSION

NEUP7-0233
CHEMOGENETIC DISSECTION OF THE CENTRAL NORADRENERGIC SYSTEM:
DISSOCIATION OF ANALGESIC AND AVERSIVE CIRCUITS
S. Hirschberg¹, Y. Li¹, A. Randall², E.J. Kremer³, A.E. Pickering¹
¹University of Bristol, Physiology- Pharmacology and Neuroscience, Bristol, United Kingdom
²University of Exeter, Medical School, Exeter, United Kingdom
³Institut de Génétique Moléculaire de Montpellier,
Centre Nationnal de la Recherche Scientifique, Montpellier, France

Background and Aims:
The Locus coeruleus (LC) is the principle noradrenergic nucleus in the CNS. Anatomical
studies suggest that the LC has distinct output modules, providing selective innervation of
some target areas. For example, there are discrete populations that innervate the prefrontal
cortex (LC-PFC) and the spinal cord (LC-SC).

We hypothesise that stress-like adverse effects and analgesic effects that are caused by
tonically increased noradrenergic activity are mediated by these distinct subpopulations of
LC neurons and their postsynaptic target cells.

Methods:
Retrogradely transported CAV2 based vectors were developed to express a genetically
"engineered" excitatory ionophore (PSAM) specifically activated by the selective agonist
(PSEM308) under a catecholaminergic neuron specific promoter. Chemogenetic activation
of LC neurons was electrophysiologically verified and subsequently employed to activate LC-
PFC and LC-SC neurons in naïve rats and rats that underwent tibial nerve transection
(TNT).

Results:
Activation of LC-SC neurons (10mg/kg PSEM308) increased thermal withdrawal latency but
had no effect in conditioned place preference (CPP) and open field experiments (N=9). In
contrast, activation of LC-PFC neurons was associated with aversive and anxiety-like
behaviour (N=7) in CPP and open field test without any analgesic effect.

In the TNT model, activating LC-SC neurons significantly reduced mechanical and cold
evoked hypersensitivity and improved incapacitance between the injured and uninjured hind
leg (N=7). The animals also exhibited conditioned place preference for the PSEM308 paired
environment.

Conclusions:
These findings demonstrate that by using a selective therapeutic strategy to activate
pontosspinal noradrenergic neurons it is possible to dissociate analgesic from aversive
noradrenergic circuits and this is effective in a neuropathic pain model.
BEST POSTER SESSION

NEUP7-0093
NEURAL ENSEMBLE ABSTRACTIONS OF NOCICEPTIVE INFORMATION IN THE AMYGDALA DRIVE PAIN AFFECTIVE BEHAVIOR
G. Corder¹, B. Ahanonu², B. Grewé³, M. Schnitzer², G. Scherrer¹
¹Stanford, Anesthesiology- Perioperative and Pain Medicine, Palo Alto, USA
²Stanford, Biology, Palo Alto, USA
³Stanford, Applied Physics, Palo Alto, USA

Background and Aims:

The experience of pain arises from the integration of somatosensory and emotional nociceptive information, which guides the selection of motivationally protective behaviors. However, the computational processing of nociception within emotional neuronal ensemble networks, such as the basolateral amygdala (BLA), is not understood. Furthermore, injury-induced neuroplasticity in the BLA may lead to miscoding of sensory information concomitant with the development of chronic pain. Here, we aim to determine the causal link between neural ensemble activities and their emergent behavioral consequences, along with the evolving co-dynamics due to nerve injury.

Methods:

We utilized recurrent in vivo calcium (Ca²⁺) imaging, chemogenetic manipulation, and trans-synaptic circuit tracing of BLA nociceptive neuronal ensembles prior to and throughout the development of neuropathic pain.

Results:

Multidimensional and population vector analyses of Ca²⁺ spike activity patterns revealed that acute, multimodal nociception (i.e. noxious heat vs. cold vs. mechanical) is innately encoded in a shared ensemble involving hundreds of BLA principal neurons, distinct from general salience and appetitive valence codes. Chemogenetic silencing of this nociceptive ensemble selectively mitigated pain affective-motivational behaviors, with no effect on anxiety, reward, or nociceptive reflex behavior. During a neuropathic state, the representation of normally innocuous stimuli overlaid the nociceptive ensemble, which can be targeted for alleviation of chronic pain affect.

Conclusions:

We have identified unique ensemble representations of abstracted noxious information in the BLA facilitating the innate, negative qualities of the pain experience. Our findings demonstrate how BLA ensemble miscoding assigns negative valence to non-noxious stimuli, which may ground the psychological and motivational dysfunctions of chronic pain.
BEST POSTER SESSION

NEUP7-0241
ENDEOGENOUS OPIOIDS RELEASE INDUCED BY ENVIRONMENTAL ENRICHMENT COMPLETELY ABOLISHES NEUROPATHIC PAIN BEHAVIOR IN RATS WITHOUT CHANGING OPIOID RECEPTORS EXPRESSION
L.F. Kimura¹, M.B.M. Sant’Anna², N.B. Teixeira², V.G.D.M. Mattaraia³, V.O. Zambelli²,
G. Picolo¹
¹Butantan Institute- Institute of Biomedical Sciences - USP,
Special Laboratory of Pain and Signaling- Department of Pharmacology, São Paulo, Brazil
²Butantan Institute, Special Laboratory of Pain and Signaling, São Paulo, Brazil
³Butantan Institute, Vivarium, São Paulo, Brazil

Background and Aims:

Environmental enrichment (EE) can alter the perception of nociceptive stimuli and the analgesic response induced by opioids. The aim of this work was to evaluate the role of animal welfare in pain sensitivity of rats against chronic noxious stimuli and the participation of opioid signaling in this effect.

Methods:

Male Wistar rats were used. Enriched group was already born in an enriched environment. Within 7 weeks of life, under EE condition, chronic constriction injury (CCI) of the sciatic nerve was surgically performed. EE effects in mechanical and thermal hypernociception or tactile allodynia were analyzed before and 7 and 14 days after surgery, using rat paw pressure, Hargreaves and von Frey hair tests, respectively. Naloxone was used to evaluate the involvement of endogenous opioids. After 14 days, animals were euthanized and serum was obtained to quantify endogenous opioid levels using EIA kit. Opioid receptors expression was evaluated by Western Blotting.

Results:

EE completely abolished allodynia, and mechanical and thermal hyperalgesia, after 14 days of CCI. Naloxone treatment reversed analgesic effect of EE. Beta-endorphin and met-enkephalin serum levels were augmented in enriched animals only in the presence of CCI, without changing opioid receptors expression in spinal cord, PAG and DRG.

Conclusions:

EE, without exercise wheel, abolish chronic pain behavior by endogenous opioid pathway activation, demonstrating that welfare per se is able to control chronic pain behavior. This work contributes for the understanding of endogenous mechanisms involved in pain control and in the diversity of responses to different treatments used for patients.

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BEST POSTER SESSION

NEUP7-0305
 USING AN ENGINEERED GLUCL CHANNEL TO SILENCE SENSORY NEURONS AND TREAT NEUROPATHIC PAIN AT THE SOURCE
 G. Weir¹, A. Clark¹, S. Middleton¹, D. Bennett¹
 ¹University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom

Background and Aims:

Following nerve injury sensory neurons develop ectopic activity thought to be critical for the induction and maintenance of peripheral neuropathic pain. Local anaesthetics and anti-epileptic drugs can suppress such hyper-excitability however these drugs are complicated by motor, CNS and cardiac dysfunction. Here we show that a glutamate-gated chloride channel (GluCl) modified to be activated by the non-toxic drug Ivermectin, but not glutamate, is highly effective in silencing sensory neurons and treating neuropathic pain related hypersensitivity.

Methods:

Using sensory neuron cultures we performed detailed in vitro analysis of GluCl efficacy and translated these findings in vivo by delivering AAV-GluCl specifically to mouse dorsal root ganglion. This allowed us to use sensory withdrawal assays to assess GluCl silencing capability in vivo, in both naïve and nerve-injured animals.

Results:

GluCl activation potently inhibited the ability of human and rodent sensory neurons to respond to electrical and algogenic stimuli, in vitro. Intrathecal delivery of AAV9-GluCl generated high levels of sensory neuron transduction (L4 DRG, 66.1 ± 9.6%) that was still evident 7 months later. This enabled reproducible and reversible increases of thermal (+48.4 ± 15.6%, P=0.007) and mechanical (+63.0 ± 20.9%, P=0.015) pain thresholds by Ivermectin, with no motor deficits. Established mechanical pain related hypersensitivity secondary to traumatic nerve injury was reversed by Ivermectin; mirrored at the cellular level with a cessation of ectopic activity.

Conclusions:

These findings emphasise the importance of aberrant afferent input in the maintenance of neuropathic pain and the potential for targeted chemogenetic silencing as a new treatment modality for neuropathic pain.
BEST POSTER SESSION

NEUP7-0199
CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY IS ASSOCIATED WITH ALTERED CONNECTIVITY IN INTEROCEPTIVE BRAIN CIRCUITRY

I. Kleckner¹, R. Aslin², C. Heckler¹, M. Janelsins³, N.Mohile³, M. Asare¹, C. Cole¹, P.J. Lin¹, K. Mustian¹, V. Rao⁴, S. Kesler⁴

¹University of Rochester Medical Center, Surgery Cancer Control Division, Rochester, USA
²University of Rochester, Rochester Center for Brain Imaging, Rochester, USA
³University of Rochester Medical Center, Neurology, Rochester, USA
⁴MD Anderson Cancer Center, Neuro-Oncology, Houston, USA

Background and Aims:

Over half of patients receiving taxane chemotherapy experience chemotherapy-induced peripheral neuropathy (CIPN), which involves numbness and neuropathic pain in the hands and feet. CIPN has no effective treatments partly because its etiology is poorly understood. We theorize that CIPN symptoms are partly caused by impairment of interoceptive brain circuitry, which processes bodily sensations via the posterior insula and anterior cingulate cortex (ACC). We investigated whether CIPN is associated with altered connectivity in interoceptive brain circuitry.

Methods:

Fifty women with breast cancer (50±9 years) reported CIPN symptoms (CIPN-20) and underwent resting fMRI one or more times: before surgery, one month after completion of chemotherapy, and one year after chemotherapy. We used an a priori seed-based investigation of connectivity between the posterior insula, subgenual ACC (sgACC), and pregenual ACC (pACC). We compared connectivity between 31 patients without CIPN symptoms (≤10 CIPN-20-Sensory), 19 patients with CIPN symptoms (>10 CIPN-20-Sensory), and 280 healthy adults (174 women, 19.3 years) from another study.

Results:

Patients with CIPN symptoms had significantly reduced connectivity between the posterior insula and both the pACC (p=0.02, Cohen’s d=0.80) and the sgACC (p=0.01, d=0.73) compared to patients without CIPN symptoms. Connectivity between the posterior insula and both regions of the ACC was negative in patients with CIPN symptoms but positive in both healthy adults and patients without CIPN symptoms.

Conclusions:

CIPN is characterized by reduced connectivity in interoceptive brain circuitry, which may be a viable treatment target. Future work will assess causal relationships between CIPN symptoms and reduced connectivity.
IMPLICATION OF RARE NAV1.7 VARIANTS IN PAINFUL DIABETIC PERIPHERAL NEUROPATHY

I. Blesneac1, A. Themistocleous1, C. Fratter2, S. Tesfaye3, P. Shillo3, J. Ramirez1, A. Rice4, D. Bennett1

1University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom
2Oxford University Hospitals NHS Foundation Trust, Oxford Medical Genetics Laboratories, Oxford, United Kingdom
3Sheffield Teaching Hospitals NHS Foundation Trust, Diabetes Research Unit, Sheffield, United Kingdom
4Imperial College London, Pain Research Group & Pain Medicine, London, United Kingdom

Background and Aims:

Neuropathy is one of the most common long-term complications of diabetes and 25% to 50% of diabetic neuropathy patients will develop neuropathic pain. Although neuropathic pain can have a major deleterious impact on quality of life its pathophysiology in the context of diabetic peripheral neuropathy is complex and not fully understood. A potential mechanism are changes in sodium channels such as Nav1.7. Loss of function mutations in this channel cause insensitivity to pain, whereas gain of function mutations have been linked with different pain syndromes. Our aim was to investigate whether mutations in Nav1.7 are associated with diabetic neuropathic pain.

Methods:

190 patients with diabetic peripheral neuropathy were screened for mutations in Nav1.7. The potentially pathogenic mutations were expressed in HEK293T cells and assessed functionally by whole cell patch clamp.

Results:

Eleven Nav1.7 variants were identified in 8 patients within a series of 111 subjects with painful diabetic neuropathy. Five of these variants were previously associated with pain disorders: V991L, M932L (Faber et al. 2011); W1538R (Cregg et al. 2013), R185H (Han et al. 2012), L1267V (Huang et al. 2014). Among the other variants two of them met the criteria of potential pathogenicity based on predictive algorithms and were further studied. Functional analysis showed that one of these variants (M1852T) drastically impairs channel inactivation by shifting the steady-state fast-inactivation towards more depolarising potentials. No rare Nav1.7 variants were found in 79 subjects with painless diabetic peripheral neuropathy.

Conclusions:

These observations suggest that mutations in Nav1.7 may contribute to painful diabetic peripheral neuropathy.
Background and Aims:

This review addresses chronic post-surgical neuropathic pain (NP) presenting as phantom limb pain (PLP) post-amputation or chronic NP post-limb sparing in pediatric and young adult patients with cancer.

Methods:

This review is based on 3 studies from our institution.

Results:

In a retrospective study of 25 children and young adults, 64% had pre-amputation pain, 76% experienced PLP during the first year, and 10% had PLP at 1 year post-amputation, indicating that PLP after cancer-related amputation is common but resolves by 1 year in most patients.

In a subsequent retrospective study of 21 children and young adults post-amputations, 85.7% experienced PLP and 38.9% of them experienced PLP at 1 year post-amputation. Of them, a group received mirror therapy (MT) in addition to the standard treatment and a group received standard treatment (non-MT), and experienced PLP at 1 year post-amputation in 11.1% and 66.7%, respectively; PLP duration was mean (SD) of 246 (200) days and 541 (363) days, respectively (P = .08). This study indicated that MT is associated with lower incidence of PLP at 1 year and shorter duration of PLP.

In a prospective investigation, of 37 patients with osteosarcoma post-limb sparing (26, 68.4%) or post-amputation (12, 31.6%), 81% developed NP for mean (SD) duration of 6.5 weeks (7.2), and 87.7% of them required NP specific medications, with gabapentin (65.4%), or gabapentin, amitriptyline and methadone (35.6%).

Conclusions:

Chronic post-surgical NP is frequent, can persist for a significant time, and NP outcomes are similar in limb sparing and amputation groups.
NEUROMAS IN PATIENTS WITH PERIPHERAL NERVE INJURY AND AMPUTATION. AN ONGOING STUDY

N. Buch¹, E. Qerama², N. Finnerup³, L. Nikolajsen¹
¹Aarhus University Hospital, Department of Anaesthesiology and Intensive Care, Aarhus, Denmark
²Aarhus University Hospital, Department of Neurophysiology, Aarhus, Denmark
³Aarhus University Hospital, Danish Pain Research Center, Aarhus, Denmark

Background and Aims:

Injury to peripheral nerves associated with trauma, amputation, or surgery may lead to the formation of neuromas that can cause severe pain. Unfortunately, neuromas are frequently refractory to medical and surgical treatment. This ongoing study examines whether neuromas are more frequent in patients experiencing pain after peripheral nerve injury or amputation than in patients without pain.

Methods:

In this observational cohort study, 80 patients with peripheral nerve injury or amputation will be recruited. Patients will answer pain questionnaires and undergo a clinical examination with quantitative sensory testing performed within the area of spontaneous pain, including areas of brush-evoked allodynia and pinprick hyperalgesia. Neuromas are identified using ultrasound.

Results:

Patient inclusion is ongoing. At present, fourteen amputees have participated in the study: nine males and five females, aged 38-77 years. Six patients had no neuromas. Stump pain in this group ranged from 0 to 8 and phantom pain from 0 to 10 on a numerical rating scale, 0-10. Eight patients had neuromas. Stump pain in this group ranged from 0 to 7 and phantom pain from 0 to 8. Further results will be presented at the congress.

Conclusions:

Because of a limited number of patients included, it is not yet possible to conclude if neuromas are more frequent in patients with pain. Hopefully, this study will increase our understanding of the role of neuromas in patients with pain after peripheral nerve injury and amputation.
Background and Aims:

Although secondary trigeminal neuralgia is usually due to tumours or multiple sclerosis, other major neurological diseases, such as aneurysms, should be taken into account when the history or the symptoms suggest a secondary origin. This is the case of a 67-year-old lady, that presented with a 6-month history of trigeminal neuralgia involving exclusively the right ophthalmic division.

Methods:

In order to exclude a trigeminal pathway damage, the patient underwent trigeminal reflex testing including the blink reflex and the masseter inhibitory reflex recordings. A dedicated MRI study with volumetric sequences and Magnetic Resonance Angiography to check for neurovascular compression, revealed a 5 mm wide-necked aneurysm of the right superior cerebellar artery. The patient underwent an endovascular treatment by stent assisted coiling.

Results:

We observed the complete relief of the neuralgic pain attacks within the first 24 hours after the aneurysm embolization. This complete, drugless pain relief, was still persisting at last follow-up visit, i.e. six months after the intervention.

Conclusions:

In this patient, the unexpected immediate remission of paroxysmal pain after the endovascular intervention, long before a remyelinating process could take place, suggests the crucial role of artery pulsation in the axon hyperexcitability and in the development of ectopic activity. This hypothesis is in line with animal studies showing that in demyelinated central axons mechanical stimulation triggers abnormal high frequency discharges.
Background and Aims:

Patients with unilateral neuropathic pain (UNP) exhibit both positive and negative sensory signs in the affected area. Published data also suggest contralateral affection in animal models of UNP and in patients with unilateral trigeminal neuralgia and postherpetic neuralgia. Aim of the study was to analyse the contralateral sensory function in a large sample of UNP patients from three multinational research networks (DFNS, IMI-Europain, Neuropain).

Methods:

Standardized quantitative sensory testing was performed in 513 patients with UNP (peripheral nerve injury, n=241; postherpetic neuralgia, n=95; radiculopathy, n=76; trigeminal neuralgia, n=101). Sensory function on the contralateral pain-free side was compared to published reference material ([1], mean=0, SD=1 z-values) and analysed also in relation to disease duration, examined body area, pain intensity and sensory changes in the most painful area.

Results:
Analysing all entities together, contralateral to UNP both thermal and mechanical detection thresholds (MDT) were shifted towards sensory loss (all p<0.01), whereas thermal pain thresholds and mechanical pain sensitivity (MPS) were shifted towards sensory gain (all p<0.05). Pain intensity in the affected area was not related to contralateral sensory function. Longer disease duration was associated with more sensory gain in cold pain threshold. An affected facial area was associated with more sensory loss for MDT and more sensory gain for pressure pain threshold (PPT), areas on the back were associated with more sensory gain for PPT and MPS.

**Conclusions:**

Contralateral sensory changes in UNP indicate complex central nervous plasticity, and should be considered when using side-to-side comparison in everyday clinical practice.

NEUP7-0173
USE OF PENNS (PERIPHERAL NERVE NEURO STIMULATION) TREATMENT FOR CHRONIC NUEROPATHIC PAIN
B. Lewinsohn, S. Ramaswamy
1 London, United Kingdom
2 Barts and the London, Anaesthesia, London, United Kingdom

Background and Aims:
Following the publication of the “gate theory” by Wall and Melzack in 1965 there has been the development of peripheral nerve neuromodulation in conjunction with spinal chord stimulators, albeit more slowly. From 1996 to 2004 there were a whole slew of papers looking in peripheral nerve stimulation for variety of conditions which lead to the 2013 NICE recommendations (Interventional procedures guidance [IPG450]).

Methods:
Following these recommendation’s, we started using the PENS in 2014. We were keen to see the outcomes, following the treatment, and whether our cohort of patients with complex chronic pain conditions benefited. We already run a Qutenza (8% capsaicin patch) clinic but some patients could not tolerate this treatment, so the second question apart from efficacy would be to see if PENS treatment would benefit this cohort of patients.

Results:
We collected 12 patients during this study. Of these 12 patients. Out of these 12 we are still awaiting follow up data from 3 patients. So far we have had successful treatment of 78% of patients listed for PENS treatment. The average length of improvement was 4 months before pain levels returned to baseline figures.

Conclusions:
Treating resistant chronic neuropathic pain can be very challenging for both patient and pain physician. From our data so far, neuromodulation using PENS treatment for peripheral neuropathic pain shows promise in treating some of these patients who have failed to gain any benefit from established existing treatments and we await further high quality research as to the long term management of this patient subgroup.
Background and Aims:

Background: Tapentadol is a centrally acting opioid acting as μ-opioid receptor agonism and noradrenaline reuptake inhibitor. It has a strong indication for neuropathic and mixed pain and a good tolerability profile. In Poland, it is refunded only for adult patients with severe chronic cancer pain not adequately controlled on morphine SR or not tolerated.

Purpose: To consider different aspects related to rotation for tapentadol in chemotherapy-induced peripheral neuropathy (CIPN).

Methods:

Case report: A 62-year-old woman with multiple myeloma suffering from intractable 8/10 pain on NRS in her both shoulders, low back, knees, and feet as CIPN and neuropathic pain as side effects of neurotoxic chemotherapy agents. She had previously been treated with different opioids, (morphine, oxycodone, transdermal fentanyl, fentanyl buccal tablets for incident btp). She suffered also from opioid-induced bowel dysfunction. She isn't tolerating a preparation combining oxycodone and naloxone. Tapentadol was started at 200 mg/day and the dose increased to 400 mg/day 3 days later. Simultaneously, the dose of oxycodone was decreased from 240 mg to 120 mg/day.

Results:

At the end of the partial rotation, pain intensity at rest was minimal (2/10). In addition, constipation was reduced.

Conclusions:

1. The broad analgesic efficacy of tapentadol PR allows the treatment of CIPN. 2. Tapentadol is associated with a low risk of pharmacokinetic interactions, which permits its use in patients who take multiple medications. 3. The favorable tolerability profile allows for easy titration and rotation from previous strong opioids. 4. It is advisable to taper the dose gradually when more than one strong opioid was used before.
IMPACT OF 5% LIDOCAINE MEDICATED PLASTER ON ALLODYNIC SYMPTOMS OF LOCALIZED NEUROPATHIC PAIN AFTER KNEE SURGERY

M. Voute¹, N. Macian¹, V. Leray¹, B. Pereira², G. Pickering¹
¹CHU Clermont-Ferrand, Centre de Pharmacologie Clinique - Inserm 1405, Clermont-Ferrand, France
²CHU Clermont-Ferrand, Délégation à la Recherche Clinique et à l’Innovation, Clermont-Ferrand, France

Background and Aims:

Chronic post-operative neuropathic pain (PONP) occurs in 30-50 % of patients after knee surgery and localized neuropathic symptoms such as “alldynia” and/or “hyperalgesia” are reported. A retrospective study (Mimassi et al., 2015) had shown that a topical 5% Lidocaine Plaster (Versatis®), has a beneficial effect over time in the disappearance of symptoms of different modalities. Our study focuses on the impact of Versatis® on alldynia, hyperalgesia and thermal stimuli in knee localized neuropathic pain.

Methods:

A double blind 2 parallel groups clinical trial (NCT02763592) took place in the Clinical Pharmacology Center/CIC, University Hospital Clermont-Ferrand, in 36 patients with knee PONP (69.4±7.3 years old). Patients received Versatis® or placebo plaster during three months. Evolution of neuropathic pain symptoms (dynamic mechanical alldynia, pressure (Von Frey®), hot and cold (Pathway Medoc®), size of the alldynic area and global pain (numerical scale) were followed at each visit (day (D) 0,7,15, month (M) 1, 2, 3).

Results:

From D7 onwards mechanical alldynia diminished significantly of more than 30% over 3 months (p=0.003) when compared to placebo. Other parameters are currently in the course of analysis.

Conclusions:

This study shows the efficacy of Lidocaine 5% plaster on mechanical alldynia and the progressive therapeutic benefits of using the plaster on a prolonged period. Chronological changes in pain modalities suggesting the implication of underlying pharmacological mechanisms on different receptors will be assessed with the ongoing analysis.
Background and Aims:

Diabetic neuropathic pain is associated with small fiber neuropathy. Assessment of small fibers is only possible with advanced techniques such as CHEPs, LEPs, microneurography or skin biopsy. Axon-reflex flare response is a vasodilatation (hyperemia) induced by stimulation of the dermal nociceptive C-fibers. In this study we assessed functions of small fibers in 3 different stages of diabetic patients.

Methods:

Patients with impaired glucose tolerance (Group 1), Diabetic neuropathic pain (Group 2), and only diabetic patients (Group 3) were included. Axon-reflex flare responses were induced by menthol, histamine and capsaicin applied both to the proximal and distal lower extremities. The pain, burning pain, painful cold and itch responses were rated.

Results:

The scores of DN4, MNPS and VAS were highest and menthol induced more lasting coolness and burning, capsaicin induced pain and burning, histamin induced more severe pain, burning pain and itch in Group 2. Axon-flare responses were prominently obtained by histamin in all groups and in the proximal leg. However these responses were most negatively affected in Group 2 and distally.

Conclusions:

Early assessment of neuropathic pain is essential. In our study we demonstrated that axon-flare responses were severely affected in Group 2 and distally. This is compatible with length-dependent neuropathy observed in diabetes.

LASCA is an easy, quick, objective, quantitative, new diagnostic method to assess small fiber functions in neuropathic pain patients.

This work is funded by The Scientific and Technical Research Council of Turkey (TUBITAK) 1001 Project No: 214S068 and Hacettepe University, Scientific Research Projects Coordination Unit, Project: 014 A 101 007-710.
Background and Aims:

Contact heat evoked potentials (CHEPs) have become an established method of assessing small-fiber sensory nerves; however, their potential as a physiological signature of neuropathic pain symptoms was unclear. The present study was aimed to investigate the diagnostic efficacy in examining small-fiber sensory nerve degeneration, the relationship with skin innervations, and clinical correlates with sensory symptoms.

Methods:

We recruited 188 patients (115 men) with length-dependent sensory symptoms and reduced intraepidermal nerve fiber (IENF) density at distal leg to perform CHEP, quantitative sensory testing (QST), and nerve conduction study (NCS). Fifty-seven age- and gender-matched controls were enrolled for comparison of CHEP and skin innervation.

Results:

Among neuropathic patients, 144 patients had neuropathic pain and 64 cases had evoked pain. Compared with QST and NCS parameters, CHEP amplitudes showed the highest sensitivity for diagnosing small-fiber sensory nerve degeneration and exhibited the strongest correlation with IENF density in multiple linear regression. CHEP amplitudes were strongly correlated with the degree of skin innervation in both neuropathic patients and controls, and the slope of the regression line between CHEP amplitude and IENF density was higher in neuropathic patients than in controls. Patients with evoked pain had higher CHEP amplitude than those without evoked pain, independent of IENF density. ROC analysis showed that CHEP had better performance in diagnosing small-fiber sensory nerve degeneration than thermal thresholds. Furthermore, CHEPs showed superior classification accuracy with respect to evoked pain.

Conclusions:

CHEP is a sensitive tool to evaluate pathophysiology of small-fiber sensory nerve, and serves as a physiological signature of neuropathic pain symptoms.
Background and Aims:

Painful polyneuropathy (PPN) is a disabling complication of diabetes. This study aims to determine its prevalence and relationship with Quality of Life (QoL) in a nationwide prospective cohort of incident recently diagnosed Danish type 2 diabetic patients.

Methods:

We sent a detailed questionnaire on neuropathy, pain and QoL to 6,726 patients prospectively enrolled from general practitioners and hospital specialist outpatient clinics into the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. Patients who reported pain in both feet and a score ≥3 on the Douleur Neuropathique (DN4) questionnaire were considered to have possible PPN. QoL and pain intensity were measured on a numeric rating scale (NRS, 0-10). The Michigan Neuropathy Screening Instrument (MNSI) was used to assess neuropathy.

Results:

A total of 5,371 (79.8 %) returned a complete questionnaire. 848 (15.8%) recently diagnosed type 2 diabetic patients reported pain in both feet. Of the 619 patients with pain who completed the DN4 questionnaire, 404 (65.2%) had a DN4 score ≥ 3, corresponding to a prevalence in the total population of possible PPN of 10.3%. Mean pain intensity was 5.2 (SD 2.2) and 89% had a MNSI score ≥ 3. Patients with possible PPN had a substantially lower QoL score than those without PPN (median QoL score 6 versus 8 (p <0.001)), also when correcting for MNSI score.

Conclusions:

Ten percent of newly diagnosed type 2 diabetic patients in Denmark had possible PPN. Patients with PPN had lower QoL than patients without PPN.
Background and Aims:

Symptoms of Diabetic polyneuropathy (DPN) follow a continuum ranging from sensory loss to neuropathic pain. Estimation of intraepidermal nerve fiber density (IENFD) from skin biopsies is useful to diagnose neuropathy, but values of IENFD are similar in patients with and without painful symptoms. Therefore, the aim of this ongoing study is to perform a detailed structural analysis of nociceptors in type 1 DPN (T1-DPN) to identify markers which may explain this discrepancy.

Methods:

Twelve T1-DPN patients with confirmed neuropathy using Michigan Neuropathy Screening Instrument (>3) and nerve conduction measurements were enrolled together with 30 healthy controls. Topical capsaicin (10%) causing nerve fiber depletion was applied for 30 min to the distal leg and biopsies were taken at three time points: at baseline 3-5 cm distal to the capsaicin site and 1 and 90 days after the application, at the capsaicin site. The biopsies were stained using PGP 9.5, GAP-43 and TRPV1 antibodies, and IENFD, nerve fiber length density (NFLD), axonal swellings, and GAP-43 and TRPV1 positive fibers were quantified.

Results:

Patients had significantly lower IENFD (fig 1), NFLD and GAP43+ and TRPV1+ IENFs than controls, slower regeneration rate post-capsaicin (fig 1), lower proportions of GAP-43+ IENFs (fig 2) and higher swelling ratios (median 0.26 and 0.00 for patients and controls, respectively (all p<0.002).
Figure 1. IENFD values.
Figure 2. Percentage of GAP-43+ fibers.

Conclusions:

Nociceptors in T1-DPN patients exhibit multiple structural changes, including lower regeneration rate and regeneration marker and higher degenerative marker. These changes may explain some of the symptoms seen in T1-DPN.
Background and Aims:

Phantom Motor Execution (PME) facilitated by Myoelectric Pattern Recognition (MPR) and Virtual Reality (VR) poses itself as an effective treatment for Phantom Limb Pain (PLP). Notably, a recent clinical control trial using the methodology on a population of 14 upper limb amputees with intractable PLP showed significant improvements (Ortiz-Catalan, 2016). The present study aims at assessing whether PME facilitated by MPR and VR can reduce PLP in lower limb.

Methods:

A 70-years-old male with trans-femoral amputation was treated for a total of 24 PME treatment sessions. Pain was assessed in terms of Weighted Pain Distribution (WDP) (Ortiz-Catalan, 2014) and Short Form of McGill Pain Questionnaire (SF-MPQ). The treatment consisted in using myoelectric signals produced by stump muscles during phantom motions in order to control a VR limb.

Results:

SF-MPQ showed a significant reduction (>50%) in the number of word chosen and in the Pain Rating Index (Figure 1). WDP (Figure 2) shows reduction of time spent in pain. Improvement of sleep (from 2h to 7h/night) was also reported.
Figure 1. Pain Rating Index at each session.

Figure 2. Weighted Pain Distribution at each session. Pain is rated from 0 (none) to 5 (excruciating).

Conclusions:

Although the results are limited to one subject, this study indicates that PME could potentially reduce PLP also in the lower limb.
**Background and Aims:**

Stronger placebo responses have been observed in pain studies, and Painful Diabetic Neuropathy (PDN) studies show a high rate of failure. Aim is to characterise the phenomena of an enhanced placebo response in PDN studies.

**Methods:**

A systematic review of PDN studies evaluating the placebo response was performed. MEDLINE/PUBMED search was utilised to identify all relevant PDN trials between 1998 to 2016.

**Results:**

A strong placebo response in PDN studies has been observed over the last two decades. Seven randomized studies with pregabalin showed a placebo response of -1.47 of the average daily pain score (ADPS) vs baseline. Within the same drug class, data with mirogabalin also showed a large placebo response of -1.87 of ADPS at five-week endpoint vs baseline. This rise in placebo response is unpredictable and can continue over time as shown from results of trial SP743 with lacosamide.

**Conclusions:**

The observed findings may be due to changing participant characteristics, increased patient expectation and high number of face to face study visits leading to enhanced positive feedback. Placebo response may be attenuated by using more innovative study designs, such as randomized withdrawal designs. Additional approaches could also be beneficial: enrolment of patients with greater baseline pain severity; minimizing the number of treatment groups; implementing strategies to decrease investigators and patient’s expectations of improvement; improving patient’s selection to pre-identify placebo and active responders. The impact of this trend in study design will need to be further assessed, including future trial sizes and duration.
Background and Aims:

This study aimed to gain patient insights into population characteristics, treatment pathways, and functional impact of neuropathic pain (NP) to develop EMA401, a potential agent for NP treatment.

Methods:

Real-world patient-reported data from the US and EU (May 2010-2016) from social media/drug-safety databases were collated using RLytics, a patient-analytics platform (Real Life Sciences Ltd., Kinapse Ltd.). Using an analytical framework, patient reports of symptoms/impairment were categorized (social, physical, emotional, cognitive, and role activity) and analyzed to generate insights on functional impairment and NP treatment pathways.

Results:

Data from 204 sources reporting 829,147 NP reports across the US and EU were collected. Overall, 75,377 patients with NP (US: 56,886, EU: 18,490) qualified for analysis; 47,272 patients had received NP treatment. Physical (71.5%) and Emotional Concepts (42.3%) were the most reported in patients with NP (n=30,717). Amongst patients with non-specified NP, hypoesthesia/hyperesthesia, paraesthesia, asthenia, and fatigue were the highest reported Physical Concept symptoms (US: 5,948, EU: 2,269). Anxiety-, depressive-, affective-disorders and stress and tension were the highest reported Emotional Concept symptoms (US: 3,148, EU: 1,135). NP treatments including anti-convulsants, anti-depressants, and analgesics were used consistently in the US and EU, with a majority of patients receiving combination therapy. Observations for sub-populations with peripheral diabetic neuropathy and post-herpetic neuralgia were consistent with those reported above.

Conclusions:

NP is associated with significant functional impact on patients. Despite some heterogeneity in treatments and need for combination therapy, commonalities in treatment pathways for NP are observed in the US and EU.
NEUP7-0250
SIGNIFICANT CHANGES IN SOMATOSENSORY PROFILES, PAIN AND FUNCTION IN PATIENTS WITH LUMBAR RADICULOPATHY PRE-AND POST MICRODISCECTOMY

B. Tampin¹,²,³,⁴, H. Slater⁵, C. Lind⁶,⁵

¹Sir Charles Gairdner Hospital, Department of Physiotherapy, Perth, Australia
²Sir Charles Gairdner Hospital, Department of Neurosurgery, Perth, Australia
³Curtin University, School of Physiotherapy and Exercise Science, Perth, Australia
⁴Hochschule Osnabrück- University of Applied Sciences, Faculty of Business Management and Social Sciences, Osnabrück, Germany
⁵University of Western Australia, School of Surgery, Perth, Australia

Background and Aims:
To date, studies using quantitative sensory testing (QST) to assess sensory nerve function in patients with lumbar radiculopathy undergoing surgery have focused on changes in the affected dermatome. Somatosensory profiles in the main pain area (MPA) have not been investigated, although this is a requirement for assessing neuropathic pain.

Aim: To establish the somatosensory profile of patients with lumbar radiculopathy at pre-and 3 months post-microdiscectomy.

Methods:
The full QST protocol of the German Research Network on Neuropathic Pain was performed in 53 patients (mean age 38±11 years, 26 females) with unilateral L5/S1 radiculopathy in their MPA, affected dermatome and contralateral mirror sides. Repeat measures at 3 months included QST, the Oswestry Disability Index (ODI), painDETECT (PD-Q) and bothersomeness of leg pain (numeric rating scale 0-10).

Results:
At baseline, there was a significant loss of function in all sensory fibre populations in the symptomatic leg compared to the asymptomatic leg in the MPA (thermal, mechanical, vibration detection, mechanical pain threshold, mechanical pain sensitivity p<0.005) and dermatome (thermal, mechanical, vibration detection p<0.024). At three months, there was a significant improvement in all pre-surgery altered dermatomal QST parameters (p<0.008) and in QST measurements in the MPA (p<0.017) except thermal detection thresholds. Clinical outcomes all improved (p<0.000) (ODI pre/post: mean 18.1±6.1/ 5.5±6.5; PD-Q pre/post: mean 16.2±5.5/5.6±6.2; leg pain pre/post: mean 5.8±1.9/1.3±1.9). Seven individuals reported little improvement post-surgery.

Conclusions:
Surgical intervention was associated with improvements in affected somatosensory parameters and clinical outcomes.

Acknowledgement: Western Australian Department of Health/Raine Medical Research Foundation; School of Physiotherapy and Exercise Science, Curtin University.
Background and Aims:

We report preliminary data for a neurosurgery patient group with radicular leg pain, with or without nerve root compression, who do not proceed to surgery for various reasons. We aimed to characterise their somatosensory and clinical profile and to monitor if their pain condition improves over time. This will enable direct comparison with patients with radicular leg pain in a parallel study who proceed to surgery.

Methods:

Twenty-three patients with unilateral L5 or S1 radicular pain (age 47±10 years, 9 females, symptom duration 20±24 months) were assessed using quantitative sensory testing (baseline only) and clinical outcomes (Oswestry Disability Index (ODI), the painDETECT (PD-Q) and pain intensity measured on a numeric rating scale (0-10)). The complete QST protocol of the German Research Network on Neuropathic Pain was performed in the patients’ main pain area and affected dermatome and the unaffected contralateral mirror sides.

Results:

At baseline, group data (n=23) indicated a significant loss of function in large and small nerve fibres in the symptomatic leg compared to the asymptomatic leg in the main pain area (mechanical, vibration, warm detection and mechanical pain threshold p<0.023) and affected dermatome (thermal and mechanical detection p<0.023). Based on preliminary data (n=17) at 3 months, clinical outcomes were not significantly changed (ODI baseline: mean 13.2±6.8, post: mean 13.6±6.3; PD-Q baseline: mean 13.9±7.2, post: mean 13.9±7.5; maximum/average pain intensity last 4 weeks baseline: mean 6.7±1.8/3.6±1.9, post mean 6.3±2.7/4.5±2.4).

Conclusions:

Trajectories for clinical outcomes were stable in short term. It is unclear if this continues at 12 months.

Acknowledgement: Funding support Arthritis Australia
Background and Aims:

Myelopathy frequently leaves chronic pain which is either nociceptive or neuropathic in nature. The aim of this study is to determine whether spinal cord MR images can predict the quality and severity of pain in non-traumatic, non-compressive (NTNC) myelopathy.

Methods:

Forty-two NTNC patients with chronic pain were recruited. The pain was assessed using The Short Form McGill Pain Questionnaire (SF-MPQ) and the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS). A clinically relevant spinal MRI including T2-weighted and gadolinium enhanced T1-weighted sagittal and axial images was taken in all patients. Lesion location on the axial images was divided into four groups; anterolateral containing the spinothalamic tract, posterior comprising the dorsal columns, both and none. Level, length and location of the lesion were analyzed how to influence the quality and pain severity.

Results:

Of the 42 patients, 25 were male. Median pain duration was 41 (3.4-166) months. The most common cause of myelopathy was acute (partial) transverse myelitis (27, 64%). Twelve (28%) patients experienced neuropathic pain. Mean score of the visual analogue scale and the present pain intensity of the SF-MPQ were 5.1 and 2.3, respectively. Any lesion variables on spinal cord image were not significantly related to neuropathic pain or pain severity except lesion length, which tends to be inversely related to pain severity.

Conclusions:

Chronic pain in NTNC myelopathy is moderate and neuropathic component is relatively common. MRI findings of the spinal cord does not seem to provide valuable information to predict occurrence of neuropathic pain or pain severity.
ABNORMALITIES OF BRAIN EVOKED POTENTIALS IN PATIENTS WITH AND WITHOUT NEUROPATHIC PAIN AFTER SPINAL CORD INJURY

E. Opsommer¹, N. Korogod¹, S. Lenka², L. Gunther²

¹University of Applied Sciences and Arts Western Switzerland HES-SO, School of Health Sciences - HESAV, Lausanne, Switzerland
²Swiss Paraplegic Centre, Centre for Pain Medicine, Nottwil, Switzerland

Background and Aims:

Neuropathic pain (NP) often affects individuals with spinal cord injury (SCI) disturbing their daily life. Here, we report the contribution of laser evoked (LEPs), ultra-late LEPs (uLEPs) and contact heat evoked potentials (CHEPs) techniques for quantifying the neurological dysfunction and identifying possible differences between persons with and without NP.

Methods:

24 SCI individuals (12 each group) were examined. Inclusion criteria: traumatic SCI below T1, at- or below-level SCI NP for pain group. Groups were matched by age, sex, lesion level and the AIS grade. LEPs and uLEPs measurements were done with Tm-YAG laser stimulation of the skin and CHEPs with heat-foil thermode, applied to: hand (control site) and test site (dermatome of altered sensation). LEPs, uLEPs and CHEPs were recorded from a single scalp electrode at Cz. Components were identified and analysed.

Results:

LEPs and CHEPs components (latencies, amplitudes) from the control site were normal and showed no differences between groups and were abnormal (absent) in 10 out of 12 patients in both groups from the test site. uLEPs reaction times (RT) analysis showed a diffuse distribution in group without pain in contrast to bimodal distribution in group with pain.

Conclusions:

LEPs, uLEPs and CHEPs methods were able to detect abnormalities of spino-thalamic tract in both groups of patients with SCI. From uLEPs RT results, we could suggest that patients with NP have partial nerve fiber preservation with abnormal functions causing pain, whereas patients without pain have total loss and therefore no pain.
Background and Aims:

Sensory profiling is suggested to understand mechanisms of neuropathic pain (NP). Therefore, quantitative sensory testing (QST) was compared in persons with spinal cord injury (SCI) with and without NP.

Methods:

QST according to the DFNS protocol was applied to 24 individuals with traumatic SCI below T1 (12 each group) on hand (control site) and test site (dermatome of altered sensation). For sensory function, loss and gain score (LOGA) was applied. Ratings are L: loss, G: gain, 1: thermal, 2: mechanical, 3: thermal and mechanical symptoms. Groups were matched by age, sex, lesion level and AIS grade.

Results:

Mean values of QST parameters did not differ between groups. At the test site, both groups showed loss of function for CDT, WDT, TSL, MDT and/or VDT. LOGA scores were abnormal in all pain patients and in 11/12 of the pain-free group. Most frequent LOGA score was L3G0 (6/12: pain group, 7/12: pain-free group). In addition, patients from both groups had also abnormalities in the control site: Loss of function for MDT and/or VDT. LOGA scores were abnormal in 11/12 (pain group) and in 9/12 (pain-free group). Most frequent LOGA score for control site was L2G0 (5/12: pain group) but very heterogeneous in the pain-free group.

Conclusions:

Our study confirms altered spinothalamic and dorsal column functions below the level of injury independent of NP. Unexpectedly, abnormalities in dorsal column functions were also detected at the control site for both groups indicating changes in sensory processing rostral to the spinal lesion.
Background and Aims:

A 69-year-old female patient with postherpetic neuralgia (PHN) in dermatome TH3 reported that touching the ipsilateral earlap (dermatome C3-2) would enhance pain and dynamic mechanical allodynia (DMA) in the affected TH3-dermatome.

The aim was to investigate possible underlying mechanisms of this phenomenon using quantitative sensory testing (QST) and functional spinal and supraspinal MRI.

Methods:

QST in the affected TH3-dermatome as well as spinal and supraspinal fMRI (3T, 10 sagittal slices, slice thickness 2mm, single-shot FSE, TR 9000 ms, TE 38 ms, FOV 288 x 144 x 20 mm, matrix 192 x 96, voxel size 1.5 x 1.5 x 2 mm) were performed before and after topical application of capsaicin (0.6%) to the ipsilateral earlap.

Results:

After application of capsaicin to the earlap DMA in TH3 increased from 43.69 to 72.48. Functional MRT demonstrated an increase of signal activity in the ipsilateral dorsal gray matter at level TH2/TH3 as well as in supraspinal pain-modulating areas including the ipsilateral periaqueductal gray, the rostral ventromedial medulla oblongata and the subnucleus reticularis dorsalis.

Conclusions:

The observed transferred pain in combination with activation of pain modulating areas upon fMRI suggests either (1) co-processing of projections of cervical and thoracal somatosensory afferents via the spinal trigeminal nucleus or (2) deafferentation-induced reorganization of somatotopic maps as ear and trunk are adjacent areas of the sensory homunculus.
Background and Aims:

Allodynia is a type of pain caused by an innocuous stimulus. The aim was to investigate potential beneficial effects of a new Manual Technique (A.DupratMT) in mechanical allodynia in patients with spinal cord injury (SCI) admitted at a Trauma Emergency Hospital (TEH) from Brazil.

Methods:

All conscious patients with SCI, complaining mechanical allodynia, admitted to the neurosurgery sector from the TEH during 2014 were included. All patients received the A.DupratMT, which consists of subtle maneuvers using light touch, pressure and sweep the painful area, according to patient's feedback. Pain level was measured using Visual Analogue Scale. The technique was complementary to medical drugs prescriptions. The study was approved by the local ethical committee.

Results:

312 patients (243M and 69F) were hospitalized with SCI and only 4 presented mechanical allodynia. All male, with thoracolumbar SCI caused by fire gun. The mean age was 23 and none underwent surgery. In all cases the pain began immediately after trauma and localized at level of the SCI. The mean days of hospitalization until the first session using the A.DupratMT was 5 days. Pain level was 10/10 in all cases before the first session, leading to significant functional limitations. After the first session the pain reduced to lower levels (mean 1.7/10) in all cases. An average of two sessions using A.DupratMT were necessary to control allodynia and recover functional abilities.

Conclusions:

Although unusual, allodynia in SCI was intense and disabling. The A.DupratMT seems to be an effective and simple technique that results in immediate positive outcomes.
Background and Aims:

**Background:** There is no study on the long-term use of prednisolone in post-stroke complex regional pain syndrome-1 (CRPS1).

**Objective:** To evaluate the efficacy and safety of long-term low dose prednisolone in post-stroke CRPS-I.

**Methods:**

58 patients with post stroke CRPS-I were included and their clinical details and CRPS, Visual Analogue Scale (VAS), modified Rankin Scale (mRS) and Barthel Index (BI) scores were noted. The patients were prescribed 40mg prednisolone for 2 weeks followed by taper in the next 2 weeks. Patients who responded were randomly assigned prednisolone 10mg daily (group I) or no prednisolone (group II). They were followed up at 1st and 2nd month of randomization and their CRPS, VAS, mRS and BI scores were noted. The primary outcome was improvement in CRPS score and secondary outcomes were VAS, mRS, BI scores and severe adverse events (SAE).

**Results:**

56/58 (96.5%) patients responded to initial high dose prednisolone and 26 each were assigned group I and group II treatment. Group I patients had further improvement in CRPS score. 50% patients in group II had deterioration at 1mo needing reinstitution of prednisolone; following which 77% of them improved in next month. The improvement in CRPS score paralleled the VAS score but not mRS and BI scores at 1st and 2nd months in group I compared to group II. There was no SAE necessitating withdrawal of prednisolone.

**Conclusions:**

**Conclusion:** In post stroke CEPS-I, continuation of low dose prednisolone for 2 months is safe and effective.
NEUP7-0272
A PILOT STUDY INVESTIGATING WHETHER QUANTITATIVE SENSORY TESTING ALTERS AFTER TREATMENT IN PATIENTS WITH FIBROMYALGIA

T. Wodehouse

1Barts Health NHS Trust, Pain and Anaesthetic Research Centre, London, United Kingdom

Background and Aims:

Background and aims

Fibromyalgia (FM) is a chronic musculoskeletal pain condition that is often associated with sleep disturbances and fatigue. The pathophysiology of FM is not understood but indirect evidence suggests a central dysfunction of the nociceptive modulating system. Efficacy outcomes in clinical trials of fibromyalgia typically employ standardised questionnaire measures but objective tests of endogenous pain mechanisms such as QST are yet to be utilised to measure efficacy of treatments. The aim of this study was to evaluate whether quantitative sensory testing (QST) detects a change in pain in FM receiving Pregabalin treatment.

Methods:

Methods

Twenty-five patients were recruited for the study and received routine Pregabalin, 14 patients completed. QST was measured at baseline and every 4 weeks for 12 weeks. Measurement of pressure pain thresholds (PPT) and conditioned pain modulation were measured. Fibromyalgia impact questionnaire (FIQ), PainDETECT and SF-12 was also completed.

Results:

Results

Patients with FM demonstrated loss of CPM at baseline. A ‘normal’ CPM was observed at one month and this was maintained until the final visit. PPT’s showed a significant improvement from baseline. Patients also reported a similar magnitude of improvements in PainDETECT and its impact on daily life by on FIQ and change in outcome for SF-12.

Conclusions:

Conclusions

The central sensitisation response particularly ‘dynamic responses’ to Pregabalin has not been reported before. This is the first study demonstrating improvement in peripheral and central sensitization as measured by QST in patients with fibromyalgia following treatment with Pregabalin.
CLINICAL: CENTRAL NEUROPATHIC PAIN - PART 2

NEUP7-0388
AGE IS ASSOCIATED WITH POSTROKE PAIN
G. Požlep¹, M. Zaletel²
¹University Clinical Centre Ljubljana, Department fo anesteziology, Ljubljana, Slovenia
²University Clinical Centre Ljubljana, Department of Neurology, Ljubljana, Slovenia

Background and Aims:

Central post-stroke pain (CPSP) is a syndrome characterized by sensory disturbances and neuropathic pain. Functional disturbances such as depression, anxiety and sleep disturbances may significantly have an influence on neuropathic pain expression. The contribution of age in CSPS is not clear.

Methods:

We randomly investigated 297 patients (mean age 72±5.4 years) with first-time stroke over a 1-year period. Patients were evaluated 6 months and 12 months following stroke onset. Pain was assessed using a visual analogue scale ranging from zero mm (no pain) to 100 mm. Using the scale, zero was defined as no pain, 10 to 30 as mild pain, and 40 to 100 as moderate to severe pain. Depression was evaluated on a depression scale. Logistic regression was used to analyse the associations.

Results:

27 (9.2%) pts developed CPSP. Factors significantly associated with an increased likelihood of having moderate to severe pain included younger age and higher scores on a depression scale (p <0.01). Pain was reported as constantly present in 37% pts, and it disturbed sleep in 67% pts.

Conclusions:

We concluded that CPSP was associated with younger age stroke patients. Depression is an important factor in CPSP.
THE PREVALENCE OF SMALL FIBER POLYNEUROPATHY IN PATIENTS WITH CHRONIC PELVIC PAIN
A. Chen\textsuperscript{1}, E. De\textsuperscript{2}, C. Argoff\textsuperscript{1}
\textsuperscript{1}Albany Medical Center, Neurology, Albany, USA
\textsuperscript{2}Albany Medical Center, Surgery, Albany, USA

Background and Aims:
This study assesses prevalence of small fiber polyneuropathy (SFPN) based upon clinical presentation and skin biopsy findings in patients with refractory CPP and concurrent pain syndromes. The average CPP patient has 2.4 comorbidities with multiple pain syndromes like irritable bowel syndrome (IBS), interstitial cystitis (IC), and fibromyalgia (FM). The lack of common etiology complicates the ability to offer effective treatment options. SFPN is emerging as a major contributor to unexplained multi-symptom syndromes involving chronic widespread pain. In our practice a significant proportion of refractory CPP patients had biopsies consistent with SFPN, a finding never reported in the literature. We propose that SFPN is a unifying underlying treatable mechanism for pain in the refractory CPP patient with comorbid pain syndromes.

Methods:
We evaluated refractory CPP patients for SFPN with 3mm punch biopsies of the lower extremity through Corinthian Reference Lab (CRL). The sensitivity and specificity are 78-92\% and 65-90\% respectively.

Results:
19/28 patients (68\%) were positive for SFPN. Comorbid conditions include migraine (39\%), IBS (36\%), endometriosis (21\%), FM (32\%), IC (14\%), GERD (50\%), vulvodynia (7\%), lower back pain (32\%), and other types of chronic pain syndrome (35\%).

Conclusions:
The prevalence of SFPN in specialty referral patients with refractory CPP is remarkably high versus population data. Consideration of SFPN shifts the focus from a syndrome to unifying treatable disorder. Many CPP patients with SFPN are undiagnosed. Making the diagnosis of SFPN should be a priority, and may result in treatments not usually offered to CPP patients such as IVIG or other immunomodulatory therapies.
Background and Aims:
Peripheral Sensory Neuropathy (PSN) is the main dose-limiting side effect of oxaliplatin treatment in cancer patients. Up to 90% of all patients experience PSN and the onset may already occur after the first infusion. Assessment of PSN is commonly based on subjective evaluation of PSN symptoms using Common Terminology Criteria for Adverse Events (CTCAE). Thus, the study aim was to assess the association of quantitative sensory tests (QST) and nerve fibre excitability assessments.

Methods:
Seventeen patients stage III colon cancer were treated with 12 cycles of adjuvant oxaliplatin-containing chemotherapy. Nerve fibre excitability using perception threshold tracking (PTT) and QST was assessed one hour prior to each infusion. QST consisted of Vibration (VTh), warmth (WDT) and cold (CDT) detection, heat (HPT) and cold (CPT) pain thresholds and ratings to 12.8g and 60.0g static mechanical stimulation (SMS). The chronaxie (Tao) and the rheobase (Rhe), 20ms (TE20dep) and 80ms (TE80dep) depolarizing and 80ms hyperpolarizing (TE80hyp) threshold electrotonus, and 20ms constant raising triangular (Tri) stimulation were assessed by PTT.

Results:
Multiple logistic regression showed that the cumulative dose of oxaliplatin, CPT, Tao, Rhe, and Tri were independently related to CTCAE. Factor analysis revealed five factors: the first mainly containing single PTT pulses, the second threshold electrotonus PTT pulses, the third painful thermal threshold, the fourth non-painful threshold, and the fifth containing SMS ratings.

Conclusions:
QST and PTT assessments are independently related to PSN during oxaliplatin treatment.
CLINICAL: DIAGNOSIS / ASSESSMENT - PART 1

NEUP7-0197
CONDITIONAL PAIN MODULATION: EFFECT OF INTERVENTIONS IN DIFFERENT CHRONIC PAIN STATES
S. Ramaswamy¹, V. Mehta¹, T. Wodehouse¹
¹Pain and Anaesthesia Research Centre, Pain and Anaesthesia Research Centre- St Bartholomew's Hospital, London, United Kingdom

Background and Aims:

Conditional pain modulation (CPM) is a psychophysical paradigm which is an objective measure of the endogenous descending pain modulation. This is thought to be one of the main driving mechanisms for the central sensitization in chronic pain.

The aim of this study is to monitor the consistency of the changes in the CPM following an appropriate clinical intervention in different chronic pain states.

Methods:

We looked at the effect of intervention on CPM in 4 different pain models as detailed below. CPM was measured using the ischaemic arm technique.

Results:

The results for the different pain models are summarized as below:

- Fibromyalgia: 14/16 patients had abnormal baseline CPM. CPM was normalized in all these patients following pregabalin.
- Chronic intractable headache: 11/14 patients had abnormal baseline CPM. Occipital nerve stimulator normalized CPM in all patients.
- Chronic radicular back pain: 16/19 patients had abnormal baseline CPM. DRG steroid injection normalized CPM in 14 of these patients.
- Osteoarthritis (OA) of the knee: 19/20 patients had abnormal baseline CPM. TKR normalized CPM in 17 of these patients.
- Control: 19/20 patients had a normal CPM.

Conclusions:

In this study we have demonstrated that CPM is abnormal in majority of patients in different chronic pain models. In contrast, CPM was found to be normal in 95% of the control group. Fortunately CPM can be normalised following an appropriate intervention and hence is a modifiable effect of chronic pain. In future, there is a need to look into the role of CPM as a phenotypic marker for chronic pain.
Background and Aims:

Currently, the gold standard to diagnose small fibre degeneration (SFD) in patients with peripheral neuropathies involves invasive skin biopsies. The development of valid cost-effective bedside tests that are readily available in clinical practice is warranted.

Aim: To assess the validity of clinical tests to assess SFD using carpal tunnel syndrome (CTS) as a model system.

Methods:

107 participants (22 asymptomatics, 85 with CTS) underwent pin prick (PP) testing over the index finger. In a sub-group (n=51), cold sensation (CS) and warm sensation (WS) were tested using coins at room temperature (cold) and body temperature (warm). SFD was established by quantifying intra-epidermal nerve fibre density (IENFD) in skin biopsies taken from the index finger. Validity of pin prick, CS and WS was tested against IENFD. A binary logistic regression model was used to identify factors most likely to predict a SFD.

Results:

The best validity occurred with clusters of tests: sensitivity 0.98 for WS OR CS, specificity 0.88 for PP.

Only PP regression results revealed a significant prediction (b= 1.92 P<0.00, Odds ratio =6.79).

Conclusions:

If neither CS or WS is positive, there is a high (98%) chance of not having SFD, if PP is positive, there is a high (88%) chance of having SFD. A positive PP means a person is 6.8 times more likely to have SFD. We therefore recommend testing WS and CS first, if both are negative, it is unlikely that SFD has occurred. If one or both are positive, then test PP; a positive PP test indicates a likely SFD.
Background and Aims:

To validate psychometric properties of Ukrainian version of DN4 questionnaire for identification of neuropathic component (NeC) of chronic low back pain (cLBP).

Methods:

103 patients with cLBP were examined clinically and neurologically, based on which all patients were divided into 4 groups according to Quebec Task Force Classification of Spinal Disorders. DN4 questionnaire was applied separately on lumbar and leg level (groups 2 to 4).

Results:

ROC-analysis between groups 1 and 4 validated psychometric properties of Ukrainian version of DN4 questionnaire for assessment of NeC of cLBP (specificity – 94.0%, sensitivity – 67.9%, Youden index – 0.62, for a cutoff of 4/10). Average DN4 score on lumbar level was 2.81 ± 1.38; 27 (26.2%) patients had a score ≥ 4/10. Average DN4 score on leg level was 4.85 ± 1.71; 65 (63.1%) patients had a score ≥ 4/10. Patients in group 4 had score ≥ 4/10 on DN4 questionnaire significantly more frequently (P < 0.05) compared to the other groups of patients. Average score on the DN4 questionnaire on the lumbar level was significantly higher (P < 0.05) in group 4 (3.33 ± 1.56) compared to group 1 (2.26 ± 1.01).

Conclusions:

This study validated psychometric properties of Ukrainian version of the DN4 questionnaire for identification of NeC of cLBP. It has been shown that application of DN4 questionnaire in two areas (lumbar and leg level) allows to determine the presence of NeC of cLBP even in atypical cases.
Background and Aims:

Small fiber neuropathy is characterized by neuropathic pain and autonomic symptoms. Recently skin biopsy and microneurography studies in fibromyalgia patients disclosed small fiber neuropathy. Here we aimed to quantify the morphology of small nerve fibers of the cornea and evaluate the ocular surface characteristics in FM.

Methods:

FM (n=34) and healthy control (n=42) were enrolled. All participants underwent ocular surface tests, tear break-up time (BUT), lissamine green (LG) staining, Schirmer I test, corneal sensitivity and ocular surface disease index (OSDI) questionnaire. Subbasal corneal nerves were evaluated by invivo confocal microscopy (ICM). Fibromyalgia patients' demographic characteristics, ACR 1990 and 2013 scores were obtained.

Results:

Duration of FM was 6.9±5.2 years. VAS:6.8±1.6, ACR 1990 scores:14.53±2.7, widespread pain index scores (WPS):21.6±5.0 and Symptom Impact Questionnaire (SIQR): 28.5±1.6. Compared to the controls, FM had higher OSDI (42.2±18.9 vs. 1.2±1.7, p<0.001), LG staining scores (0.5±0.5 vs. 0.05±0.2, p<0.001), and lower BUT scores (9.0±3.6 vs. 10.3±1.5, p=0.03). Schirmer test results (16.1±8.2 vs. 17.7±7.2, p=0.390) were similar. Corneal sensation was 0.96 g/mm² in all eyes. FM had lower total nerve density (1562.6±620.3 vs. 2544.6±973.0, p<0.001), lower long nerve fibers (3.4±1.3 vs. 4.5±1.0, p<0.001) and lower number of nerves (5.0±1.8 vs. 10.3±2.1, p<0.001). There was statistically significant negative correlation between WPS and Schirmer test results (rho=-0.374, p=0.030).

Conclusions:

To the best of our knowledge, this is the first study which evaluated ocular surface alterations and corneal nerves in FM. FM patients should be evaluated in terms of ocular surface diseases. ICM may be used in FM to assess small fiber neuropathy.
Background and Aims:

Pain is a complex medical problem. Monitoring nociception remains challenging in critically ill intensive care (ICU) patients as a consequence of sedation need. The negative consequences of unrelieved pain or excessive opioid use are significant (neuropathic pain, hyperalgesia).

In this proof of concept study we aimed to test the clinical feasibility of the pupillary dilation reflex (PDR) and the nociception flexion reflex (NFR) in mechanically ventilated ICU patients treated with different opioid analgesics.

Methods:

- Nine non-communicative ICU patients
- Analgesedation protocol: propofol and ultra short-acting opioid remifentanil or long acting sufentanil
- Validated behavior pain scale (BPS)

PDR by Algiscan® (IDMed, France): tetanic stimulations over median nerves were administered starting at 10 mA up to 60mA. Pupillary diameter was measured before, during and after stimulation.

NFR by Paintracker® (Dolosys GmbH, Germany): similar tetanic stimulations over ipsilateral sural nerve starting via an automated inbedded RIII threshold tracking model.

Results:

Patients’ characteristics did not differ between groups. RASS measurement was in all cases -4 prior to study inclusion.
Pupillometry enables a fast, straightforward and easy to use device to assess the autonomic nociceptive processing.

NFR measurement in order to assess the ascending component of the somatosensory system gives the health care provider a more challenging method to perform.

Vital signs remained unchanged during measurements

**Conclusions:**

PDR and NFR may help to evaluate the antinociceptive-nociceptive balance in deeply sedated ICU patients in whom no self-reported pain scales are achievable. The ability of both innovative devices to predict nociceptive status in non-communicative sedated critically ill requires further research.
**Background and Aims:**

Prolonged exposure to vibrating tools can cause *Hand Arm Vibration Syndrome (HAVS)* a condition with neuropathy, Raynaud’s phenomenon and musculoskeletal involvement. HAVS is one of the most frequent diagnosis for workers’ compensation among Swedish men. Therefore we wanted to identify the damage scenario among construction workers, a highly exposed group.

**Methods:**

98 carpenters at two construction companies answered a questionnaire on symptoms and were examined by Quantitative sensory testing (QST) and nerve entrapment tests (Phalen’s, Tinel’s). They were also asked about the company’s safety strategies.

**Results:**

Preliminary results from 98 construction workers shows; 42% reported increased feeling of coldness in the fingers/hands, 38% pain in fingers/hands when exposed to cold. 10% had Raynaud’s phenomenon. 39% experienced tingling and numbness in their hands and 25% reduced sense of touch. 15% had reduced perception thresholds (Semmes-Weinstein monofilament >4, 31) in at least one of the tested fingers. 10% had positive nerve entrapment tests for carpal tunnel syndrome. More than half of the construction workers reported neck pain over the past year; 38% from hands and 37% from elbows. One third had never or seldom got any information from the employer about the risk with vibrating tools.

**Conclusions:**

These preliminary results shows that many construction workers have neuropathy to such degree that preventive measurements are urgently needed. A high rate of pain from the neck and elbows also reveals a substantial coexisting ergonomic exposure. By informing the construction sector about the results we hope to influence decision makers for better preventive strategies.
CLINICAL: DIAGNOSIS / ASSESSMENT - PART 1

NEUP7-0425
PAINDETECT DID NOT DETECT NEUROPATHIC PAIN IN PAINFUL LOW BACK RADICULOPATHIES
E. Hasvik¹, L. Grøvle², A. Julsrud Haugen², J. Gjerstad³
¹Østfold Hospital Trust, Department of Physiotherapy, Grålum, Norway
²Østfold Hospital Trust, Department of Rheumatology, Grålum, Norway
³The National Institute of Occupational Health, Department of Work Psychology and Physiology, Oslo, Norway

Background and Aims:

Low back related leg pain with corresponding disc herniation is conceptually regarded as a neuropathic pain condition. One questionnaire developed to detect neuropathic components is the painDETECT (Freynhagen et al. 2006). We aimed to assess the performance of painDETECT in a population with low back related leg pain compared to the 2016 NeuPSIG grading system for neuropathic pain (Finnerup et al. 2016) as reference standard.

Methods:

We recruited 50 participants with low back related leg pain and MRI confirmed lumbar disc herniation corresponding to signs and symptoms. Participants were assessed with sensory examination and painDETECT. After completed recruitment, we calculated percentage agreement between classifications, and used Bayesian estimation to assess range of uncertainty with 95% credible intervals.

Results:

Nine participants (18%) had painDETECT scores indicating likely neuropathic pain, mean score 21.3 (SD 2.8). 44 participants (88%) fulfilled criteria for probable- (based on sensory abnormalities) and definite neuropathic pain (based on MRI findings).

Agreement for the grading as definite/likely neuropathic was 18% (9-31%), and agreement for possible/uncertain was 14% (4-32%). Of those graded as definite neuropathic pain by the 2016 system, 48% (33 - 62%) was classified with unlikely neuropathic component by PainDETECT.
Conclusions:

Compared to the NeuPSIG grading system, the ability for painDETECT to classify neuropathic pain components in patients with painful low back radiculopathies is questionable.

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Table 1 - Demographics split by painDETECT groups

<table>
<thead>
<tr>
<th></th>
<th>Unlikely NP</th>
<th>Uncertain</th>
<th>Likely NP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=23 (46%)</td>
<td>N=18 (36%)</td>
<td>N=9 (18%)</td>
</tr>
<tr>
<td>Age</td>
<td>42 (10.5)</td>
<td>44.6 (7.8)</td>
<td>41.1 (5.8)</td>
</tr>
<tr>
<td>PainDETECT score</td>
<td>9 (2.9)</td>
<td>15.1 (1.2)</td>
<td>21.3 (2.8)</td>
</tr>
<tr>
<td>Duration of LBP (weeks)</td>
<td>14.5 (8-40)</td>
<td>13 (7.5-38.5)</td>
<td>30 (28-34)</td>
</tr>
<tr>
<td>Duration of leg pain</td>
<td>14 (7.5-21.5)</td>
<td>12 (7-20)</td>
<td>10 (5-27)</td>
</tr>
<tr>
<td>Low back pain intensity</td>
<td>4.4 (3.1)</td>
<td>5.4 (2.3)</td>
<td>5.3 (3.5)</td>
</tr>
<tr>
<td>Leg pain intensity</td>
<td>6.5 (1.9)</td>
<td>6.8 (1.6)</td>
<td>7.7 (2.1)</td>
</tr>
<tr>
<td>Hopkins symptom checklist-25</td>
<td>1.36 (0.3)</td>
<td>1.36 (0.3)</td>
<td>1.71 (0.3)</td>
</tr>
<tr>
<td>Oswestry disability index</td>
<td>37.7 (15.4)</td>
<td>38.4 (14.7)</td>
<td>55.2 (11.2)</td>
</tr>
</tbody>
</table>

Values as mean (SD). *Median values (IQR).
NP – neuropathic pain. LBP – low back pain.

Table 2 - Agreement between painDETECT and 2016 NeuPSIG system

<table>
<thead>
<tr>
<th></th>
<th>2016 NeuPSIG grading system of neuropathic pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definite</td>
</tr>
<tr>
<td>painDETECT</td>
<td></td>
</tr>
<tr>
<td>Likely NP</td>
<td>8</td>
</tr>
<tr>
<td>Uncertain</td>
<td>15</td>
</tr>
<tr>
<td>Unlikely NP</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
</tr>
</tbody>
</table>

NP - neuropathic pain.

Conclusions:

Compared to the NeuPSIG grading system, the ability for painDETECT to classify neuropathic pain components in patients with painful low back radiculopathies is questionable.
CLINICAL: DIAGNOSIS / ASSESSMENT - PART 2

NEUP7-0051
THE EFFECTIVENESS OF QUANTITATIVE SUDOMOTOR AXONAL REFLEX TEST IN THE DIAGNOSIS OF COMPLEX REGIONAL PAIN SYNDROME

B.K. Cheon¹, J.H. Kim¹, Y.C. Kim², P.M. Lee¹
¹Konkuk University Medical Center, Department of Anesthesiology and Pain Medicine, Seoul, Republic of Korea
²Seoul National University Hospital, Department of Anesthesiology and Pain Medicine, Seoul, Republic of Korea

Background and Aims:
Quantitative sudomotor axonal reflex test (QSART) is known to be useful to evaluate the sudomotor function to some extent. The aim of this study was to evaluate the usefulness of a QSART for making the diagnosis and the treatment strategy of Complex regional pain syndrome (CRPS).

Methods:
We conducted a retrospective study on 92 patients who had been diagnosed with CRPS (CRPS group) and 94 patients who had been diagnosed with neuropathy without CRPS (non-CRPS group). The diagnostic value of QSART for CRPS was evaluated. The abnormality of QSART, the relationship of symptom/sign and QSART, each parameter of the QSART were analyzed between CRPS group and non-CRPS group.

Results:
Eighty-one patients (88.0%) in CRPS group and seventy-six patients (80.9%) in non-CRPS group presented the abnormal results of QSART. There was no significant difference of abnormal QSART results.

Only 48.3% of patients (43/89) reported their abnormal sweating and physician detected abnormal sweating in 65.2% (58/89) of CRPS group. In the results of QSART, there was no difference of total volume and baseline of sweating. However, the response latency (227.56 ± 86.97 sec) of CRPS group was longer than that of the control group (190.97 ± 100.55 sec, $P = 0.01$), and the ending offset of CRPS group (65.08±65.53 nl/min/cm²) was higher than the control group(46.74 ± 52.87 nl/min/cm², $P = 0.04$).

Conclusions:
CRPS cannot be diagnosed only by QSART. However, QSART showed objective and quantitative abnormality of sweating in CRPS patients.
Background and Aims:

There is little knowledge about possible orofacial somatosensory consequences of diabetic polyneuropathy (DPN). The aim of this study was to evaluate the trigeminal nociceptive function and the oral somatosensory profile of patients with DPN.

Methods:

This case-control study enrolled twenty-four participants: DPN patients (n=12) and healthy controls (n=12). The nociceptive blink reflex (nBR) was recorded applying an electrical stimulation over the entry zone of the right supraorbital (V1R), infraorbital (V2R) and mental (V3R) and left infraorbital (V2L) nerve. The outcomes were: individual electrical sensory ($I_0$) and pain thresholds ($I_P$); root mean square (RMS), area-under-the-curve (AUC) and onset latencies of R2 responses. Furthermore, a standardized full battery of quantitative sensory testing (QST) was performed on the oral mucosa. ANOVA, T-test for independent samples and X² test were applied to compare, respectively, the nBR parameters, QST values and the distribution of somatosensory abnormalities between groups.

Results:

The mean age (SD) of the DPN patients (8 women, 4 men) and healthy controls (7 women, 5 men) were, respectively 63 (7.0) and 59.5 (9.1) (p=0.307). The DPN patients presented higher $I_0$, $I_P$, RMS and AUC values (p<0.027) and lower warm detection threshold (WDT) (p=0.004) and higher number of paradoxical heat sensation (PHS) (p=0.027) than the healthy controls. However, the distribution of somatosensory abnormalities was not significantly different between the groups (p>0.050).

Conclusions:

It seems that the trigeminal nociceptive function but not the oral somatosensory profile is changed in DPN patients.
NEUP7-0235

DIAGNOSTIC ACCURACY OF LASER EVOKED POTENTIALS IN DIABETIC NEUROPATHY

S. La Cesa1, G. Di Stefano1, C.M. Leone1, E. Galosi1, A. Pepe1, A. Biasiotta1, G. Cruccu1, A. Truini1

1“Sapienza” Università di Roma, Neurology and Psychiatry Neuromuscular disease, Roma, Italy

Background and Aims:

Although the most widely agreed neurophysiological tool for investigating small fibre damage is laser evoked potential (LEP) recording, no study has documented its diagnostic accuracy. In this clinical, neurophysiological and skin biopsy study we collected age-corrected LEP normative ranges, verified the association of LEPs with pinprick sensory disturbances in the typical diabetic mixed-fibre polyneuropathy, affecting large fibres and small fibres, and assessed the sensitivity and specificity of LEPs in diabetic small-fibre neuropathy.

Methods:

From 288 LEP recordings from the face, hand and foot in 73 healthy subjects we collected age-corrected normative ranges for LEPs. We then selected 100 patients with mixed-fibre diabetic neuropathy and 25 patients with possible small-fibre diabetic neuropathy. In the 100 patients with mixed-fibre neuropathy we verified how LEP abnormalities were associated with clinically evident pinprick sensory disturbances, and in the 25 patients with possible pure small-fibre neuropathy, using the skin biopsy as a reference standard, we calculated LEP sensitivity and specificity.

Results:

In healthy participants, age strongly influenced normative ranges for all LEP variables. By applying age-corrected normative ranges for LEPs, we found that LEPs were strongly associated with pinprick sensory disturbances. LEPs yielded 78% sensitivity and 81% specificity in the diagnosis of diabetic small-fibre neuropathy.

Conclusions:

Our study, providing age-corrected normative ranges for the main LEP data and their diagnostic accuracy, helps to make LEPs more reliable as a clinical diagnostic tool, and proposes this technique as a less invasive alternative to skin biopsy for diagnosing diabetic small-fibre neuropathy.
Background and Aims:

Different courses of disease are observed in complex regional pain syndrome (CRPS). The aim was to characterize symptoms in the course of disease using established and validated self-reported questionnaires at baseline and after 12-24 months.

Methods:

Data collected from CRPS patients routinely seen between 2005 and 2013 by pain specialists or general practitioners in Germany was analyzed retrospectively. Data included information about medical history, pain intensity and the following questionnaires: PainDetect questionnaire (PD-Q), Mainz Pain staging system, Hannover Functional ability questionnaire and Patient Health Questionnaire 9.

Results:

Upon baseline a neuropathic pain component was observed in 56/82 (68.3%) of the patients, the average pain intensity was 60.8 (NAS, 0-100) and 43/60 (71.7%) of patients reported an impaired functionality in daily routine tasks (of which 19/60 (31.7%) reported significant impairment). 77/91 (84.6%) scored positive for presence of depression.

In the course of the disease there was an improvement of the mean (60.8 vs. 52.9; p ≤ 0.01) and maximal (81.2 vs. 73.1; p ≤ 0.01) pain intensity. The frequency of a neuropathic pain component as well as pain characteristics upon PD-Q were unchanged. There was a tendency towards an improvement of functional limitations and depressive symptoms (p = n. s.).

Conclusions:

Despite an improvement of pain intensity within the course of the disease, changes regarding presence of a neuropathic pain component, functional limitations, and depressive symptoms were not observed. This suggests to include improvements of functionality and emotional well-being into treatment evaluation.
Background and Aims:

There is little information on the usefulness of magnetic resonance imaging (MRI) in the management of neuropathic pain (NP). We compared prospectively the contribution of MRI to the management of patients with and without NP.

Methods:

Patients (n=81): Mean age 56 ±14, 42 females. Standardised neurological examination in all. PNS (n=40) (32 polyneuropathies, 8 focal PNS), CNS (n=25) and combined CNS&PNS disease (n=16), each subdivided in painful (NP+) and painless (NP-). Total NP+ (n=59), NP- (n=22).

MRI (n=81): Siemens Symphony 1.5T. Sequences were T1, T2, DP, FLAIR, Diffusion and T2GRE for the brain and T1, T2, T2STIR, and T2CISS for the whole spine, with and without Gadolinium.

MRI classification: Concordant when diagnostic/consistent with the initial clinical diagnosis, Discordant when findings not pathologically significant, unexpectedly normal, or when unsuspected significant pathology was found related or unrelated to the neurological diagnosis.

Fisher’s exact test was used to compare proportions. Ethical Committee approvals were obtained.

Results:

Concordant MRIs (n=62): NP+ 79%, NP- 77% (p=1.0); proportions of NP+ and NP- also not significantly different within the PNS, CNS and CNS&PNS groups.

Discordant MRIs (n=26): NP+ 30.5% NP- 36.3% (p=0.8); proportions also not significantly different within the above groups. 13/26 showed significant unsuspected pathology (10 NP+, 3 NP-). MRI changed the initial anatomopathological diagnosis in 11/81 patients (13.5%) (8NP+, 3NP-).
Conclusions:

22% of patients with neuropathic pain had complex pathology involving both the PNS and CNS. The diagnostic yield of MRI was similar in NP+ and NP- patients. Neurological and MRI assessments are essential for patients with neuropathic pain, as for patients with painless but similar pathology.

*Fondecyt1120339: LAcevedo, GBarraza, MCampero, JLCastillo, GCavada, RGuiloff, JHoneyman, RHughes, JMMatamala EMullins, POrellana, CRamirez, HRojas, CRomero, ISazunic, RVVerdugo, YWang
Background and Aims:

Pain diagnostic relies nearly exclusively on self-reports of patients. This is prone to large variations even for testing of one and the same patient. A method to visualize peripheral correlates of pain, its location, and/or its intensity is currently missing. The "prostate specific membrane antigen (PSMA)" is widely used for prostate cancer diagnostics. But beyond cancer, PSMA is an enzyme, which increases the local concentration of the neurostimulatory transmitter, glutamate, and has been shown to be involved in chronic pain. If PSMA tracer can detect peripheral pain states is unknown.

Methods:

Positron Emission Tomography (PET) scanning; mouse models of complete Freund adjuvant (CFA)-induced inflammatory as well as spared nerve injury (SNI) induced neuropathic pain;

Results:

We find the PSMA-tracer to localize to the side of CFA-induced inflammatory as well as SNI-induced neuropathic pain in mice. Beyond the enrichment of the tracer at the location of pain, the tracer intensity was highly correlated with mechanical hypersensitivity of the hindpaw. Application of the tracer to an anecdotal pain patient revealed strong enrichment in the affected lumbar dorsal root ganglia as well as elucidated a previously unreported chronic pain in the upper trunk.

Conclusions:

Our animal study as well as the patient data suggest that the PSMA-tracer might be used to detect the localization and the intensity of peripheral inflammatory and neuropathic pain.
Background and Aims:

Introduction In the neurophysiological assessment of patients with neuropathic pain, laser evoked potentials (LEPs) and contact heat evoked potentials (CHEPs) are widely agreed as nociceptive specific responses; conversely, the nociceptive specificity of evoked potentials by surface concentric electrode (PREPs) is still debated.

Methods:

Methods In this neurophysiological study we aimed at verifying the nociceptive specificity of PREPs. We recorded LEPs, CHEPs and PREPs in ten healthy participants, before and after epidermal denervation produced by prolonged capsaicin application. We also used skin biopsy to verify the capsaicin-induced nociceptive free nerve ending loss.

Results:

Results We found that while LEPs and CHEPs were suppressed after capsaicin-induced free nerve ending loss, PREPs did not significantly changed. Skin biopsy proved epidermal denervation due to capsaicin application.

Conclusions:

Conclusion The suppression of LEPs and CHEPs after epidermal denervation confirms that these techniques are selectively mediated by nociceptive pathways. Conversely, the lack of PREP changes after free nerve ending loss suggests that PREPs do not provide selective information on nociceptive system function.
Impact of Complex Regional Pain Syndrome on the Quality of Life

R. Cerny¹, J. Kozak², H. Marcisova¹
¹2nd Faculty of Medicine- Charles University, Neurology, Prague, Czech Republic
²2nd Faculty of Medicine- Charles University, Department of Rehabilitation and Sports Medicine, Prague, Czech Republic

Background and Aims:

Complex Regional Pain Syndrome (CRPS) is a debilitating chronic disease. Recent hypotheses consider this syndrome as a form of central neuropathic pain in which remodeling of cortical somatosensory and pain-related areas leads to abnormal pain perception and severe autonomic dysregulation. The aim of the study is to evaluate its impact on the quality of life (QOL).

Methods:

QOL in a group of 40 chronic patients treated at the Center for Treatment and Study of Pain, University hospital Motol was measured by European Quality of Life scale EQ-5D-3L. EQ-5D evaluates 5 parameters in 3 level each and visual analogue scale.

Results:

We found profound decrease of QOL in CRPS. In comparison with control group, QOL index and visual analogue scale decreases of more than 50%.

Further, two distinct groups of patients could be differentiated — more affected group with QOL index < 0.4 and less affected one with QOL index > 0.5. Worse patients had longer duration of disease and higher overall pain intensity. Linear model analysis of the five QOL parameters (motion, self care, activities of daily living, pain, anxiety/depression) showed, that pain and self care parameters have highest impact on the QOL in this disease.

Conclusions:

There is a great and statistically significant decrease of QOL in CRPS patients.

The results suggest, that more affected patients should be actively searched for.

Their identification is not possible without implementation of QOL assessment in the practice. There is a CRPS subgroup with a need for more intensive treatment and support.
Background and Aims:

Laser-evoked brain potentials (LEPs) are used extensively as a clinical tool to diagnose neuropathic pain. Yet, the obtained responses are related exclusively to the perception of transient pain triggered by the activation of quickly-adapting thermonociceptors. In fact, except if the activation of A-delta fibers is avoided or if A fibers are blocked, LEPs reflect only activity related to the activation Type 2 quickly-adapting A fiber mechano-heat nociceptors (AMH-2). Here, we propose a novel non-invasive electroencephalographic (EEG) method to isolate brain activity related to the activation of C fibers and the perception of tonic heat pain.

Methods:

Surface EEG was recorded in 14 healthy participants. A temperature-controlled laser stimulator was used to generate long-lasting (75 s) very slow (0.2 Hz) periodic rises and decreases of skin temperature between baseline and 50°C. The stimuli were applied to the left and right hand dorsum.

Results:

In a first experiment, we show that the sustained periodic activation of thermonociceptors generates a robust periodic EEG response at 0.2 Hz and its harmonics (Fig. B), as well as a
periodic modulation of theta, alpha and beta-band oscillations. In a second experiment, we show that these EEG responses are unaffected by an A-fiber block and thus demonstrate that they are predominantly conveyed by unmyelinated C fibers.

Conclusions:

The proposed EEG frequency-tagging technique constitutes a novel mean to study C fiber function in humans, and to explore the cortical processing of tonic heat pain in physiological and pathological conditions.
Background and Aims:

The aim of this study was to explore the spectrum of sensory abnormalities in clinically diagnosed adults with neuropathic pain (NP) using quantitative somatosensory testing methods followed by Somatosensory Rehabilitation Protocol (SRP) developed by Spicher.

Methods:

A retrospective analysis of 31 subjects with NP associated with varying sensory abnormalities was conducted. Somatosensory Testing and Rehabilitation (SSTR) was performed by a specially trained Physiotherapist. The subjects were assessed and diagnosed using Somatosensory Qualifiers, McGill Pain Questionnaire, DN4 Questionnaire, VAS and other specific tests for mapping the prognosis and were characterized under 4 different Neuropathic Dysfunctional Categories (NDC) followed by SRP based on the type of NDC. Tests were repeated 1 month post rehabilitation for determining the reduction of symptoms and follow up after 3 months.

Results:

Among 31 patients, 75% of the participants were male and in the age group of 30-40 years. 71% reported pain as the major symptom, 32% burning sensation, 20% tingling sensation, 163% pulling, electric shock like, parenthesis and hypersensitivity. 96.8% of the patients had involvement of lower extremity (LE). Posterior antebrachial cutaneous nerve was the most commonly affected nerve in the upper extremity and medial cutaneous nerve was most commonly affected in the LE. The most commonly affected regions were foot, heel and sole with burning, tingling and numbness as major symptom and elbow and ankle least affected. 18 subjects recovered completely post rehabilitation and maintained the progress during the follow-up, while 13 recovered partially.

Conclusions:

SSTR is an effective diagnostic and rehabilitation method for individuals with NP.
A COMPARATIVE STUDY ON THE HIGHEST EDUCATIONAL STATUS AMONG PATIENTS WITH CHRONIC NON-MALIGNANT PAIN AND THE GENERAL POPULATION IN DENMARK - IS THERE A DIFFERENCE?

A. Bendiksen¹, K. Bested¹
¹Friklinikken, Pain Clinic, Give, Denmark

Background and Aims:

Denmark is a country with good educational possibilities. According to Danish Statistics (2003-2015) 28% of the population between 30 and 69 years have primary and lower secondary school, 6% high school, 37% a vocational education and 28-35% a further education (unspecified). With this study we want to analyze whether the educational level differs for patients with chronic pain referred to a free pain clinic compared to the general population. Multidisciplinary pain treatment in Denmark is without cost to the patient.

Methods:

All patients referred to The Multidisciplinary Pain Clinic, Give, Region South Denmark, December 2016 were included. 204 patients, 72 men, 132 women, average age 48 years (19-90), 89% ethnic Danish. The highest completed education for each patient was registered.

Results:

No schooling 2%, primary or lower secondary school 28%, high school 5%, vocational education (e.g. carpenter) 24%, other short-term education of 1-3 years (e.g. clerk) 20%, further education of 3-5 years (e.g. nurse, teacher) 18%, university education of more than 5 years (e.g. doctor, lawyer) 3%.

Conclusions:

No significant difference was seen in educational level between patients with chronic pain and the general population in Denmark. The proportion of unskilled persons without further education was the same. At Danish Statistics further education was unspecified which made the comparison somewhat difficult. Moreover we had a wider range of age, though with almost the same average. Anyway our conclusion is: The educational status of patients with chronic non-malignant pain in Denmark is comparable to the general population.
Background and Aims:

Oxaliplatin-induced peripheral neuropathy (OIPN) affects the health-related quality of life (HR-QOL) of patients during chemotherapy, but little data are available on the prevalence of OIPN after the end of the chemotherapy. This study aimed to assess the prevalence of OIPN and consequences until 5 years after the end of an adjuvant oxaliplatin based chemotherapy.

Methods:

This cross-sectional study was deployed in 4 hospital centers in France. Patients were selected according to the following inclusion criteria: FOLFOX adjuvant chemotherapy stopped since 1 to 5 years and colorectal cancer. OIPN was assessed with the QLQ-CIPN20, HR-QOL with the QLQ-C30 and anxiety and depression with the HADS questionnaire. Neuropathic pain was assessed with the DN4 interview questionnaire (pain score ≥4/10).

Results:

On the 3rd January 2017, 114 patients accepted to answer the questionnaires. Between 10% (5th year) and 38.9% (1st year) of patients suffered of sensory neuropathy. Scores of sensory neuropathy were correlated with scores of HR-QOL (global health, functional and symptoms dimension) and with scores of anxiety. Neuropathic pain was identified in 0% (5th year) and 16.7% (1st year) of patients, and was related to scores of sensory neuropathy. No patient was treated by pregabalin nor duloxetine. One patient was treated by gabapentin.

Conclusions:

Even if neuropathic symptoms seemed to improve with time, these preliminary results have demonstrated a high prevalence of OIPN until 5 years after the end of chemotherapy. This study is still ongoing and should emphasize the strong consequences of OIPN and the emergency to find effective management strategies.
Background and Aims:

Neuropathic pain (NeuP) is a major health burden affecting approximately 8% of the population. Smoking, obesity, older age, and genetic factors have all been linked to NeuP, but not everyone with these factors develops the disorder and those that do have variable severity and response to treatment. Therefore, the aim of this study is to identify demographic, clinical, psychosocial and genetic risk factors associated with NeuP and examine their exact contribution to disease onset.

Methods:

Participants in two cohorts, Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS – a diabetic population, n = 5,242) and Generation Scotland: The Scottish Family Health Study (GS:SFHS – recruited from the general population, n =~15,000), with rich phenotypic and GWAS data already available, were posted a self-complete questionnaire. The questionnaire contains the DN4 and S-LANSS for NeuP assessment, as well items relating to pain severity and catastrophizing, neuropathy, alcohol, smoking, depression, anxiety, sleep disturbance, trauma and personality traits.

Results:

In an initial pilot study of 196 randomly selected GoDARTS participants, 71 participated (36%). Response rates for all questionnaire items were >80%. For the main mailing of the GoDARTS cohort, 1,743 (33%) have returned completed questionnaires. Mailing for GS:SFHS is currently ongoing and main results from analyses of the questionnaire data for both cohorts will be presented.

Conclusions:

This study will help identify individuals at increased risk of developing NeuP, contribute to the development of NeuP specific drugs which target the identified biological pathways and in turn ease the burden on patients and healthcare services.
Background and Aims:

Refractory neuropathic inguinodynia following inguinal herniorrhaphy is a common and debilitating complication. This prospective study evaluated clinical and neurophysiological outcomes associated with laparoscopic retroperitoneal triple neurectomy (LRTN).

Methods:

Sixty-two consecutive chronic postherniorrhaphy inguinal pain (CPIP) patients (51 male; mean age, 47); all failing pain management; prior reoperation in 35, prior neurectomy in 26; average follow-up 681 days (range, 90 days to 3 years); were included. Pain severity, activity level, analgesic consumption and complications were assessed longitudinally. A subset of 10 patients underwent comprehensive longitudinal quantitative sensory testing (QST).

Results:

LRTN provided robust group-level improvements of all clinical measures. Mean numerical pain scores were significantly decreased (baseline, 8.6) at all postoperative time points (postoperative day (POD) 1, 3.6; P < .001: POD 90, 2.3, P < .001) with durable efficacy from POD 90 to 3 years (P < .001). QST revealed marked increases in mechanical, pressure, thermal, and pain thresholds in the areas with maximum pain prior to LRTN surgery for the immediate (p<0.01) (160.9 min (range, 103-255 min) after extubation) and late postoperative (p<0.05) (27.9 days (range, 14-78 days) after surgery) assessments compared to baseline. Wind-up phenomena were eliminated postoperatively. No preoperative QST variables were found to be predictive of surgical outcomes.

Conclusions:

Retroperitoneal triple neurectomy is an effective and durable treatment for refractory neuropathic inguinodynia. LRTN may produce immediate and consistent positive effects across multiple mechanical, pressure, and thermal QST variables; these data contribute to the understanding of mechanisms involved in the success of LRTN.
TREATMENT OF TRIGEMINAL NEURALGIA IN PATIENTS WITH MULTIPLE SCLEROSIS BY RADIOFREQUENCY ON THE GASSER’S GANGLION

C. Batet¹, M. Lleixa², R. Armand¹, C. Gracia¹, S. Marmaña¹, P. Magalló¹, M. Moncho¹, R. Chacón¹, E. Mora², J. Masdeu²
¹SJD Moisés Broggi Hospital, Anaesthesiology, Barcelona, Spain
²SJD Moisés Broggi Hospital, Neurology, Barcelona, Spain

Background and Aims:

Multiple Sclerosis (MS) is a demyelinating and inflammatory disease affecting the central nervous system. Danesh-Saini et al, evaluated 500 patients with MS, of whom 88.6% had orofacial affections and 7.9% presented Trigeminal Neuralgia (NT). Pain in the NT follows the distribution of one or more divisions of the nerve and the type of pain is acute, superficial and stabbing. The prevalence range is from 1.9% to 4.9%, but patients with MS have a 20% higher risk of developing NT, compared to the general population.

Methods:

We studied 2 patients with long-term MS who had NT. The first was a 66-year-old male (1P) and the second was a female of 51 (2P). We applied Thermal RF (RFT) for 3 minutes. One minute 60 and two minutes 70 degrees. We have controlled the patients the 1st, 3rd, 6th month and 1 year after treatment.

Results:

<table>
<thead>
<tr>
<th></th>
<th>1º P before Treatment</th>
<th>2º P before Treatment</th>
<th>Post-RF Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVS</td>
<td>10</td>
<td>10</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Difficulty talking</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Difficulty washing</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Difficulty chewing</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Pain in the touch</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>NEU medication</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Global Rating

It values from 0 to 10: 0 no difficulty and 10 maximum pain or difficulty Persist > 1year

Conclusions:

Patients with Multiple Sclerosis have an increased risk of Trigeminal Neuralgia. The application of Thermal Radiofrequency on the Gasser’s Ganglion, allows to quickly decrease the medication with Carbamazepine and improve the symptomatology quickly.
Background and Aims:

Most patients treated with TENS therapy for chronic neuropathic pain complain of discomfort related to the device and their skill to adjust the available stimulation programs. The aim of the “actiTENS®” is to be most easy to use for patients every day.

Methods:

The actiTENS®, thin (8mm) and flexible device is directly worn on the skin. Miniaturized and smartphone controlled, it adapts itself at the morphology of each patient and deliver 9 different stimulation programs allowing both direct gate-control stimulation and endorphinic diffuse stimulation. When the program selected by the therapist is not sufficiently satisfactory, the patient is allowed to follow a next choice along an algorithm including the characteristics of pain, the duration of sessions, the intensity and setting of stimulation. The changes of settings are recorded and availables in the smartphone.

Results:

We conducted twelve interviews with patients suffering from neuropathic pain, twenty with doctors or nurses specialized in chronic pain management, ans 28 head of pain clinics. The goal was to assess the different points of satisfaction or dissatisfaction for patients and caregivers. Both patients and caregivers emphasize a great interest in the actiTENS® presented at the end of the interview, and particularly the possibility of modifying the program by a customised algorithm is much appreciated.

Conclusions:

The actiTENS® lets avoid the physical and psychological discomfort usually encounter with classical TENS devices. After an education session, the patient will be totally independent and will be able to return to a comfortable everyday life.
Background and Aims:

Spinal cord stimulation (SCS) is a proven and effective treatment for neuropathic pain conditions, such as failed back surgery syndrome (FBSS). Recently a novel form of neurostimulation has emerged: “high density” (HD) stimulation, which is characterized by subthreshold stimulation with higher energy (pulses/second) and pulse density compared to conventional SCS. The main objective of High-Density In Spinal Cord stimulation: Virtual Expert Registry (DISCOVER) is to evaluate safety and efficacy of HD-SCS.

Methods:

Participants who will be included are (1) patients with insufficient pain relief from conventional SCS and (2) patients suited for neurostimulation according to good clinical practice (GCP). HD stimulation settings are used as prescribed for each individual neurostimulator. Assessments will occur at 1 month, 3 months, and 12 months after implantation of the internal pulse generator (IPG). Patients with already an IPG will be assessed 1, 3 and 12 months after conversion to HD stimulation parameters. Each patient visit will include: a numerical rating scale (NRS), oswestry disability index (ODI), Pittsburgh sleep quality index (PSQI), a pain map, SCS settings, SCS parameters and a list of used pain medication.

Results:

Main results are anticipated in 2019.

Conclusions:

DISCOVER will be the largest prospective, multicenter HD-SCS study conducted to date. The study results will provide insight about the clinical efficacy and safety of HD SCS. Along with the primary outcome (NRS) and secondary outcomes (PSQI, EQ-5D, ODI, pain medication, pain map), feasibility endpoints will be noted during the one year follow-up.
Background and Aims:

Repetitive transcranial magnetic stimulation (rTMS) of the motor cortex has been proposed as a novel method for pain relief in neuropathic pain. We studied the safety and tolerability of long-term rTMS in a group of chronic neuropathic pain patients.

Methods:

21 patients with chronic neuropathic pain entered a long term open label study. All had previously participated in efficacy trials and were considered responders. Each patient filled in a 10-item graded adverse effects questionnaire at regular intervals and reported any adverse effects they had noted since the previous rTMS session.

Results:

14 of 21 continue to attend rTMS sessions at intervals of their choosing. The median follow up of these 14 patients so far is 18 months (range 6–20), and the median number of sessions per patient 24 (range 19-71). 4 of the initial 21 patients withdrew early because of lacking efficacy. 3 benefited from rTMS but withdrew: 1 because of tinnitus (no causality shown); 1 to have neurosurgical treatment for her pain, and 1 because of travelling difficulties.

Adverse effects were transient and usually developed immediately after a session for 1-3 days. One-half of the patients reported worsening of their pain after one or more sessions, while 42% reported somnolence and 38% headache. No patient suffered a seizure.

Conclusions:

Long-term rTMS treatment for chronic neuropathic pain is safe and well tolerated. The majority of responders also comply well with the requirement of regular attendance.
INVESTIGATION OF SENSORY SYMPTOMS ASSOCIATED WITH TRANSCRANIAL MAGNETIC STIMULATION (TMS) IN HEALTHY VOLUNTEERS AND PATIENTS WITH NEUROPATHIC PAIN.

B. Frank¹, T. Nurmikko², P. Sacco², A. Mavrianou²

¹The Walton Centre NHS Foundation Trust, Pain Medicine, Liverpool, United Kingdom
²University of Liverpool, Pain Research Institute, Liverpool, United Kingdom

Background and Aims:

We compared subject experiences to stimulation from a commercially available sham TMS coil with that of a real coil, identical in appearance, specifically designed for double-blind studies (Nexstim).

Methods:

14 pain patients and 10 controls attended two randomised sessions of real or sham navigated rTMS over 4 sites (500 pulses): (1) 1Hz left M1 90% RMT, (2) 10Hz right M1 90% RMT, (3) 10Hz left DLPFC 110% RMT and (4) 10Hz right S2 110%. Participants rated sensations in the scalp, face and neck on a Numerical Rating Scale (0-10).

Results:

Scalp and facial sensations were reported for 89% of stimulation sites, but rarely in the limbs (3%). After 1Hz M1 stimulation, 22 subjects reported mild/no pain for real vs 24 with sham coil (p=0.49, Fisher's exact test). After 10Hz M1 stimulation, mild/moderate pain was reported by 20 receiving sham and 24 real stimulation (p=0.11). Conversely, scalp sensations differed significantly when rTMS was applied over the two other sites, with severe pain reported more frequently after real DLPFC (8 vs 1, p=0.02) and S2 (9 vs 0, p=0.001) stimulation.

Conclusions:

Blinding is secure in parallel group study participants naive to rTMS if stimulation is applied at 1Hz, 90% RMT over M1 (stroke rehabilitation) and 10Hz, 90% RMT over M1 (pain). The participants should be instructed to expect slightly or moderately painful scalp sensations. In cross-over studies blinding is likely to be lost. High levels of pain associated with DLPFC and S2 stimulation are problematic, and adjustment of stimulation parameters is needed for adequate blinding.
Background and Aims:

Motor cortex stimulation (MCS) was introduced as a last-resort treatment for chronic neuropathic pain. Over the years, MCS has been used for the treatment of various pain syndromes but long-term follow-up is unknown.

Methods:

To report the results of MCS in patients with chronic neuropathic pain with a 3-year follow-up. The analgesic effect was determined as successful by decrease in pain-intensity on the visual analog scale (VAS) of at least 40%. The modifications in drug regimens were monitored with use of the medication quantification scale (MQS). Stimulation parameters and complications were also noted. Interference of pain with quality of life (QoL), the Quality of Life Index (QLI), was determined with use of a specific subset of questions from the MPQ-DLV score.

Results:

Eighteen patients were included. Mean pre-operative VAS changed from 89.4 ± 11.2 to 53.1 ± 25.0 after three years of follow-up (z = −3.628; P < 0.000). A successful outcome was achieved in seven responders (38.9%). All patients in the responder group suffered from pain caused by a central lesion. With regard to all the patients with central pain lesions (n=10) and peripheral lesions (n=8), a significant difference in response to MCS was noticed (P = 0.002). Analgesic use, especially opioids (Figure 1) and the QLI diminished during follow-up in all patients.
Figure 1. Differences in the cumulative, total intake of pain medication in milligram per day before and after MCS

Conclusions:

MCS seems a promising therapeutic option for patients with refractory pain syndromes of central origin.
Background and Aims:

Spinal cord stimulation (SCS) often loses treatment effectiveness over time. However, pain assessment resolution is limited because different locations are often grouped in the same category. For instance, “low back pain” may refer to several locations within the lower back. Thus, long term increases in pain ratings may not be due to increasing pain in the same initial location(s). Therefore, we sought to determine the extent that existing pain patterns change over time and degree to which new patterns develop.

Methods:

The RELIEF registry is on-going at multiple sites globally to evaluate real-world outcomes for commercially-approved Boston Scientific SCS systems. At baseline and at follow-up visit, patients use a highlighter to mark areas on a standardized anatomical template where they experienced pain within the past 7 days. Analyses were carried out on these drawings for >100 patients to investigate shifts over time. All drawings were completed using a standardized template, and pixel-by-pixel comparisons were made to examine where pain remained constant, was relieved, and newly appeared.

Results:

We determined how frequently new areas of pain arose within our patient sample. We also characterized the spatial distribution of this new pain, including its distance from areas of preexisting pain, in order to determine how often treatment of new pain would require re-targeting of therapy provided by SCS.

Conclusions:

New areas of pain occurred within 1 year for a subset of patients suggesting that SCS devices with highly adaptable features are likely necessary to address evolving changes in the perception of pain over time.
Background and Aims:

Spinal cord stimulation (SCS) is one of the most evidence-based treatments for patients with radicular type back pain. But the effect of SCS on the sensory profile as well as the peripheral and central sensitisation still remains to be quantified.

Aim: To characterize the sensory profile of patients with FBSS undergoing SCS.

Methods:

A battery of Quantitative Sensory Tests and psychometric measurements using validated questionnaires (PainDetect) were collected before SCS and also at 18 days and 3 months following SCS.

Results:

Twenty three patients underwent the SCS trial of which 20 patients had a successful permanent implant. At baseline, there was high incidence (>50% incidence of moderate to severe sensation) of burning, tingling, electric shock, numbness and pressure pain sensitivity. Following SCS there were significant improvements in burning, tingling, electric shock and pressure pain both at 18 days and 3 months and significant improvement in numbness at 3 months. There was a significant improvement in CPM as measured both at 18 d and 3 months post-SCS. Following SCS there was a normalisation of the CPM. There was also a significant improvement in PPT’s measured at the painful leg.

Conclusions:

In this study we have demonstrated a definite shift in the phenotypic profile of patients following SCS. For the first time we have reported that, following SCS, there was a significant improvement in a number of sensory characteristics including burning, tingling, electric shock, numbness and pressure pain sensitivity. Also following SCS the pronociceptive profile becomes antinociceptive profile based on CPM measurements.
NEUROPATHY — PART 1

NEUP7-0086
TEMPORAL DEVELOPMENT OF OPIOID INDUCED HYPERALGESIA IN SURGICAL PATIENTS
A. Rolle¹, M. Calvo², L.I. Cortinez¹, A. Fernando¹
¹Facultad de Medicina. Pontificia Universidad Católica de Chile, Anestesiología, Santiago, Chile
²Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Neurofisiología, Santiago, Chile

Background and Aims:

Opioid induced hyperalgesia (OIH) is an increased sensitivity to noxious stimuli that occurs after opioids exposure. Effective treatment of postoperative pain can be hindered by the occurrence of OIH. Not much is known about the occurrence of OIH following use of opioids in surgery.

Aim: To characterize the time-course presentation of OIH after surgery.

Methods:

Patients with indication of laparoscopic cholecystectomy were enrolled at the University Hospital (UC Christus) with ethical approval from the local institutional review board. Mechanical Pain threshold (MPT) and Wind-Up ratio (WUR) were measured using PinPrick® stimulators (MRC, Germany) at baselines, and at 2, 4 and 24 hours after surgery. Both tests were applied at the dorsum of the hand, a site far from the operative site. Remifentanil (0.3 mcg/kg/min) was given during surgery, and a bolus of 0.15 mg/kg of morphine was given at the end of surgery. Statistical analysis was done using Quade test.

Results:

We enrolled 8 patients (100% females, ages 23-57 years). We found no difference in MPT after opioids exposure (p=0.55). However, we observed an increase in the WUR, which was apparent at 4 and 24 hours after surgery/opioids exposure (p=0.01).

Conclusions:

We observed hypersensitivity following the use of remifentanil, in a site far from the operative area. We only observed increase in the WUR but not in the MPT, but this can be due to the small size of our sample. However, the appearance of increased WUR suggests that mechanisms of central sensitization are probably responsible of OIH.
IMPACT OF TIMING OF SPINAL CORD STIMULATION ON MEDICATION UTILISATION LONG-TERM: RESULTS FROM THE CPRD DATABASE

N. Hall¹, M. Zahra², S. Eldabe³
¹James Cook University Hospital, Pain Management, South Teeside, United Kingdom
²Medtronic International, Statistics, Tolochenaz, Switzerland
³James Cook University Hospital, Pain Management, South Teeside, United Kingdom

Background and Aims:

Failed back surgery syndrome (FBSS) is the persistence of back and or leg pain 6 months following on from an anatomically successful surgery. Patients with FBSS typically experience pain, disability and reduced quality of life.

The initial management of FBSS is conventional medical management (CMM). This includes pharmacological management through the use of opioids, NSAIDs, antidepressants, and anticonvulsants/antiepileptic. There are many problems associated with long-term use of these medications.

We aim to determine if spinal cord stimulation (SCS) and the timing of therapy initiation reduces patient medication use.

Methods:

Data was analysed from primary and secondary care from the UK (CPRD and HES).

A retrospective cohort study was undertaken identifying 10,901 patients with at least 24 months of follow-up data. 20.8% of these patients had FBSS with 85 patients receiving SCS therapy.

FBSS patient’s criteria included:

Additional lumbar surgery (6-24 months post-index)  AND/OR

Pain-related physician visits (spanning 6 months from 6-24 months post-index) AND/OR

Another surgical intervention to address pain (any time post-index)

The impact of SCS on medication use was measured in pre- and post-SCS patients between a 2 and 4 year period.

85 SCS patients were sorted by time to treatment. Following exclusion of patients at extreme and near mean time intervals, two distinct cohorts of 30 patients each were obtained

Results:
A declining drug use at 2 and 4 years post implant:

Early SCS therapy shows a significant declining drug use:

Conclusions:

SCS therapy reduces medication use in SCS patients. There is a steady rate of decline in use over 4 years demonstrating a sustained impact of SCS therapy.
Background and Aims:

This study characterizes the impact of increasing-intensity treadmill exercise (iTR) on noradrenergic (NE) and serotonergic (5HT) modulation of neuropathic pain. Spared nerve injury (SNI) and sciatic nerve transection and repair (SNTR) were used as different neuropathic pain models.

Methods:

iTR was performed during the first 2 weeks after injury. The effects of iTR were analyzed after SNTR at both lumbar spinal cord and midbrain periaqueductal grey (PAG), locus coeruleus (LC), raphe magnus (RM) and dorsal raphe nucleus (DRN).

Results:

Mechanical and thermal hyperalgesic responses at medial injured paw territory were rapidly reduced by iTR in both models. The expression of α1A- and β2-, 5HT2A but not α2A-adrenergic receptor, was strongly decreased in dorsal horn at 2 weeks after SNTR, iTR significantly recovered it. iTR increased 5HT2A-receptor expression in PAG, DRN and RM. iTR also strongly increased the expression of α1A-receptors in LC and DRN, and β2-receptors in LC. iTR hypoalgesia was antagonized by pharmacologically blockade of β2- and 5HT2A-receptors. DSP4, a neurotoxin for LC NE neurons, worsened mechanical hyperalgesia, but iTR similarly produced hypoalgesia in DSP4-injected rats. 5HT2A-receptor is increased in LC by iTR, this effect unchanged after Butoxamine treatment but strongly increased after DSP4 injection. iTR reduced microglial reactivity in LC, along to increasing non-microglial BDNF expression, and this was reverted by Butoxamine.

Conclusions:

Failing 5HT and NE descending inhibition under neuropathic conditions can be reactivated by iTR to restore spinal pain inhibition, and modifications in BDNF regulation may be also implicated in the central 5HT-NE actions on chronic pain and inflammation.
Background and Aims:

Repetitive transcranial magnetic stimulation (rTMS) is a safe and noninvasive method to stimulate specific areas of the cerebral cortex. rTMS has been proven to relieve neuropathic pain when applied on the primary motor (M1) or secondary somatosensory cortex (S2). At the Turku University Central Hospital, electric field navigated rTMS has been used to treat severe cases of chronic pain since 2011. The object of this study was to analyse the usefulness of rTMS in a clinical setting and to find factors predicting good or poor clinical response.

Methods:

All patients who had undergone therapeutic rTMS for pain in the hospital district of Southwest Finland (n=46) were included in the study. A 3-tesla MRI of the head was taken of each patient in order to acquire a three-dimensional model of the brain. The stimulation was given by an electric field navigated NBS system 4.0 (Nexstim Ltd, Helsinki, Finland). A visual analogue scale (VAS) was used to evaluate the intensity and interference of the pain after each treatment session.

Results:

39% of the patients reported a significant (over 30%) reduction in both the intensity and annoyance of pain. In addition 51% of the patients reported a positive global impression of change (GIC) score.

Conclusions:

Our data suggests that in addition to neuropathic pain, rTMS is viable for CRPS and other chronic pain types. In addition it seems that with navigated rTMS it is possible to get better results compared to non-navigated rTMS.
Background and Aims:

Spinal cord stimulation (SCS) has been proven to be an effective therapy for failed back surgery syndrome when conservative treatment has failed. The descending nociceptive inhibitory pathways, commonly investigated through the conditioned pain modulation (CPM) paradigm, are often malfunctioning in chronic low back pain patients and could possibly be a sustaining factor for chronic pain states. The question arises whether SCS is able to induce an influence on the working mechanisms of the pain system and to restore the possible altered functioning of the descending inhibitory pathways. Therefore the objective is to evaluate the functioning of the descending nociceptive inhibitory pathways in patients with SCS.

Methods:

Four failed back surgery syndrome patients with predominant leg pain (VAS-score >5/10) were included. Electrical stimulations were applied on the Sural nerves at painful intensities and thereafter CPM was induced by the cold pressor test (12°C). Electrical evoked potentials (ERPs) were recorded in the week before surgery and after one month of conventional SCS, using a 32 channel EEG.

Results:

Pre-implantation, endogenous pain inhibition was present in only one patient at the painful side. After one month of conventional SCS, CPM was present in two more patients. ERP analyses before SCS and after one month of SCS revealed no significant differences in amplitude between both assessments during CPM.

Conclusions:

Descending nociceptive inhibitory pathways seem to “fail” in Failed Back Surgery Syndrome, but conventional Spinal Cord Stimulation may influence this top down pain inhibition mechanism.
Background and Aims:

BACKGROUND

Persistent Atypical Facial Pain with no characteristics of cranial neuralgias and not associated with physical signs or a demonstrable organic cause. Usually severe, continuous and persistent from weeks to years. Laboratory tests and scans do not reveal any pathological abnormality.

AIM

The aim of this study was to evaluate effects of rTMS on chronic atypical facial pain.

Methods:

METHODS

35 outpatients raped during childhood and/or adolescence were compared to non-raped 50 outpatients, aged 25 - 40, with chronic atypical facial pain, associated with predominant depression or anxiety, treatment-free for one month.

They were randomly assigned to receive rTMS active (A) or sham-stimulation (S). They were treated during 12 weeks Monday-Friday with high-frequency 10 Hz stimulation applied to the left dorsolateral prefrontal cortex (DLPFC), train duration 4.2 s, intensity of 120%, and 75 trains per treatment session.

Symptoms were measured by Numeric Rating Scale, Hamilton Depression Rating Scale-17 and Hamilton Anxiety Rating Scale, during baseline (T0), after 6th (T1) and 12th (T2) weeks of treatment. Sham-conditions involved lifting the coil off the person’s head, thereby generating sound but no tactile sensation.

Results:

RESULT

Only Atypical Facial Pain and predominant depression remitted in 7 women and 9 men non-raped outpatients that received active rTMS after 12-weeks treatment.

CONCLUSIONS

rTMS high-frequency 10Hz stimulation applied to DLPFC was effective in relieving Atypical Facial Pain associate with predominant depression in our not-raped patients. This treatment is ineffective for anxiety. Raped-patients do not obtain improvement if the trauma caused by rape isn't treated.
Utilization of Multiple Spinal Cord Stimulation Waveforms in Chronic Pain Patients

A. Berg¹, D. Huynh², R. Jain²
¹Spine Team Texas, Pain Management, Rockwall, USA
²Boston Scientific Corporation, Clinical Research, Valencia, USA

Background and Aims:

The variability and plasticity inherent in chronic pain disorders suggests that spinal cord stimulation (SCS) systems must be flexible to modify targets, provide alternative stimulation patterns to the same target, or both. To evaluate the extent chronic pain patients take advantage of the availability of multiple neurostimulative waveforms provided from a single device, we performed a real-world device utilization registry study of patients implanted with a device capable of delivering multiple waveforms in addition to standard rate stimulation.

Methods:

Data was derived from a database of clinical programmers from an unbiased cohort of 250 patients. Variables collected included: stimulus amplitude, frequency, current fractionalization, patient program usage, and program changes. Patients had access to three non-standard waveform programming types in addition to standard rate stimulation including at least 1000 pulses per second (1 kilohertz stimulation), fractionalization of cathodic current away from target while intensifying anodal current near target region (anodal intensification [AI]), and burst stimulation.

Results:

Approximately 60% of patients utilized multiple, non-standard waveforms at least 20% of the time, with 11 different waveform combinations observed. Not all patients used standard rate stimulation when using available non-standard waveforms, as some reported using 1 kHz stimulation combined with AI as well as 1 kHz stimulation combined with burst stimulation. We also observed sustained usage of multiple waveform programming, as patients on average reported using 3 programs out to 1 year.

Conclusions:

The results obtained demonstrate that SCS patients do utilize non-standard waveforms for notable proportions of stimulation time if provided the opportunity.
Different approaches to spinal cord stimulation (SCS) can facilitate greater individualization of therapy including higher frequencies \(\text{(e.g., 1 kHz, 10 kHz)}\), other waveforms/modes of neurostimulation \(\text{(e.g., burst, anode intensification)}\), and advances in neural stimulation field targeting \(\text{(e.g., anatomically-guided 3D neural targeting)}\). Utilization of multiple waveforms \(\text{(multiple waveform SCS)}\) is likely to be increasingly important for managing chronic pain given the diversity of patients that benefit from SCS treatment. To examine how chronic pain patients respond when treated using a multiple waveform SCS system, we conducted a case series analysis that is representative of our clinical experience.

**Methods:**

All patients have been implanted with a commercially-approved, multiple waveform SCS system \(\text{(Precision Spectra, Boston Scientific)}\) equipped with multiple independent current control \(\text{(MICC)}\), anatomically-guided \(\text{(3D)}\) neural targeting, and waveform programming options for different modes of stimulation \(\text{ (>1 kHz, burst, and anode intensification)}\) as well as standard rate stimulation.

**Results:**

The results will present baseline information, clinical outcomes, and utilization data of patients implanted with a device capable of multiple waveform SCS.

**Conclusions:**

Patient variability and/or neural plasticity can complicate the effectiveness of SCS. Adaptable SCS systems that deliver different modes and approaches to neurostimulation offer a strategy for addressing patient variation and progression of pain disease. Greater customization of therapy may help improve overall patient experience with SCS. This study thus serves as a preliminary examination of a multiple waveform SCS system in order to contribute to the emergent understanding of how best to personalize SCS treatment in the real world clinical setting.
Background and Aims:

Spinal Cord Stimulation (SCS) trial outcomes have a profound impact on the decision to proceed with permanent implantation or not. To begin investigating the effect of a system capable of Multiple Waveform SCS on the patient trial experience, we examined a cohort who, after enduring a trial failure using an SCS system with stimulation held constant at 10 kHz only, were subsequently switched to a system capable of delivering multiple stimulation waveforms.

Methods:

Patients who failed an SCS trial at 10 kHz stimulation only (Senza, Nevro Corp.) were analyzed after interchanging to a trial stimulator providing standard rate stimulation, anatomically-guided 3D neural targeting, and available programming capabilities using multiple stimulation waveforms (Multiple Waveform SCS) such as 1 kHz, burst, and anode intensification (Precision Spectra, Boston Scientific). Percent pain relief (PPR) as calculated using baseline and post-trial pain scores is reported following trials using both systems. Patient preference data was also collected.

Results:

To date, 20 patients who failed the 10 kHz trial and attempted the Multiple Waveform SCS system trial were analyzed. 40% of patients reported experiencing back pain only. In those patients where NRS/PPR data was available (n = 14), 50% reported ≥50% improvement in pain relief as measured by PPR when using a Multiple Waveform SCS System. Of all the 20 patients, 65% preferred Multiple Waveform SCS.

Conclusions:

An SCS system capable of multiple waveforms and precise targeting capabilities offers the potential for more personalized optimization of stimulation thereby helping to improve trial outcomes in chronic pain patients.
CLINICAL: NEUROMODULATION - PART 2

NEUP7-0061
CHRONIC PAIN PATIENT OUTCOMES USING A NEUROMODULATION SYSTEM WITH AVAILABLE MULTIPLE WAVEFORM PROGRAMMING IN AUSTRALIA

J. Monagle¹, D. Holthouse², N. Christelis³, P. Georgius⁴, M. Russo⁵, M. Green⁶, S. Gupta⁷, K. Lechleiter⁸, R. Jain⁹

¹Berwick Pain Management, Pain Management, Berwick, Australia
²Green Lizard Science, Pain Management, Cottesloe, Australia
³Victoria Pain Specialists, Pain Management, Richmond, Australia
⁴Sunshine Coast Clinical Research, Pain Management, Birtinya, Australia
⁵Hunter Pain Clinic/Genesis Research, Pain Management, Broadmeadow, Australia
⁶Pain Medicine of South Australia, Pain Medicine, Marion, Australia
⁷Boston Scientific Corporation, Clinical Research, Sydney, Australia
⁸Boston Scientific Corporation, Clinical Research, Valencia, USA

Background and Aims:

Spinal Cord Stimulation (SCS), Peripheral Nerve Stimulation (PNS), Peripheral Nerve Field Stimulation (PNFS), Occipital Nerve Stimulation (ONS), and Sacral Nerve Stimulation (SNS) are treatment modalities in which developments are enabling for more patient customization. This is reflected in different approaches to neuromodulation thought to be clinically useful including 1 kHz and 10 kHz rates of stimulation, alternative waveforms of stimulation such as burst and anode intensification, and improved methods of targeting as exemplified by the recent introduction of anatomically-guided (3D) neural targeting technology. This analysis presents a real-world, case series examination of patients using a neuromodulation system with available multiple waveform programming capability on a wide group of patients.

Methods:

This is an observational case series of patients implanted with a multiple waveform SCS system (Precision Spectra, Boston Scientific). The following different modes of stimulation programming are available to the analyzed patients using this device: 1 kHz, variations of burst programs, anode intensification, standard rate stimulation, multiple independent current control (MICC) and an available 3D neural targeting algorithm.

Results:

This study is currently on-going at multiple centres across Australia. Finalized results to be presented and will include clinical outcomes of patients implanted with a multiple waveform neuromodulation system.

Conclusions:

This data seeks to help inform on how SCS, PNS, PNFS, ONS, and SNS can be applied in a more personalized manner and contribute to how future studies and devices might be designed. Results should reflect what outcomes might be reasonably expected when using a multiple waveform SCS system to treat chronic pain patients.
Background and Aims:

Clinical evaluation of a transverse tripole using a 3-column, 4-contact lead suggests an improved usage range in which shielding from lateral anodes was thought to increase dorsal root thresholds compared to dorsal column thresholds (Oakley, et al., 2006). We hypothesize that transverse fields can enable many paresthesia states at a single level on the lead, which may increase ability to optimize coverage. Here we characterize transverse steering in a 4-column, 32-contact lead with a spinal cord stimulation (SCS) system using multiple independent current sources.

Methods:

This study is a prospective, on-label, multi-center, non-randomized, exploratory, single-arm, single visit study. All subjects were previously implanted with an SCS system and 32-contact paddle. The lead level closest to midline was selected for programming and paresthesia distributions were collected. A finite element model with a surgical lead was constructed based on spinal cord anatomy. The spinal cord finite element model was coupled with dorsal column and dorsal root fiber models to predict neural excitation. Computer modeling was used to predict anode and cathode configurations.

Results:

Paresthesia drawings from each configuration were collected and converted to binary images for analysis. When comparing configurations within patients at a single rostral-caudal level, paresthesia area changed at least 17% and up to 100%. Anode-cathode assignment was constant, but percentages of current were changed, paresthesia area changes of at least 37% and up to 100% were observed.

Conclusions:

Initial observations show a large range of possible paresthesia coverage using transverse current steering, and are consistent with the hypothesis and published theory.
Background and Aims:

Low back pain/paresthesia concordance likely occurs when stimulating electrodes are placed at T6 - T7 versus T8 – T12. However, the abdomen is likely uncomfortably stimulated at T6 - T7. Advances in recent spinal cord stimulation (SCS) systems now allow for different waveforms/modes of neurostimulation as well as precise neural stimulation field targeting (e.g. anatomically-guided 3D neural targeting). In this study, we examined how chronic pain patients respond to electrodes/contacts placed at T7 and using a multiple waveform SCS system.

Methods:

Patients underwent an SCS trial and subsequent implant using a multiple waveform SCS system (Precision Spectra, Boston Scientific) with the distal lead placed midline at top of T7. 3D neural targeting was used to maximize pain/paresthesia overlap at standard rates with pulse widths ranging from 210-250 μs. For first 24 hours of the trial, a standard rate program (30-60 Hz) was given. For the second 24 hours of the trial, a new program was created using the 3D algorithm. The program was next simplified, typically using a guarded cathode configuration (2-4 cathodes). Once desired coverage achieved, rate was increased to 1000 Hz, pulse width was decreased to 130 μs, and amplitude was adjusted to a comfortable level using patient feedback. During the final 24 hours of trial, the preferred waveform was used.

Results:

Results will be presented for this ongoing study for consecutively enrolled patients who underwent a trial with a multiple waveform SCS device.

Conclusions:

We postulate that SCS adaptability will be key to improving and maintaining chronic pain patient outcomes.
Background and Aims:

Successful spinal cord stimulation (SCS) therapy in patients with chronic pain may not only improve pain intensity but may also reduce disability and increase activities of daily living (ADL). A recently introduced SCS paradigm using a 3-dimensional (3D) algorithm to customize stimulation (anatomically guided3D Neural Targeting SCS) has enabled SCS treatment of pain areas which have historically been challenging, such as low-back pain. We undertook a large observational study to characterize real world disability and physical, functional outcomes using 3D Neural Targeting SCS out to 2 years post-implant.

Methods:

This is a multi-center, consecutive, observational assessment of 100 subjects using Precision Spectra SCS (Boston Scientific Corporation) for chronic, intractable pain of the low back and/or legs out to 2 years post-implant. The majority of subjects in this sub-study (72%) reported severe pain at baseline. In addition, 62% percent of subjects reported only experiencing low back pain at baseline.

Results:

To date, significant reductions in Oswestry Disability Index (ODI) scores and Numeric Rating Scale(NRS) scores, with increases in walking tolerance and an approximately 90% satisfaction rate, have been observed. Relative to baseline, these reductions include a 21.3 point decrease in mean ODI score, a 51% increase in mean walking time, and a 3.3 point drop in mean NRS score.

Conclusions:

In subjects with moderate or severe low back and/or leg pain, 3D Neural Targeting SCS provided reduced disability (as measured by ODI and walking tolerance), reduced pain intensity (as measured by NRS), and produced generally high satisfaction rates.
A DATA ANALYTICS APPROACH TO IDENTIFY FACTORS UNDERLYING PATIENT SATISFACTION FROM THE RELIEF GLOBAL REGISTRY

R. Woon¹, D. Huynh¹, R. Jain¹
¹Boston Scientific Corporation, Clinical Research, Valencia, USA

Background and Aims:

Many studies report only weak to moderate correlation between pain ratings and patient satisfaction. This suggests that pain ratings may not account for all facets of the patient experience. In this analysis, we examine the relationships between pain ratings and quality of life responses following spinal cord stimulation (SCS) to identify key factors driving patient satisfaction.

Methods:

Data analyzed was obtained as part of a prospective, multi-center, global registry (RELIEF, Boston Scientific) that collects on-label patient outcomes for SCS systems with at least one post-implantation visit (6, 12, 24, or 36 months). Data was combined across a series of questionnaires (e.g., NRS Pain Intensity, Oswestry Disability Index, etc). Subjects with < 20% data missing were analyzed (N = 319). Machine learning feature selection algorithms were used to compute the assessment (CART, ReliefF, Information Gain) and develop the final model.

Results:

This analysis found that while increases in pain reduction are associated with increases in patient satisfaction, roughly ~50% subjects with ≤ 1 NRS point pain reduction are still satisfied with SCS therapy. The results indicate that satisfaction with paresthesia coverage as well as with comfort of stimulation, clinical global impression of change, moderate pain, and simple tasks such as usual activities/mobility were most influential on patient satisfaction.

Conclusions:

For subjects with ≤ 1 point NRS pain reduction, satisfaction with paresthesia coverage and comfort of stimulation as well as improvement in the ability to perform simple tasks are most highly related to patient satisfaction. The way these features interact with subjects’ overall satisfaction warrants future investigation.
Background and Aims:

The evolution of dorsal root ganglion (DRG) spinal cord stimulation (SCS) has improved the efficacy and applicability of SCS. It is demonstrated as effective in etiologies including failed back surgery syndrome (FBSS), complex regional pain syndrome (CRPS) and chronic postsurgical pain. We share results and lessons learned through our first 100 consecutive cases.

Methods:

Technological and practitioner led advances, along with patient outcomes to treatment (baseline, end of trial (EOT), 6 and 12 month follow-up) will be presented:

- Pain (NRS)
- Disability (ODI)
- Activities of Daily Living (ADL)
- Psychometrics (DASS21)
- Complications/adverse events.

Results:

There have been 102 DRG trials; 78 permanent implants and 24 unsuccessful trials (1 abandoned due to anatomical difficulties). Fifteen lead revisions, one complication (infection). Three deaths recorded (unrelated).

The mean baseline NRS was 7.28 (sd 1.17, n= 78), and dropped significantly at EOT to 3.34 (sd 1.9, n=46). It remained lower at 6 months (3.45, sd 1.94, n=41) and 12 months (NRS 3, sd 1.6, n=16).

The mean baseline ODI was 44.73% (sd 15.2, n=57, severe disability). It dropped significantly at EOT to 32.5% (sd 16.8, n=42, moderate disability). The ODI remained lower at 6 (33.4%, sd 16.79, n=32) and 12 months (31.4%, sd 14.2, n=18).

Mean DASS21 scores decreased. ADL also improved following DRG therapy.

Conclusions:

Continual refinements in DRG SCS have led to compelling outcomes in a challenging cohort. The importance of clinicians identifying vulnerabilities, refining techniques, influencing technological developments and importantly, sharing their knowledge is where our greatest lessons are learnt.
Background and Aims:

Clinical studies have demonstrated that stimulation of the dorsal root ganglion (DRG) can significantly reduce chronic intractable pain with an enhanced ability to target difficult-to-reach regions. Preliminary data suggest that paresthesias related to the treatment can be eliminated by decreasing the stimulation amplitude below the perceptual level (subthreshold). This study investigates the efficacy of subthreshold DRG stimulation in a cohort of subjects being treated for intractable chronic pain.

Methods:

A retrospective review was performed at a single site with selection criteria including diagnosis of chronic intractable pain for a period of at least six months. Forty-one subjects had DRG leads placed between spinal levels C6-S1. Thirty-six of the subjects reported no paresthesias at all of the follow-up visits (subthreshold group). Changes in overall pain were compared between baseline measurements and those made at the end of trial, 3, 6, and 12-month follow-ups.

Results:

Overall responder rate in the subthreshold group, as defined by a successful trial with ≥50% pain reduction, was 88.9%. On average, these subjects reported a reduction of overall pain of 77.5% at the conclusion of the trial stimulation phase. Subjects reported continued pain relief of 71.1%, 55.5% and 62.8% at the 3, 6, and 12-month follow-ups, respectively.

Conclusions:

Clinically significant and sustained paresthesia-free pain relief over a period of 12 months was achieved with DRG stimulation. The beneficial clinical outcome of subthreshold DRG stimulation suggests several mechanisms of action, including the inhibition of supraspinal regions involved in somatic paresthesia sensation. Future prospective study of subthreshold DRG stimulation is warranted.
Background and Aims:

Neuralgic amyotrophy (NA) is one of the brachial plexus syndrome, characterized by severe pain in the shoulder and arm, and the effective management is not established. Although spinal cord stimulation (SCS) has been known as an effective therapy for patients with chronic neuropathic pain such as CRPS type I, efficacy against the pain of NA is not well known. We provide a case report of a 28-year-old woman who suffered with NA, and the SCS was remarkably effective.

Methods:

The patient had complained of persistent pressing, crushing, dull and heavy pain of her right shoulder and upper limb. Her first symptoms occurred 3 years ago followed by patchy weakness of scapula and upper limb. After steroid pulse therapy, her upper limb recovered 80% of normal strength. Two times of stellate ganglion block were not effective, but ultrasound guided brachial plexus block was effective. She continued brachial plexus block for 12 times during 13 months. She hoped more effective procedure and we applied SCS.

Results:

Before SCS operation, her Numerical Rating Scale (NRS), EuroQol-5D (EQ-5D), Pain Self Efficacy Questionnaire (PSEQ) and Pain Disability Assessment Scale (PDAS) was 7, 0.61, 24 and 12, respectively. SCS was performed with dual cylindrical electrode (Boston Scientific, ILLUMINA 3D™) at level of C6-Th1. SCS (1000Hz) decreased her pain (NRS 1). One month after operation, her NRS, EQ-5D, PSEQ and PDAS was 2, 0.77, 31 and 15, respectively. At home, she adjusted SCS to her pain grade by herself.

Conclusions:

SCS might be a clinical option for NA.
CLINICAL: NEUROPATHIC CANCER PAIN

NEUP7-0076
EXPLORING ADDITIONAL COMPLEXITY TO BREAKTHROUGH CANCER PAIN USING THE EDMONTON CLASSIFICATION SYSTEM FOR CANCER PAIN

J. Canals Sotelo¹, J. Lopez Ribes², R. Gonzalez Rubio³, N. Arraràs Torrelles², E. Barallat Gimeno³, J. Trujillano cabello⁴
¹Hospital Universitario de Santa Maria, Supportive Palliative Care Team, Lleida, Spain
²Hospital Universitario de Santa Maria, Home care Team, Lleida, Spain
³Universitat de Lleida, Departament d'Infermeria, Lleida, Spain
⁴Universitat de Lleida, Departament de Cirurgia, Lleida, Spain

Background and Aims:
Breakthrough cancer pain (BTcP) is highly prevalent in advanced cancer patients with significant differences regarding the place where the study has been carried out. The Edmonton Classification System for Cancer Pain (ECS-CP) helps to detect other aspects of pain (neuropathic, addictive behaviour, psychological distress) and can contribute to add complexity to BTcP symptom

Methods:
An observational, retrospective, non-interventional and single center study of advanced cancer patients was designed. We reviewed clinical files of the patients attended at the outpatient clinic over the period 2014-2015. BTcP was diagnosed using the Davies algoritm. The ECS-CP was applied to any patients with pain. Neuropathic pain (NP) is diagnosed according to the NEUPsig criteria and supported by the DN4 questionnaire

Results:
277 patients were included. 63, 9% were man. Mean of age was 68, 2 years. Main diagnosis was lung cancer (31, 6%). Metastases were diagnosed in the 83% of the sample. BTcP prevalence was of 39.34%. 488 different types of BTcP were detected. Mean of 1,75 per patient. Altogether with the incidental component of cancer pain, 244 types of BTcP (50%) presented a component of NP. The addictive behavior (alcohol) was present in 130 types of BTcP (26,64%) and the Psychological distress was present in 222 types on BTcP (45,49%)

Conclusions:
NP is present in up to 50% of types of BTcP detected by using the ECS-CP. Hovewer BTcP is treated with rapid onset opioids, mainly fentanyl. further research is needed in order to determine the role of NP component in BTcP
Background and Aims:

The aim of this study was to examine efficacy and safety of calcium channel blocker (pregabalin) in patients already accepted opioid with neuropathic cancer pain (NCP).

Methods:

A prospective randomized controlled multicenter trial was conducted in National Pain Center. From January 2015 to January 2016, one hundred and twenty two eligible inpatients and outpatients were divided into pregablin group (n=60) and control group (n=62). Pregablin group added pregablin to opioid background analgesia. Meanwhile control group raised opioid dose instead. The primary end point was NRS score. Secondary end points assessed paraesthesia score, HAMD score, analgesia dose, patients satisfaction, The secondary outcomes included 30% pain relief rate, 50% pain relief rate and adverse events. Study period was two weeks.

Results:

There were significant differences in average pain variation (2.3 vs.1.3, P=0.037), paraesthesia pain variation (1.6 vs. 0.4, P<0.001) There were differences in mood (4.4 vs. 2.4, P<0.001) and satisfaction (P<0.05) between arms. Morphine dose for breakthrough pain were less in pregabalin group (30.6mg/d vs. 70.9mg/d, P<0.05). With increasing dose of pregabalin, the 30% pain relief and 50% pain relief on the 14th day reached 85.7% and 58.9% (P=0.022, 0.015), respectively. Pregabalin had less severe adverse effects (3/56, 5%, P<0.05).

Conclusions:

Our findings support the role of pregabalin in patients with NCP already receiving opioid. Pregabalin has better pain controlled and mood improved.
Background and Aims:

Over half of patients receiving taxane chemotherapy experience chemotherapy-induced peripheral neuropathy (CIPN), which involves numbness and neuropathic pain in the hands and feet. CIPN has no effective treatments partly because its etiology is poorly understood. We theorize that CIPN symptoms are partly caused by impairment of interoceptive brain circuitry, which processes bodily sensations via the posterior insula and anterior cingulate cortex (ACC). We investigated whether CIPN is associated with altered connectivity in interoceptive brain circuitry.

Methods:

Fifty women with breast cancer (50±9 years) reported CIPN symptoms (CIPN-20) and underwent resting fMRI one or more times: before surgery, one month after completion of chemotherapy, and one year after chemotherapy. We used an a priori seed-based investigation of connectivity between the posterior insula, subgenual ACC (sgACC), and pregenual ACC (pACC). We compared connectivity between 31 patients without CIPN symptoms (≤10 CIPN-20-Sensory), 19 patients with CIPN symptoms (>10 CIPN-20-Sensory), and 280 healthy adults (174 women, 19.3 years) from another study.

Results:

Patients with CIPN symptoms had significantly reduced connectivity between the posterior insula and both the pACC (p=0.02, Cohen’s d=0.80) and the sgACC (p=0.01, d=0.73) compared to patients without CIPN symptoms. Connectivity between the posterior insula and both regions of the ACC was negative in patients with CIPN symptoms but positive in both healthy adults and patients without CIPN symptoms.

Conclusions:

CIPN is characterized by reduced connectivity in interoceptive brain circuitry, which may be a viable treatment target. Future work will assess causal relationships between CIPN symptoms and reduced connectivity.
Background and Aims:

Chemotherapy-Induced Peripheral Neuropathy (CIPN) is a significant cause of persistent neuropathic pain in cancer survivors.

We collect patient-level data on all Pain Clinic outpatients and present a descriptive analysis of pain characteristics and morbidity associated with this population.

No neuropathic pain assessment is accepted for CIPN assessment, although Douleur Neuropathique-4 (DN4) has sensitivity for cancer neuropathic pain. Here, we examine the sensitivity of DN4 in this population.

Methods:

All patients who consented to provide data between January – December 2016 were included. Numerous, pain and CIPN-specific assessment tools were recorded including The following data were extracted:

- Peripheral Neuropathy Questionnaire
- Brief Pain Inventory
- DN4
- Hospital Anxiety and Depression Scale
- Patient Global Impression of Change scale
- Patient satisfaction

Results:

See table 1. DN4 was positive in 88.7% of patients with CIPN. Whilst only 31% reported significant improvement, the mean satisfaction score was 87%. There was no significant difference in pain intensity, interference, anxiety, depression or patient-reported improvement between CIPN and non-CIPN patients.
Conclusions:

Contrary to perception of neuropathic pain, patients with CIPN did not report greater levels of pain interference/severity than patients with non-CIPN cancer pain.

This first analysis of the sensitivity of DN4 in CIPN patients shows similar sensitivity to other neuropathic cancer pain. Although this sample is too small to draw firm conclusions, continuing data collection on all attending CIPN patients will enable reporting a significantly larger number in the near future.
Background and Aims:

Neuropathic pain is a major challenge in the palliative care of cancer patients. Opioids are of limited benefit in treating neuropathic pain, and can produce substantial adverse effects. The aim of this paper is to present cases that highlight the value of selected medications and modes of delivery to better manage neuropathic cancer pain.

Methods:

A series of selected cases will be presented.

Results:

Cases of coeliac plexus irritation due to pancreatic head tumour or lymphadenopathy caused severe, episodic epigastric pain which radiated to the lower thoracic region. Opioids were not effective, whereas Pregabalin provided good relief of pain with less side effects. Cases of brachial and lumbar plexus pain responded to subcutaneous lignocaine infusions that did not cause drowsiness, delirium, constipation, or other significant adverse effects. A case involving refractory lumbar plexus pain was helped with an intrathecal infusion of marcaine, morphine and midazolam.

Conclusions:

Clinicians should be aware of medication options to palliate neuropathic pain syndromes. Pregabalin can be helpful for coeliac plexus syndrome as well as a range of other neuropathic pains. Lignocaine subcutaneous infusions are under-utilised in treating neuropathic pain. Intrathecal systems have an important role in treating refractory pain. Clinical trials are needed to better establish the evidence for the use of these treatments of neuropathic pain.
Background and Aims:

Vitamin D deficiency is linked to the development of many musculoskeletal conditions like non-specific chronic low back pain (NSCLBP). The objective of this study was to determine association of vitamin D deficiency and low back pain in women.

Methods:

With a case control study, 182 women participated in study. Serum vitamin D was assessed by electrochemiluminescence method. Levels <20ng/ml were considered as vitamin D deficiency. Data analyzed with SPSS 21. Mann-Whitney U test and chi square test was used for analysis.

Results:

All participants completed the study. Mean (±SD) age of 81 patients and 101 matched-controls people were 35.1±8.14 and 37.4±7.9 years respectively. Serum 25-OHD deficiency was observed in 57(70.4%) patients versus 47(46.5%) controls (p=0.001). Median serum 25-OHD concentration in patients was significantly lower than control group (p=0.003). There was a significant association between serum 25-OHD deficiency and low back pain (OR=2.72, 95%CI, 1.47-5, p=0.001). 25-OHD deficiency was significantly correlated with low back pain (r=0.239, p=0.001).

Conclusions:

This study indicates a significant association between vitamin D deficiency and NSCLBP in women and justifies serum 25-OHD assessment in women with low back pain.
Background and Aims:

Back pain is one of the most common pain conditions. Over 10% of acute cases continue to have refractory and persistent symptom. Unlike the nociceptive component, neuropathic back pain is relatively under-recognized, under-treated and difficult to manage even when recognized. Previous studies on neuropathic back pain were predominantly done in the west and the prevalence varied from 15% to 55%. This pilot study aimed to find out the prevalence of neuropathic characteristics and psychological distress in chronic low back pain patients attending a musculoskeletal rehabilitation clinic in Singapore.

Methods:

Patients with chronic back pain of over 3 months duration were screened for neuropathic pain with the interview and clinical examination version of Douleur Neuropathique 4 (DN4) and psychological distress with the Kessler Psychological Distress Scale 6 (K6). The DN4 and K6 findings were analyzed with patient demographic and clinical features.

Results:

30 patients, 10 males and 20 females, age 20 to 80 years (mean 54.2, median 56.0, SD16.6) were studied. 9 patients had neuropathic back pain and 5 of them had psychological distress. Neuropathic back pain patients were more likely to have radicular pain (p < 0.05), higher pain intensity on the Numerical Rating Scale (NRS) and suffer psychological distress (p < 0.05).

Conclusions:

Prevalence of neuropathic back pain in this study was 30%. It was associated with pain radiating down the lower limb, higher pain intensity and psychological distress. Further studies will help to improve understanding and recognition, better diagnosis and management.
Background and Aims:

There is limited data about the clinical course of neuropathic pain in low back-related leg pain (LBLP) patients who consult in primary care. This research aimed to describe the clinical course of those with and without neuropathic pain in terms of pain intensity, leg and back-related disability over three years.

Methods:

This study was a prospective observational treatment cohort of 606 LBLP patients consulting in primary care. The following outcome measures were collected at baseline, 4-months, 12-months and 3-years: neuropathic pain (Self report version of Leeds Assessment for Neurological Symptoms and Signs (S-LANSS) - score of ≥12 indicates neuropathic pain); pain intensity (highest of mean leg or mean back pain - 0-10 numerical rating scale (NRS)); leg and back-related disability (RMDQ - 0-23 NRS). Mixed effect models were used to compare pain intensity and disability over the three-year follow-up between those with and without neuropathic pain at baseline.

Results:

48.3% (293/606) of patients presented with neuropathic pain at baseline, this reduced to 25.0% (94/376) at 4-months, 22.6% (79/349) at 12-months and 21.6% (58/268) at 3-years. Patients with neuropathic pain at baseline reported significantly higher pain intensity (figure 1) and disability (figure 2) at all time points compared to those without.
Conclusions:

LBLP patients with symptoms indicative of neuropathic pain at baseline report more severe pain intensity and more leg and back-related disability over a period of 3 years compared to those without. These findings show the clinical course of neuropathic pain in LBLP and will
inform research investigating prognosis of persistent neuropathic pain.

**Acknowledgments**

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THE EFFECT OF ADDITIONAL WHOLE BODY VIBRATION EXERCISES ALONG WITH HOME EXERCISE PROGRAM ON MUSCLE STRENGTH IN PATIENTS WITH POSTPOLIO SYNDROME

M. Topaloğlu¹, A. Ketenci¹

¹Istanbul University- Istanbul Faculty of Medicine, Istanbul, Turkey

Background and Aims:

The aim of this study is to investigate the effectiveness of whole-body vibration (WBV) exercises with home exercise program and patient education; on muscle strength, fatigue and quality of life in patients with postpolio syndrome (PPS) in comparison to home exercise program and patient education.

Methods:

We conducted a prospective, randomized, controlled trial involving 14 patients with PPS. Patients were randomized to two group: the first group (WBV group, n=7) whole body vibration, home exercise program and patient education; the second group (control group, n=7) home exercise program and patient education. Patients were evaluated knee isometric extension peak torque (IMEPT), isokinetic extension peak torque (IKEPT), isokinetic flexion peak torque (IKFPT), fatigue severity scale (FSS), fatigue impact scale (FIS), Nottingham Health Profile (NHP), serum creatine kinase (CK), aspartate aminotransferase (AST) ve alanine aminotransferase (ALT) between baseline and end of the treatment. Statistical tests were conducted at the 0.05 significance level for all outcome measures.

Results:

At the end of treatment; home exercise program was effective to increase the muscle strength of the knee with PPS patients (p <0.05). In the intervention group that WBV exercises with home exercise program was no significant difference on muscle strength parameters, fatigue and quality of life score. Muscle damage markers (CK, AST and ALT) were no significant difference in both group.

Conclusions:

In our study, both exercise group increased the muscle strength. There is no significant difference between the two group. The long term follow-up studies are need for assess the effectiveness of WBV exercises with PPS patients.
CLINICAL: OTHER TREATMENTS - PART 1

NEUP7-0394

HYPONATREMIA AND PAIN RELIEF AFTER RADIOFREQUENCY THERMOCOAGULATION FOR IDIOPATHIC TRIGEMINAL NEURALGIA

S. Kamath

National Institute of mental Health and Neuro Sciences, Neuroanaesthesia, Bangalore, India

Background and Aims:

Radio-frequency thermocoagulation (RFT) is a standard treatment for idiopathic trigeminal neuralgia (TN). When correctly performed, patients obtain immediate pain relief after RFT. We report unusual pattern of pain relief after RFT for TN due to hyponatremia.

Methods:

A 57-year-old lady was referred to us for RFT for her TN. Her baseline serum sodium was 125 mEq/L. She underwent fluoroscopic-guided RFT of left gasserian ganglion and reported immediate pain relief. Two days later, she complained of pain recurrence for which duloxetine was added. She was admitted for exhaustion 2 days later, when severe hyponatremia was detected. Following sodium correction, pain improved dramatically and she currently remains pain-free.

Results:

Medications used for pain management for TN (carbamazepine and duloxetine) can produce hyponatremia. Hyponatremia enhances tetrodotoxin-sensitive sodium current, activates c-fibres via prostaglandin-E2 and enhances pain perception.

Conclusions:

Awareness among pain physicians about this pathophysiological aspect of hyponatremia-associated pain enhancement is necessary for optimal pain management.
Background and Aims:

Reduced exercise induced hypoalgesia (EIH) is repeatedly reported in patients with central sensitization pain. This study examined the acute effects of morphine and placebo on EIH, in patients with chronic fatigue syndrome and comorbid fibromyalgia (CFS/FM), and patients with rheumatoid arthritis (RA). Healthy volunteers underwent the same procedure with naloxone.

Methods:

A randomized, double-blind, placebo-controlled cross-over trial was set. Ten CFS/FM patients, 11 RA patients and 20 controls were randomly allocated to the experimental (10 mg morphine and 0.2 mg/ml Naloxone) or placebo (2 ml Aqua) group. Temporal summations at the Trapezius and Quadriceps were assessed by algometry. Condition Pain Modulation (CPM) efficacy was assessed by adding ischemic occlusion at the opposite upper arm. Deep Tissue Pain pressure was measured accordingly by ischemic occlusion. The aerobic power index was taken as submaximal exercise protocol.

Results:

Temporal summation at the Quadriceps in RA and pressure pain at the Trapezius in CFS/FM were significantly decreased following submaximal exercise after morphine administration. However, the effect of morphine did not significantly differ from placebo. EIH was objectified in the control group and the effects of naloxone were comparable to nocebo. CPM efficacy in the control group decreased after exercise.

Conclusions:

This study revealed small EIH effects after morphine in CFS/FM and RA patients, however, these effects were comparable to placebo. Besides, naloxone did not significantly differ from nocebo on EIH in controls. These results suggest that the opioid system is not dominant in EIH following submaximal exercise.
CLINICAL: PERIPHERAL NEUROPATHIC PAIN - PART 1

NEUP7-0112
USING INTEGRATED RANDOMIZED CLINICAL TRIALS (RCTS) AND OBSERVATIONAL STUDY DATA AND SIMULATION TO PREDICT RESPONSES TO PREGABALIN IN PATIENTS WITH PAINFUL DIABETIC PERIPHERAL NEUROPATHY (PDPN)
J. Alexander Jr, R. Edwards, A. Savoldelli, L. Manca, R. Grugni, B. Emir, E. Whalen,
S. Watt, M. Brodsky, B. Parsons

1 Pfizer, Internal Medicine, New York, USA
2 Health Services Consulting Corporation, Corporate, Boxborough, USA
3 Fair Dynamics Consulting, Corporate, Milano, Italy
4 Pfizer, Clinical Statistics, New York, USA
5 Pfizer, Medical Affairs, New York, USA

Background and Aims:
Clinicians rely on randomized and observational evidence for treatment decisions. Directly integrating both data types can inform choices by leveraging advantages of each. Our goal was to integrate such data for pDPN patients treated with pregabalin in a predictive analytics/simulation platform that utilizes on-treatment and baseline variables to predict pain.

Methods:
Active treatment pDPN patients from 9 RCTs (1,320 patients worldwide) and a large observational study (OS) (2,642 German patients) were integrated by: (1) OS cluster analysis and coarsened exact matching of RCT patients, creating a matched dataset; (2) development of cluster-specific autoregressive moving average models (ARMAXs) to predict 6-week pain levels in this matched dataset; (3) ARMAX model validation using OS patients that had not matched with RCT patients; (4) evaluation of a novel patient’s response via cluster assignment and simulation of 1000 virtual patients, examining probability distributions of outcomes.

Results:
The combined dataset covered 80.2% of the 192 possible combinations of gender, four age groups, three BMI groups, insulin use, pregabalin monotherapy, and moderate or severe pain. The matched dataset for model development included 2,843 patients, reflecting matching of 37.9% of RCT patients with 62.1% of OS patients. Covariate bias was reduced by 51.5% (Table 1). Validation of models was performed using the 1,119 unmatched patients. Cluster-specific ARMAX results performed well (Table 2). Simulation results showed high accuracy (Table 3).
### Table 1: Coarsened Exact Matching Results

<table>
<thead>
<tr>
<th></th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
<th>Cluster 5</th>
<th>Cluster 6</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>N that matched</td>
<td>542</td>
<td>487</td>
<td>473</td>
<td>419</td>
<td>470</td>
<td>452</td>
<td>2843</td>
</tr>
<tr>
<td>Global imbalance~ before CEM</td>
<td>0.66</td>
<td>0.69</td>
<td>0.58</td>
<td>0.77</td>
<td>0.76</td>
<td>0.64</td>
<td>0.68 (mean)</td>
</tr>
<tr>
<td>Global imbalance~ after CEM</td>
<td>0.32</td>
<td>0.34</td>
<td>0.31</td>
<td>0.36</td>
<td>0.33</td>
<td>0.32</td>
<td>0.33 (mean)</td>
</tr>
<tr>
<td>% Reduction in Global Imbalance~</td>
<td>51.50%</td>
<td>50.70%</td>
<td>46.50%</td>
<td>53.20%</td>
<td>55.70%</td>
<td>50.00%</td>
<td>51.5% (mean)</td>
</tr>
<tr>
<td>% female</td>
<td>0%</td>
<td>46.8%</td>
<td>30.1%</td>
<td>100%</td>
<td>30.4%</td>
<td>34.1%</td>
<td>39.7%</td>
</tr>
<tr>
<td>% 45-64 years old</td>
<td>62.6%</td>
<td>65.5%</td>
<td>52.0%</td>
<td>63.5%</td>
<td>65.4%</td>
<td>50.7%</td>
<td>60.6%</td>
</tr>
<tr>
<td>% obese</td>
<td>25.3%</td>
<td>51.1%</td>
<td>40.2%</td>
<td>44.4%</td>
<td>36.8%</td>
<td>32.1%</td>
<td>38.0%</td>
</tr>
<tr>
<td>% baseline severe pain (7-10)</td>
<td>42.6%</td>
<td>63.2%</td>
<td>55.4%</td>
<td>44.9%</td>
<td>52.6%</td>
<td>39.2%</td>
<td>49.7%</td>
</tr>
<tr>
<td>% average daily treatment dose of 150 mg</td>
<td>71.4%</td>
<td>52.8%</td>
<td>74.8%</td>
<td>66.1%</td>
<td>47.0%</td>
<td>63.3%</td>
<td>62.7%</td>
</tr>
</tbody>
</table>

~The degree of imbalance represents the level of bias in the distributions of covariates for a given sample. An imbalance of 0 in a given cluster means that the empirical distributions of the covariates of the observational study dataset are equivalent to those in the RCT datasets; an imbalance of 1 means that the empirical distributions are completely different.

### Table 2: ARMAX Regression Results

<table>
<thead>
<tr>
<th>Cluster</th>
<th>n</th>
<th>ARMAX Model Equations~</th>
<th>R-squared</th>
<th>Root-Mean-Square Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>542</td>
<td>Pain level:0.018302400.589377&quot;Sleep at lag 1&quot;=0.751677“[Pain at lag -1]=0.0371126“[Pain at lag -2]=0.043026”[Sleep at lag -1]=0.000701“[Treatment at lag -1]=0.007739“[Treatment at lag -2]=0.06792 &quot;[GF Sad at lag -1]=0.045746&quot;[GF Sad at lag -2]</td>
<td>0.90</td>
<td>0.44</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>467</td>
<td>Pain level:0.0146350.452215&quot;Sleep at lag 1&quot;=0.029310&quot;[Pain at lag -1]=0.001493&quot;[Pain at lag -2]=0.339795&quot;[Sleep at lag -1]=0.000567“[Treatment at lag -1]=0.00182“[Treatment at lag -2]=0.065110“[GF Sad at lag -1]=0.049327&quot;[GF Sad at lag -2]</td>
<td>0.91</td>
<td>0.48</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>473</td>
<td>Pain level:0.1964100.563955&quot;Sleep at lag 1&quot;=0.523975&quot;[Insulin use at 0]=0.797999“[Pain at lag -1]=0.013945“[Pain at lag -2]=0.055259“[Sleep at lag -1]=0.000836“[Treatment at lag -1]=0.00255“[Treatment at lag -2]=0.093942“[GF Sad at lag -1]=0.063902“[GF Sad at lag -2]</td>
<td>0.93</td>
<td>0.49</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>419</td>
<td>Pain level:0.019200.529119&quot;Sleep at lag 1&quot;=0.00241&quot;[Pain at lag -1]=0.002475“[Pain at lag -2]=0.077161“[Sleep at lag -3]=0.394667“[Sleep at lag -1]=0.031157“[Sleep at lag -2]=0.000249“[Treatment at lag -1]=0.000417“[Treatment at lag -2]=0.096419“[GF Ene at lag -1]=0.112920“[GF Ene at lag -2]</td>
<td>0.91</td>
<td>0.41</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>470</td>
<td>Pain level:0.067400.627592&quot;Sleep at lag 1&quot;=0.010491“[Age]=0.793532“[Pain at lag -1]=0.043824“[Pain at lag -2]=0.496101“[Sleep at lag -1]=0.000231“[Treatment at lag -1]=0.00175“[Treatment at lag -2]=0.039307“[GF Ene at lag -1]=0.080968“[GF Ene at lag -2]</td>
<td>0.93</td>
<td>0.42</td>
</tr>
<tr>
<td>Cluster 6</td>
<td>452</td>
<td>Pain level:0.2492640.618117&quot;Sleep at lag 1&quot;=0.763885“[Age]=0.793532“[Pain at lag -1]=0.043824“[Pain at lag -2]=0.496101“[Sleep at lag -1]=0.000231“[Treatment at lag -1]=0.00175“[Treatment at lag -2]=0.039307“[GF Ene at lag -1]=0.080968“[GF Ene at lag -2]</td>
<td>0.91</td>
<td>0.42</td>
</tr>
</tbody>
</table>

~Possible variables that could be included in the ARMAXs, depending on the cluster, were: Gender (male or female), Age group (0-44, 45-64, 65-74 or over 74), BMI group (normal, overweight or obese), pDPM duration in years (0-5, 6-10, 11-15, 16-20, 21-25 or over 25), Insulin use (true or false), PGB monotherapy (true or false), Prior Gabapentin use (true or false), Depression in medical history (true or false), Treatment from lag -5 weeks to lag 0 (every dose), Pain level from lag -5 weeks to lag 0 (from 0 to 10), Sleep interference level from lag -5 weeks to lag 0 (from 0 to 10), and the following General Feelings from lag -5 weeks to lag 0 (always, mostly, fairly often, sometimes, seldom or never)— calm & relaxed, sad & discouraged, full of energy.
Conclusions:

Patient and on-treatment response variables based upon integrated RCT and OS data can be utilized in pDPN to predict pain response to pregabalin in novel patients.

Table 3: Simulation Results of Virtual Patients

<table>
<thead>
<tr>
<th>Accuracy at 30% threshold of pain reduction from Baseline to Week 6</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
<th>Cluster 5</th>
<th>Cluster 6</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>93.9%</td>
<td>86.5%</td>
<td>92.6%</td>
<td>93.4%</td>
<td>89.7%</td>
<td>89.6%</td>
<td>91.55%</td>
<td></td>
</tr>
<tr>
<td>Accuracy at 50% threshold of pain reduction from Baseline to Week 6</td>
<td>80.4%</td>
<td>59.4%</td>
<td>77.0%</td>
<td>80.6%</td>
<td>69.1%</td>
<td>68.3%</td>
<td>74.20%</td>
</tr>
</tbody>
</table>

*Summary of Steps for Predicting Novel Patient Outcomes: 1) Cluster analyses are implemented to identify patient subgroups. 2) A candidate novel patient is assigned to one of the identified clusters from the prior step based on that patient’s alignment with the patients in the various clusters. 3) After the novel patient is assigned to a cluster, 1,000 instances of the ‘novel’ patients are created to reflect the various possible trajectories of outcomes according to what could occur based on the ARMAX regression model for that cluster. The ARMAX regressions are applied every week over the 6-week period with outcomes generated by the previous week used as the starting outcome for the current week. 4) The distributions of virtual patient pain levels and responder status, along with the different trajectories, are displayed at the end of the 6-week simulation.
WEARABLE TENS BAND FOR CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY: A FEASIBILITY STUDY

J. Gewandter¹, J. Chaudari², R. Kitt¹, C. Ibegbu³, J. Serventi³, J. Burke³, M. Tejani⁴

¹University of Rochester, Anesthesiology, Rochester, USA
²University of Rochester, Anesthesiology, Rochester, USA
³University of Rochester, Neurology, Rochester, USA
⁴University of Rochester, Hematology/Oncology, Rochester, USA

Background and Aims:

Forty to 80% of cancer patients report neuropathic symptoms 3 months after neurotoxic chemotherapy. No evidence-based, non-pharmacologic treatments exist. This single-arm, feasibility study evaluated the efficacy of a Transcutaneous Electrical Nerve stimulation (TENs) band.

Methods:

Eligible patients completed chemotherapy at least 3 months prior to enrollment and reported at least 1 of the following neuropathy symptoms as ≥4 on a 0 – 10 numeric rating scale: pain, numbness, tingling, cramping. Participants wore a TENs band on their lower extremities for a minimum of 6 weeks and up to 6 months. They wore the band twice a day for the first 3 weeks and subsequently as frequently as they wanted. Participants’ attitudes toward the band and study were assessed in a qualitative interview at 6 weeks.

Results:

Eleven of 18 eligible patients received the TENs band; 8 completed the initial 6-week treatment phase. Five participants decided to continue using the band after the 6-week phase. All participants found the study length and number of visits acceptable; 4 of 8 participants preferred having a daily symptom diary over less frequent ratings. Six of 8 participants reported experiencing some benefit from the band, noting improvement in tingling, pain, swelling, and/or cramping. Only one person reported improvements in numbness.

Conclusions:

This feasibility study indicates that (1) the majority of the participants found a 6-week study with daily symptom diaries acceptable and was able to operate the band and (2) outcome measures for a TENs band should evaluate positive neuropathy symptoms. Supported by Neurometrix.
Conditioned pain modulation (CPM) is the experimental human correlate of diffuse noxious inhibitory control. CPM efficiency is impaired in several neuropathic pain conditions, but no studies have examined CPM efficiency in HIV-associated sensory neuropathy (HIV-SN). Aim: To determine the CPM response in patients with painful HIV-SN.

Methods:

Patients were recruited from Chelsea & Westminster Hospital. Painful HIV-SN was defined as a positive CHANT score (Clinical HIV-associated Neuropathy Tool), and scoring greater than three on Douleur-Neuropathique-4-interview. Comparison of CPM response (CPM paradigm - Figure 1) between healthy volunteers and HIV patients with and without painful neuropathy was performed. Twice the standard error of measurement (2xSEM) of the pressure pain threshold was used as a cut-off to define that a change was likely to be due to a true CPM response rather than measurement error. (Ethical approval: NRES14/LO/1574).
Results:

(Preliminary)

Fifty healthy volunteers and 39 HIV patients (15 with painful neuropathy) were recruited. Eleven patients could not tolerate the conditioning stimulus. Similar proportions of each group did not exhibit a CPM response outside 2xSEM (p=0.523). Comparison of CPM response showed a difference between groups that neared significance (p=0.063); patients with painful neuropathy showed the largest CPM response and the largest range of values (Figure 2.)
Conclusions:

CPM response was not impaired, but enhanced in patients with painful HIV-SN. This contrasts with the majority of studies in chronic pain which demonstrate impaired, or "normal", CPM. This may be due to cognitive or psychological factors, or a true difference descending inhibitory control in patients with HIV.
Background and Aims:

Gender differences in pain thresholds have been studied mostly in healthy subjects with inconclusive results. We investigated for the first time pain thresholds using quantitative sensory testing (QST) before and after age-/gender-adjusted z-transformation in patients with CRPS or neuropathic pain, e.g. polyneuropathy (PNP) or peripheral nerve injury (PNI). Aim was to analyse whether gender differences are even stronger than in healthy.

Methods:

Using the DFNS, IMI European and Neuropain database our analysis comprised QST data of 1316 patients (f: 719, m: 597) with CRPS (n=403), PNI (n=342) and PNP (n=571). Absolute and z-values of pain thresholds were compared (independent t-test, P<.05).
Results:

In all syndromes, females demonstrate significantly lower absolute cold (=hypoalgesia), heat (HPT; = allodynia), mechanical and pressure pain thresholds (PPT; \( p < .04; \) =allodynia). After z-transformation all gender differences disappeared except PPT in CRPS (\( P = .001 \)) and HPT in PNI (\( P = .04 \)).

Conclusions:

There are some small but significant gender differences in patients with CRPS or PNI. However, using correction factors evaluated in healthy subjects [1] most differences disappear. Thus, gender-specific differences seem to be of minor relevance within the particular neuropathic pain aetiologies except the significantly decreased PPT in CRPS, most similar to an evoked suprathreshold pain. Therefore, pain tolerance, which is more often reduced in females [2], may explain this gender difference better than subthreshold pain differences.

References


Acknowledgements

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Background and Aims:

The presence of pain after injury to the brachial plexus is known to be a predictor of disability. We aim to ascertain which factors are associated with pain and disability in patients with traumatic plexus injury.

Methods:

Patients with traumatic plexus injury with flail arm were evaluated. The outcome measures included visual analog scale (VAS), Disabilities of the Arm, Shoulder, and Hand (DASH), and British Medical Research Council (BMRC or MRC) muscle grading scale. Preoperative continuous variables were compared and a multivariable regression analysis was performed.

Results:

The study group included 139 patients. The mean age at the time of injury was thirty one years (range, twenty one to forty eight years). One hundred and twenty nine right arms and eighteen left arms were involved. Sixty four patients (46 %) had involvement of the dominant arm. Hundred and fifteen (83.33%) patients had associated injuries included ninety four (68.12%) closed head injuries. Average pain intensity was 5.37 (SD = 2.89), with 94% endorsing some pain (>0/10), 24% (0-3/10) reporting mild pain, 28% moderate pain (3.1-5.4/10) and 48% severe pain (≥7/10). The mean DASH score according to pain severity were 42 (SD=18.8), 47 (SD=15), and 52(SD=17.7), respectively. A statistically significant positive correlation was found between the DASH and VAS score (p < 0.001, r= 0, 27)

Conclusions:

Pain is highly prevalent in patients with total brachial plexus injury and is predictive of disability (high DASH scores).
Background and Aims:

Hypersensitivity to painful stimuli and reduced endogenous pain inhibition are associated with various chronic neuropathic pain states, but no differences in structural and somatosensory function were previously demonstrated between the patients with neuropathic pain and neuropathy without pain.

Methods:

Demographic data and self-completed questionnaires as Hospital Anxiety and Depression Scale (HADS), the RAND-36 questionnaire, concerning questions of health and quality of life and the QuickDASH questionnaire, evaluating symptoms and function of the upper extremity were obtained from 50 patients with neuropathic pain and 20 subjects with neuropathy without pain after nerve suture surgery. Quantitative sensory testing (QST), conditioned pain modulation (CPM) responses complemented the physical examination. All subjects with pain had peripheral neuropathy as defined by abnormal QST. Cold pain task was measured by immersion of the non-operated hand into a cold-water bath.

Results:

No statistical differences were found between HADS scores between the two groups. The QuickDASH test revealed higher scores in patients with pain. The difference was statistically significant (p=0.01). The findings revealed lower scores for RAND 36 in patients with pain (p=0.02), but no difference in pressure pain threshold and tolerance (p>0.05), cold pressor pain tasks, endogenous modulation in patients with pain compared to adults without pain.

Conclusions:

The subjects with persistent neuropathic pain after nerve suture surgery had lower scores in health and quality of life and lower function in the injured upper extremity, but no differences in HADS, CPM and QST in comparison with the subjects with neuropathy without pain.

Acknowledgement: Annika Gunnarsson, Marie Essemark, Torsten Gordh, Rolf Karlsten
Background and Aims:

Axonal regeneration capacity in humans remains elusive, specifically the fate of small nerve fibres. This project uses carpal tunnel syndrome (CTS) as a human model system that allows the prospective evaluation of neural regeneration in the context of neuropathic pain.

Methods:

Sixty patients with CTS undergoing surgery as well as 13 non-operated patients participated in the study. Quantitative sensory testing (QST) and electrophysiology were used to evaluate the function of large and small fibres at baseline and 6 months after surgery/baseline measurement. Structural integrity of small fibres was evaluated on serial skin biopsies of the hand (intraepidermal nerve fibre density, IENFD). RNA sequencing of the skin was performed to determine a potential molecular signature associated with neural regeneration.

Results:

At 6 months post surgery, neurophysiological (p<0.0001) as well as somatosensory recovery of both small and large nerve fibers was apparent (p<0.041). In contrast, neurophysiological and sensory function remained unchanged without surgery (p>0.351). IENFD showed a progressive decline in patients who did not undergo surgery (p=0.021). After surgery, IENFD increased (p=0.001), but failed to reach normal levels despite the normalised sensory function. RNAseq revealed that >500 genes were dysregulated following surgery, with a highly enriched cluster being related to axonogenesis.

Conclusions:

CTS as a model system revealed significant postoperative improvement in small and large fibre function. Structural regeneration of small fibres was also apparent, but failed to reach normal levels despite symptom resolution. We are currently validating the identified genes and their association with neural regeneration.
Background and Aims:

Although it is usually a self-limited dermatomal rash with pain, VZV infection can be far more serious. It can cause a spectrum of complications, including VZV vasculopathy. Acute cases can further lead to sequela of events, postherpetic neuralgia (PHN) included.

To describe in details a rare case with atypical VZV presentation. A special emphasis is given to therapeutic options for persistent PHN.

Methods:

Clinical data, cranial CT, MRI, TCD results were analyzed. In order to scrutinize the pain, the patient was interviewed by Digital Analogue scale (DAS), DN4 questionnaire, and HAD scale.

Results:

On the 22nd March 2016, a 76-year-old otherwise healthy lady felt a sharp-sudden-onset-intensive pain on the left side of face (Figure 1). CT revealed pontine lesion on the left, which was differentiated between cavernoma and arteriovenous malformation. Then, MRI was performed and a focal haemorrhagic lesion was found in the left middle cerebellar peduncle (Figure 2). TCD was normal. In October, due to gradually-increasing-in-intensity, burning-shooting-in-manner facial pain, the patient was consulted in Pain Clinic. Pain characteristics were: duration-7months, DAS–9points, DN4–5points, HAD–7/15points, left hyperalgesia and mechanical alodynia in CN5 V1-V2. Re-collected anamnesis revealed that vesicular rash appeared a week after pain started, and then patient received Acyclovir for 7-days as if for HSV infection. The prescribed analgesics are given in Table 1.

Conclusions:

Recognition of VZV vasculopathy and accurate treatment are extremely important to prevent PHN. However, once PHN has developed, various treatments are still available, which can possess effective analgesic properties.
INTERCOSTOBRACHIAL NERVE RESECTION IN BREAST CANCER SURGERY: PATIENTS WITH AND WITHOUT NEUROPATHIC PAIN 4 TO 9 YEARS AFTER TREATMENT
L. Mustonen¹,², H. Harro¹,², E. Kalso²
¹University of Helsinki and Helsinki University Hospital, Clinical Neurosciences- Neurology, Helsinki, Finland
²University of Helsinki and Helsinki University Hospital, Division of Pain Medicine- Dept. of Anaesthesiology- Intensive Care and Pain Medicine, Helsinki, Finland

Background and Aims:
Neuropathic pain (NP) is an important component in persistent postsurgical pain (PPSP). In a cohort of 1000 women operated for breast cancer 13.5% had at least moderate pain in the surgical area one year after surgery (Meretoja T et al. JAMA 2014;311:90). In the current study we analyzed the role of nerve injury in PPSP in the same cohort.

Methods:
All patients who reported pain in the surgical area or who had peroperative intercostobrachial nerve (ICBN) injury as reported by the surgeon, were invited to a clinical examination to establish the relationship between nerve injury and PPSP. A neurologist blinded to the ICBN resection status assessed the patients according to the updated NP clinical grading criteria (Finnerup N et al. Pain 2016;157:1599). The patients answered the Brief Pain Inventory (BPI).

Results:
The patients were examined on average 6.5 years (range 4-9) from surgery. 55 patients had pain in the surgical area without surgeon verified nerve injury. 251 patients had surgeon-verified ICBN resection (partial or total) and of these 152 had definite NP. 22% of the patients with definite NP reported pain intensity of ≥ 4/10 in the surgical area. There was no statistically significant difference in pain intensity between the two types of nerve injury.

Conclusions:
This study suggests that about 61% of patients with surgeon-verified peroperative injury of the ICBN have NP after 4-9 years and 22% of the definite NP patients have at least moderate pain.
Background and Aims:

Adjuvant chemotherapy with docetaxel and oxaliplatin increases survival in patients with high-risk breast and colorectal cancer, respectively, but may induce acute and chronic neurotoxicity. This study is a 5-year follow-up of chronic chemotherapy-induced peripheral neuropathy (CIPN).

Methods:

In 2011-2012 74 patients with high-risk colorectal cancer and 100 patients with high-risk breast cancer answered a questionnaire before, during and one year after receiving adjuvant chemotherapy with oxaliplatin and docetaxel, respectively. In 2016, a 5-year follow-up with the same questionnaire was performed in survivors.

Results:

Fifty-two (36.5% women) of 57 eligible patients (91%) treated with oxaliplatin and 80 (100% women) of 94 eligible patients (85%) treated with docetaxel answered the questionnaire. The most common symptoms of CIPN were tingling in the hands (44.2% in the oxaliplatin (CI95% 30.5; 58.7) and 36.3% in the docetaxel group (CI95% 25.8; 47.8)) and feet (52.0% in the oxaliplatin (CI95% 37.6; 66.0) and 37.5% (CI95% 29.9; 49.0) in the docetaxel group) and numbness in the feet (34.6% in the oxaliplatin (CI95% 22.0; 49.1) and 17.5% (CI 95% 9.9:27.6) in the docetaxel group). Pain was present in the hands or feet in 28.9% of patients treated with oxaliplatin (CI95% 17.12; 43.0) and 31.3% of patients treated with docetaxel (CI95% 21.3; 42.6).

Conclusions:

The results showed no major change in symptoms of neuropathy or pain from 1 to 5 years after chemotherapy. Symptoms of neuropathy were more common in patients treated with oxaliplatin.

Funding: The European Union’s Horizon 2020 research and innovation programme under grant agreement No 633491 (DOLORisk) and Aarhus University.
Background and Aims:

Small fiber neuropathy (SFN) is a condition that affects the small Aδ- and C-fibers, leading to neuropathic pain and autonomic dysfunction. Several sodium channel gene mutations have been found in patients with SFN, with SCN9A-gene mutations being the most frequent. Current available sodium channel blockers are not selective for Na\textsubscript{v}1.7, and often result in severe side effects. Lacosamide targets specific sodium channels with a slow-inactivation state, while sparing those with normal activity. An impaired slow-inactivation of Na\textsubscript{v}1.7 has been found in several patients with SFN. The primary objective of this study is to determine the effect of lacosamide versus placebo on pain in subjects with SCN9A-associated SFN. Secondary objectives are to determine the effect on autonomic symptoms, sleep interference, and quality of life, and to examine the safety and tolerability.

Methods:

The Lacosamide-Efficacy-'N'-Safety in SFN (LENSS) study is a randomized, placebo-controlled, double-blind, crossover-design study. Subjects were randomized to start with lacosamide and end with placebo or vice versa. During both of the two phases, the subjects were treated for a period of eight weeks of 200mg BID, preceded by a titration period, and ended by a tapering period. Patients filled in a pain diary twice daily and scored a set of validated questionnaires on autonomic symptoms, sleep interference, and quality of life at multiple study visits.

Results:

Twenty-five patients with SCN9A-associated SFN were included between November 2014 and February 2017. At the moment the results are analyzed.

Conclusions:

The final results of the study will be presented.
Background and Aims:

To evaluate the effectiveness of regional nerve blocks in the treatment of acute herpes zoster and early post herpetic neuralgia compared to a group with medical treatment along.

Methods:

We treated 20 patients (8 female), age ranging 25-70 years During July 2015-June 2016 in the pain clinic of Northern International Medical College. Inclusion criteria herpetic zoster within 3 months. Exclusion criteria patients refusal, unconsciousness, Bleeding disorders. Patients were randomly divided into group A treated with systemic antiviral, anti-depressant and anti-convulsive drugs. Group B treated with sympathetic blocks. Pain intensity were assessed by Verbal Rating Score (VRS) and visual Analogue Scale (VAS).

Results:

In Group A - 3 of 10 had pain relieved within 4-5 days. VRS and VAS score reduced to 1-2. Two cases responded slowly and reduced pain in 2 weeks, 5 cases pain persisted beyond 2 weeks. In group B - 7 cases had rapid relieved of pain and eruption within 3-5 days of block. VAS and VRS score reduce to 1-2 after 7 days. 3 cases required 8-10days for satisfactory pain relief. It was observed that recovery rapid in those who started treatment early. No significant complication during treatment in both the Groups.

Conclusions:

Regional Sympathetic Blocks with local anesthetic agents is a suitable technique for the pain management of acute and early post herpetic neuralgia either alone or in combination with medical treatment. Early onset of treatment required for early recovery. Further studies with large samples are required for better result.
CLINICAL: PERIPHERAL NEUROPATHIC PAIN - PART 2

NEUP7-0155
MIXED PERIPHERAL NEUROPATHIC + NOCICEPTIVE PAIN: INTERDEPENDENCE AND DE-SENSITIZATION BY LOCAL PROCEDURES - A CASE REPORT OF DISTAL RADIUS FRACTURE
U. Kock¹, S. Sator-Katzenschlager¹, H.G. Kress¹
¹Medical University of Vienna,
Department of Anaesthesia- Critical Care and Pain Medicine- Division of Specialist Anaesthesia and pain Medicine, Vienna, Austria

Background and Aims:

Complex Regional Pain Syndrome (CRPS) occurs subsequently in about 3.8% of displaced radial fractures (DFR)* **. Ulnar Impaction Syndrome (UIS) (ulnar wrist pain, swelling, limitation of motion)***, often due to posttraumatic ulna-plus variance resulting in malunion of the radius*** ****, may be even more frequent, but not less debilitating. We report a case of UIS involving neuropathic and nociceptive symptoms, where rehabilitation was significantly supported by analgesics in combination with local non-invasive procedures.

Methods:

Displaced left DRF in a 56yr old patient required reduction (brachial plexus block) with immobilisation, followed by good fracture healing, but also by radial shortening, ulna-plus variance of +3mm, clinical UIS, neuropathic pain (burning sensation NRS 7-8, glove-like up to the elbow, allosthenia at back of hand/r superfic.n.rad.), and burning pain over the distal ulna NRS 5-6, both aggravating on supination. Medication (NSAID) alone decreased both pain intensities by 50%, whereas additional application of 5% lidocaine medicated plaster (LMP) (Versatis®) to the distal half of the ulna also extinguished the whole "burning glove" temporarily, and markedly reduced intensity of the allosthenia. The "burning glove" was permanently extinguished by wearing a protective splint (left) and additionally wearing a glove and forearm-cuff of the right side for one week. Grip strength improved, and tingling (entangling of nerves) declined.

Results:

Treatment remained conservative, and rehabilitation started to restore function. If persisting, allosthenia will be treated by 8% capsaicin-medicated patch (CMP) (Qutenza®).

Conclusions:

Nociceptive trigger zones as well as their antinociceptive treatment might strongly interact with peripheral neuropathic phenomena.
Background and Aims:

Neuropathic Pain (NP) is a challenge in the rehabilitation of peripheral nerve injuries. NP includes both spontaneous and evoked painful sensations. Mechanical allodynia (MA) is a painful sensation evoked by a mechanical stimulation that is normally not painful (e.g. touch). The Somatosensory Rehabilitation of Pain Method (SRPM) is a non-pharmacological treatment that aims to decrease NP by normalizing cutaneous tactile sensibility. So far, no study has investigated the duration of treatment with SRPM that is required to relieve MA. The aim of this study was to assess the duration of SRPM required to relieve MA and whether this duration is correlated with initial pain intensity in patients with nerve injuries affecting the hand.

Methods:

A retrospective case series of patients treated with SRPM at the Fribourg’s Centre de rééducation sensitive (from 2004 to 2013) for MA in the hand after nerve injury.

Results:

16 patients (50.6 ±14.7 years) meet the inclusion criteria (nerve injury with allodynia in the hand). SRPM relieved MA in the hand (pain less than 3/10 on VAS with 15g pressure) in all but 1 patient. The mean duration of SRPM required to relieve MA was 5.5 (±4.5) months (median = 3.3 months; range: 1.3 to 18.0 months). The duration of SRPM required was correlated (Spearman R=.55; p=.03) with the pain intensity (McGill pain questionnaire) before SRPM initiation.

Conclusions:

The time required to relieve MA with SRPM is related with the intensity of pain at the initiation of the method.
Background and Aims:

Pain from peripheral neuropathy is poorly treated even with the most effective pharmaceutical agents (Finnerup NB et al. Lancet Neurol 2015). The goal of this study is to assess the safety and effectiveness of 10 kHz spinal cord stimulation (SCS) in the treatment of chronic intractable pain from peripheral polyneuropathy.

Methods:

Subjects with chronic, intractable pain of ≥5 cm (on a 0-10 cm visual analog scale [VAS]) of the upper or the lower limb from peripheral polyneuropathy were enrolled in a prospective, multi-center study following Institutional Review Board approval. After successful trial stimulation, each subject was implanted with a pulse generator and two epidural leads spanning C2-C6 or T8-T11 vertebral bodies for upper limb pain and lower limb pain, respectively. Safety and effectiveness endpoints were captured up to 12 months post-implant. Results are presented as mean ± standard deviation.

Results:

A total 28 subjects have been enrolled in the study of whom two failed additional screening criteria. Twenty six subjects were trialed of whom 21 had a successful trial (81% trial success rate) and 18 received a permanent implant.

All three procedure related adverse events (infection [1], implant site extravasation [2]) were resolved. Baseline pain scores of 7.5 ± 1.4 cm (N=18) improved to 2.2 ± 1.8 cm (N=11) at 3 month follow-up (69.7% ± 24.5% pain relief). Responder rate at 3 month follow-up was 63.6% (7/11).

Conclusions:

Preliminary results from a multicenter, prospective study using 10 kHz SCS to treat chronic intractable pain from peripheral polyneuropathy are promising.
Background and Aims:

Outcome of ultrasound-guided Intercostal Nerve Block (ICNB) to neuropathic pain outpatients have not been sufficiently investigated. This retrospective study was evaluate the treatment period of repeated ICNB combined with pharmacological therapy.

Methods:

A total number of 314 blocks were evaluated in 39 outpatients. Acute pain involved 19 cases of herpes zoster, 5 cases of chest radiculopathy. Chronic pain involved 6 cases of post-herpetic neuralgia, 3 cases of post-thoracotomy pain, and 2 cases of peripheral neuropathic pain with subcutaneous tumor. 4 cases were cancer pain. All patients decided their schedule and termination of this treatment by themselves. Median and interquartile range of number of times and treatment period of ICNB was evaluated. Outcome of ICNB termination was divided into successful group and no changed group.

Results:

Acute pain patients finished ICNB after 4 times in 4 weeks. Chronic post-herpetic neuralgia patients required 25 times of ICNB in 43 weeks, and post-thoracotomy patients required 4 times in 7 weeks. Success rate of this treatment was lower in chronic pain. Allodynia in herpetic neuralgia-associated pain did not affect the number and the treatment period. Chronic pain patients required longer treatment period. A single pneumothorax was diagnosed 3 days after ICNB.

Conclusions:

Ultrasound-guided ICNB combined pharmacotherapy may be effective for acute herpes zoster associated pain. For chronic neuropathic pain patients, repetitive ICNB could be clinical option, but this treatment cannot guarantee permanent pain reduction in all patients.
Background and Aims:

Neuropathy is one of the most common long-term complications of diabetes and 25% to 50% of diabetic neuropathy patients will develop neuropathic pain. Although neuropathic pain can have a major deleterious impact on quality of life its pathophysiology in the context of diabetic peripheral neuropathy is complex and not fully understood. A potential mechanism are changes in sodium channels such as Nav1.7. Loss of function mutations in this channel cause insensitivity to pain, whereas gain of function mutations have been linked with different pain syndromes. Our aim was to investigate whether mutations in Nav1.7 are associated with diabetic neuropathic pain.

Methods:

190 patients with diabetic peripheral neuropathy were screened for mutations in Nav1.7. The potentially pathogenic mutations were expressed in HEK293T cells and assessed functionally by whole cell patch clamp.

Results:

Eleven Nav1.7 variants were identified in 8 patients within a series of 111 subjects with painful diabetic neuropathy. Five of these variants were previously associated with pain disorders: V991L, M932L (Faber et al. 2011); W1538R (Cregg et al. 2013), R185H (Han et al. 2012), L1267V (Huang et al. 2014). Among the other variants two of them met the criteria of potential pathogenicity based on predictive algorithms and were further studied. Functional analysis showed that one of these variants (M1852T) drastically impairs channel inactivation by shifting the steady-state fast-inactivation towards more depolarising potentials. No rare Nav1.7 variants were found in 79 subjects with painless diabetic peripheral neuropathy.

Conclusions:

These observations suggest that mutations in Nav1.7 may contribute to painful diabetic peripheral neuropathy.
Background and Aims:

We recently published a new way of stratifying patients with neuropathic pain based on Quantitative Sensory Testing (QST) profiles [1] in three distinct sensory phenotypes, characterized mainly by sensory loss, thermal hyperalgesia or mechanical hyperalgesia. Aim of this study was to deliver an algorithm for individual allocation of single patients to these phenotypes and determine the frequency of each phenotype in a population of patients with painful diabetic polyneuropathy.

Methods:
For each QST z-value of each patient, a probability can be calculated for a phenotype to be present based on the density function of said phenotype. The formula can be normalized so that a value that is equal to the mean of the phenotype is 100%. The resulting value can be calculated averaged over the 13 QST parameters, resulting in a probability for a patient to belong to the given phenotype.

**Results:**

151 patients with painful diabetic polyneuropathy could be included from various European studies [1,2,3]. Almost two thirds (96, 64%) were mainly characterized by sensory loss, 25 (17%) by thermal hyperalgesia and the remaining 30 (20%) by mechanical hyperalgesia (Figure 1).

**Conclusions:**

The QST profile reproduces the clusters as expected, showing that the algorithm is working properly. All three phenotypes were present in a relevant frequency in diabetic polyneuropathy, which is a remarkable difference to the former stratification method by the presence or absence of irritable nociceptors, the former virtually not present in diabetic polyneuropathy [3].

**References**

Background and Aims:

Over the last 30 years, no novel analgesics developed for the treatment of peripheral neuropathic pain (NeP) have reached the market. New NeP drugs are studied in patient populations defined by the underlying aetiological indication. However, this does not take into account chronic NeP as a disease in its own right with distinct disease mechanisms common across aetiological indications. By stratifying patients into sensory profiles-based, mechanism-related phenotypes, it may be possible to observe greater efficacy and detect greater precision, increasing the assay sensitivity of clinical trials.

Methods:

The IMI Europain consortium sought advice from the European Medicines Agency (EMA) on stratifying NeP patient populations in clinical trials. The three methods suggested are quantitative sensory testing (QST), electrophysiology (nerve excitability (NE) and microneurography (µNeG)) and corneal confocal microscopy (CCM).

Results:

The EMA considered QST adequate for determining sensory phenotypes of patients in exploratory trials on NeP. It was agreed that µNeG might have the potential to provide efficacy endpoint in proof of concept clinical trials. CCM was acknowledged to confirm a small fiber neuropathy diagnosis in diabetes.

Conclusions:

A mechanism-related classification scheme based on sensory profiles has the potential to minimize pathophysiological heterogeneity within NeP patient groups and to increase the power to detect a positive treatment result in subgroups. Although NE, µNeG and CCM show great potential, their sensitivity and specificity in detecting NeP has to be further investigated. Still, all three methods will help paving the way to a mechanism-related classification and treatment of NeP.
Background and Aims:

Dynamic mechanical allodynia is a painful sensation from normally innocuous mechanical stimulation. It is generally considered the most distressing and debilitating component of postherpetic neuralgia (PHN). In the elderly, pain treatment is challenging due to frequent comorbidities and polypharmacy. We investigated short- and long-term effects of the topical analgesic 5% lidocaine medicated plaster on allodynia severity in elderly PHN patients (≥70 years of age).

Methods:

Alldynia data from three European clinical trials were pooled and stratified by age (elderly patients and patients <70 years). Dynamic mechanical alldynia testing was performed by either using a standardized brush (N12) or a von Frey Hair (26 g) to stimulate the painful PHN area. Alldynia severity was rated by patients on a 4-point categorical scale.

Results:

Baseline data were available for 284 elderly and 154 younger patients: alldynia was present in 93.3% of elderly and 87.7% of patients <70 years of age. At baseline, the majority of patients rated alldynia severity as painful or extremely painful (elderly patients 63.4%, <70 group 57.1%). Numbers were markedly reduced by 5% lidocaine medicated plaster treatment. In treatment week 3-4, painful or extremely painful alldynia was documented for 37% of elderly and 22% of <70 patients, in week 9-12 for 25.6% and 14.9% of patients, respectively, and in week 40-52 for 8.7% and 3.7% of patients, respectively.

Conclusions:

The 5% lidocaine medicated plaster provided marked reductions in alldynia severity in elderly PHN patients indicating its usefulness for short- and long-term PHN treatment of the elderly.
Background and Aims:

Abnormal activation of the adaptive immune system, and non-inflammatory autoantibodies may contribute to cause persistent Complex Regional Pain Syndrome (CRPS). Mycophenolate is a disease modifying anti-rheumatic drug with established efficacy in autoantibody-mediated neurological and rheumatological disorders. The study aim was to gain first data on mycophenolate efficacy, and on the feasibility of conducting a larger trial.

Methods:

Patients fulfilling Budapest Research Criteria for CRPS, and with a duration of >2 years and average daily pain intensity >5NRS were eligible. Following consent, twelve patients were randomized (05.2015-05.2016) through an independent online system to receive first mycophenolate over 5.5 months, followed by usual-practice treatment, or vice-versa. Mycophenolate was uptitrated openly to effect at 1-1.5g BD. Assessment was with pain diaries, and mechanical QST, and questionnaires at both baseline and study-end.

Results:

One patient dropped out after the control-treatment. 11 patients were titrated on mycophenolate. Of these, four (mean duration 6 years) had between 2-5 NRS points pain relief versus baseline, and near-complete normalisation of both sleep and mechanical sensitivity, without side effects. Two patients had 2-6 points pain relief, but stopped due to severe nausea, or skin-cryptitis. Four patients without pain relief dropped out before full up-titration due to i) worsening depression, ii) worsening pain (x2), iii) skin itchiness; side effects resolved off-drug. One patient had no pain relief and no side effects.

Conclusions:

Mycophenolate treatment of CRPS may be efficacious in 33-50% of patients with severe, longstanding CRPS. Conduct of a phase III trial appears feasible, and is now required.
THE IMPACT OF SERUM DRUG CONCENTRATION ON THE EFFICACY OF IMIPRAMINE, PREGABALIN AND THEIR COMBINATION IN PAINFUL POLYNEUROPATHY

J.V. Holbech¹, S.H. Sindrup¹, F.W. Bach², N.B. Finnerup³, K. Brøsen⁴, T.S. Jensen⁴

¹Odense University Hospital, Neurology, Odense, Denmark
²Aarhus University Hospital, Neurology, Aarhus, Denmark
³Aarhus University Hospital, Danish Pain Research Center, Aarhus, Denmark
⁴Institute of Public Health- University of Southern Denmark, Clinical Pharmacology, Odense, Denmark

Background and Aims:

The serum concentration-effect relation was explored for first line drugs in neuropathic pain and aimed to determine if efficacy could be increased.

Methods:

Data from a randomized, placebo-controlled, cross-over trial on imipramine, pregabalin and their combination in painful polyneuropathy were used. Treatment periods were of 4 weeks’ duration, outcome was the weekly median of daily pain rated by a 0-10 numeric scale, and drug concentrations were determined by high-performance liquid chromatography.

Results:

In 47 patients pain was reduced -1.0 (95% CI -1.5:-0.6) by imipramine, -0.4 (95% CI -0.9: 0.1) by pregabalin and -1.6 (95% CI 2.1:-1.1) by combination therapy. On monotherapy, there was no difference between responders and non-responders with respect to concentrations of imipramine (mean 161 nmol/L vs. 229 nmol/L, p = 0.129) and pregabalin (mean 9.8 µmol/L vs. 11.7 µmol/L, p = 0.178). There was no correlation between drug concentration and pain reduction for imipramine (r= 0.17, p = 0.247), whereas there was a marginally, positive correlation for pregabalin (r = 0.28, p = 0.057). There was no interaction between treatment and concentration classes (imipramine < or ≥ 100 nmol/L, pregabalin < or ≥ 10 µmol/L) neither for mono-therapy nor for combination therapy (p = 0.161-0.797). Isobulographic presentations of responders with imipramine and pregabalin concentrations during combination therapy did not indicate synergistic interaction.

Conclusions:

There were no important relations between drug concentrations and efficacy, or indication of synergistic interaction between the drugs. It was not implied that treatment can be improved by measurement of drug concentration of pregabalin.
COMPARISONS BETWEEN THE EFFICACY OF LIMAPROST ALFADEX AND PREGABALIN IN CERVICAL SPONDYLOTIC RADICULOPATHY

A. Onda¹, M. Kimura¹
¹Zenshukai Hospital, Orthopaedic Surgery, Maebashi, Japan

Background and Aims:

Cervical spondylotic radiculopathy (CSR) is a relatively common neurological disease caused by the mechanical compression of nerve roots. Limaprost, a prostaglandin E₁ analogue, is a vasodilator that has been used in the treatment of lumbar spinal stenosis in Japan. However, the effect of limaprost on CSR has not been investigated. Our aim was to compare the efficacy of limaprost with pregabalin, which is widely used for the treatment of neuropathic pain.

Methods:

In this randomized trial, patients with CSR received either limaprost or pregabalin orally for 8 weeks, along with nonsteroidal anti-inflammatory drugs (NSAIDs). The primary outcomes were assessed using a numerical rating scale (NRS) of pain and numbness, both at rest and on movement. Secondary outcomes were assessed using provocation tests, pain DETECT questionnaire, Short Form (SF)-36, and subjective global assessment. The obtained data were evaluated according to the per-protocol analysis principle.

Results:

A total of 34 patients were enrolled in this study. A greater reduction in pain was observed in the pregabalin-treated group up to 4 weeks. In the limaprost-treated group, numbness of the arm on movement showed marked alleviation compared to the pregabalin-treated group at final follow-up (8 weeks). There were no apparent differences between the two groups for the secondary outcomes.

Conclusions:

Although pregabalin provided a greater pain relief than lomaprost, limaprost was superior to pregabalin in treating arm numbness. An improvement in blood flow to the nerve root might be one of the therapeutic targets for CSR-associated symptoms.
METHYLCOBALAMIN AS AN ADJUVANT ANALGESIC REDUCED PAIN SCALE OF PAINFUL DIABETIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS PATIENTS

T.E. Purwata, M. Rudy, I. Purna Putra, A. Nuartha, D. Purwa Samatra, A. Laksmidewi, P.E. Widyadharma

'Udayana University, Neurology, Denpasar, Indonesia

Background and Aims:

Painful diabetic neuropathy (PDN) is one of the most common complications of diabetes mellitus (DM) in peripheral nervous system. To date, most treatments of PDN only reduce pain symptoms with unsatisfying outcome. Methylcobalamin (MeCbl), in addition of its neuroprotective and neuroregeneration effects, also possesses analgesic effect, thus giving a new hope in the long term therapy of PDN. The aim of this study is to prove that MeCbl can reduce the pain scale in type 2 DM (T2DM) patients.

Methods:

We conducted a single blind randomized controlled trial on 28 T2DM outpatients in neurology and diabetes clinic of Sanglah General Hospital for 3 months (February until April 2016). Subjects were divided into 2 groups, intervention (using oral amitryptiline and intravenous MeCbl) and control group (oral amitryptiline and intravenous aquabidest), 14 subjects in each group. We use oral amitryptiline 12.5 mg twice daily for 10 days and intravenous MeCbl 500 μg every 2 days for 5 times. Pain intensity was measured with Numeric Pain Rating Scale (NPRS) at the beginning and end of study. We compared the mean of NPRS reduction between 2 groups with paired Student t test.

Results:

At the end of the study there was a significant difference of NPRS reduction between intervention and control group when compared with the beginning of the study, which were 5.14 (SD 1.79) or 79% and 2.64 (SD 0.84) or 48% reduction, respectively (p<0.01).

Conclusions:

Methylcobalamin reduced pain scale of PDN in T2DM patients
NEUP7-0016
CORTICOSTEROIDS AS A REAL TIME TREATMENT OF COMPLEX REGIONAL PAIN SYNDROME AFTER FRACTURE. THE EVIDENCE SUPPORTS THAT WE ACT BEFORE IT IS TOO LATE.

P. Winston

1University of British Columbia, Division of Physical Medicine and Rehabilitation, Victoria, Canada

Background and Aims:

Fracture has been implicated in the development of Complex Regional Pain Syndrome (CRPS) in up to 46% of cases of CRPS. Failure to treat may result in contracture, deformity, chronic pain, disability or amputation. Differing diagnostic criteria have made applying a universal diagnostic paradigm challenging. Many tests proposed to diagnose the condition may be expensive, require long waits and have limited access. Clinical practice, however, reveals the frequent occurrence of a painful swollen wrist and hand and possibly shoulder, with limited range, painful contracting joints and loss of use in joints including those not affected by the fracture. Though no large scale randomized control trial has endorsed the efficacy, clinical practice is supported by numerous studies showing rapid resolution of the CRPS after the introduction of corticosteroids.

Methods:

Numerous papers have shown efficacy with international experts endorsing the use of corticosteroids for early intervention to halt and reverse the effects of CRPS. Level 1 evidence has been proposed by some authors, while other note it is the only anti-inflammatory drug with direct clinical evidence. A 60 mg prednisone taper has been a common dosage.

Results:

Early treatment with acute CRPS of the upper extremity allows for real time monitoring of the course of CRPS with improvements in pain levels, gain of function and reversal of deficits noted within days to weeks.

Conclusions:

Early use of corticosteroids has been noted to be safe and may offer reversal or resolution of pain, disability and disuse. Clinical acumen allows for inexpensive, rapid and effective treatment.
Background and Aims:

Chronic pain impacts many aspects of a patient’s quality of life (QOL). Ketamine is a NMDA receptor antagonist that has been used for neuropathic pain. This study aims to: (1) evaluate the impact of outpatient ketamine infusions on pain and QOL with a larger sample size than our previous study; (2) determine if there is a difference in results in patients receiving 1 or 3-day infusions; and, (3) examine whether repeated infusions have an influence on outcomes.

Methods:

118 GW pain clinic patients completed the basic pain inventory (BPI) to rate, on a scale from 0 to 10, their pain and the degree pain interfered with QOL (general activity, walking, work, relationships, mood, sleep, and enjoyment of life). The BPI was completed prior to 1 or 3-day infusions and was repeated 2-4 weeks after infusions. Paired two tailed t-tests and random effect mixed models were used to compare post and pre-infusion scores.

Results:

There was statistically significant improvement (p<0.05) in pain, enjoyment of life, general activity, mood, work, relationships, and sleep, but not walking (p= 0.2419). There was no significant difference in outcomes between 1 and 3-day infusions. However, this may be overstated, as there was a small sample of 1-day infusions. Repeaters had significant cumulative improvement in enjoyment of life (p= 0.0132) and relationships (p= 0.0092) with increased number of repeated infusions.

Conclusions:

1-day or 3-day outpatient ketamine infusions improve pain levels and QOL and may provide cumulative benefits with repeated infusions in patients with chronic neuropathic pain.
NEUP7-0052
RELATIONSHIP BETWEEN EUPHORIA AND EARLY TREATMENT RESPONSE TO PREGABALIN IN PATIENTS WITH CHRONIC PAIN
B. Parsons1, E. Whalen1, P. Bhadra Brown1, M. Ortiz1, L. Knapp1, R. Behar1
1Pfizer, Pfizer, New York, USA

Background and Aims:

Euphoria has been reported in placebo-controlled trials of CNS-acting medications, including pregabalin. It is a complex multifactorial phenomenon, but the association with treatment response has not been examined. This study assessed the relationship between euphoria and pregabalin early treatment responses.

Methods:

Data were from placebo-controlled trials with pregabalin in patients with chronic pain due to diabetic peripheral neuropathy, postherpetic neuralgia, spinal cord injury, fibromyalgia, chronic low back pain, or osteoarthritis. Treatment-emergent euphoria was reported based on MedDRA 19.0 (preferred term ‘euphoric mood’). Pregabalin doses (75–600mg/day) were pooled. Early treatment response was 30% pain score improvement, on an 11-point numeric rating scale, for weeks 1–3 of treatment.

Results:

7322 patients were analyzed (pregabalin, 5017; placebo, 2305); 199 patients reported euphoria (pregabalin, 188; placebo, 11). Median duration of euphoria was 8 days (95% CI, 6–14) for pregabalin and 3 days (1–32) for placebo. For week 1, 43.5% of pregabalin-treated patients with euphoria had an early pain response versus 25.3% without euphoria. For weeks 2 and 3, the proportions of early responders with and without euphoria showed a similar relationship (week 2: euphoria 58.3% vs no-euphoria 36.9%; week 3: euphoria 60.5% vs no-euphoria 42.5%). The proportions of placebo early responders with and without euphoria were quantitatively similar to pregabalin at week 1. Too few placebo patients had euphoria in weeks 2 and 3 to report (n=1 for each week).

Conclusions:

In some patients with chronic pain, euphoria occurrence may be associated with early pain relief.
NEUP7-0053
COMPARISON OF THE EFFICACY AND SAFETY OF PREGABALIN FOR POSTHERPETIC NEURALGIA IN CHINESE AND INTERNATIONAL PATIENTS
B. Parsons¹, L. Xie², M. Ortiz¹, E. Whalen¹
¹Pfizer, Pfizer, New York, USA
²Peking Union Medical College, Peking Union Medical College, Beijing, China

Background and Aims:
To compare the efficacy and safety of pregabalin for postherpetic neuralgia (PHN) in Chinese and International patients.

Methods:
Data from 2 Chinese and 4 International placebo-controlled trials of pregabalin (150–600mg/day) were analyzed. Efficacy endpoints were mean pain score and sleep quality score, both based on a 11-point numeric rating scale, ≥30% or ≥50% pain responder rates, and Patient Global Impression of Change (PGIC) responder rate (symptoms much or very much improved). AE incidence assessed safety.

Results:
1181 patients were analyzed; 313 Chinese (172 pregabalin, 141 placebo) and 868 International (593 pregabalin, 275 placebo). Pregabalin significantly improved mean pain score versus placebo in Chinese (LS mean difference [95% CI]: –0.8 [–1.2, –0.5]; p<0.0001) and International patients (–1.4 [–1.7, –1.1]; p=0.0001). For pregabalin versus placebo, Chinese and International patients showed significant improvement in 30% (53.5% vs 31.9%, p=0.0007; 40.6% vs 19.3%, p<0.0001) and 50% (30.2% vs 11.3%, p=0.0007; 28.2% vs 12.4%, p<0.0001) responder rates, and PGIC responder rates (42.4% vs 17.7%, p<0.0001; 36.1% vs 16.4%, p<0.0001). Pregabalin significantly improved sleep quality versus placebo in Chinese (–0.7 [–1.0, –0.3]; p=0.0006) and International patients (–1.4 [–1.7, –1.1]; p<0.0001). Endpoint results were numerically similar between patient groups for all measures. Dizziness, somnolence, and peripheral edema were the commonest AEs. Pregabalin AE profiles versus placebo were similar between patient groups, but rates were somewhat higher in International patients.

Conclusions:
The therapeutic response and safety profile of pregabalin is comparable in Chinese and International PHN patients
Background and Aims:

Achieving target response to pregabalin in pDPN patients requires adequate upward titration. Our goal was to utilize evidence from a large German Observational Study (OS) to identify relationships between titration and response using cluster analyses to identify patient subgroups.

Methods:

We performed hierarchical cluster analysis in the OS of pDPN patients taking pregabalin (N=2,642) with the following clustering variables: patients with 0-1 dose change or 2+ dose changes, gender, medical history of depression, insulin use, prior use of gabapentin, and severe or moderate baseline pain.

Results:

The OS dataset covered 74.0% of the 192 possible combinations of gender, four age groups, three BMI groups, insulin use, pregabalin monotherapy, and moderate or severe pain. Table 1 shows subgroups for baseline pain and change in pain categories based on number of dose changes. Mean Gower Distance showed separation of nine clusters. Figures 1 and 2 show pain trajectories week-by-week. Final pain levels were significantly different based on dose changes (p<0.0001), with greater proportions improving with upward titration regardless of baseline pain. Patient satisfaction with tolerability (‘satisfied/very satisfied’) was similar across baseline pain category, regardless of number of titrations or degree of improvement (90.3-96.2%) suggesting that tolerability did not influence treatment response patterns.
Conclusions:

Upward dose titration reduced pain; however, the decision to titrate appeared limited by factors other than tolerability. Future analyses of OS data will utilize data from randomized studies to reduce covariate bias in models that predict pain levels for pregabalin-treated patients, enabling exploration of drivers underlying treatment patterns.
THE NMDA RECEPTOR MODULATOR NYX-2925 SHOWS THERAPEUTIC POTENTIAL IN PRECLINICAL MODELS FOR THE TREATMENT OF NEUROPATHIC PAIN.

N. Ghoreishi-Haack, J. Priebe, C. Cearley, T.M. Madsen, J.R. Moskal

1Aptinyx- Inc, Research, Evanston, USA
2Aptinyx- Inc, Clinical, Evanston, USA
3Northwestern University,
Falk Center for Molecular Therapeutics- McCormick School of Engineering., Evanston- IL, USA

Background and Aims:

NYX-2925 is an oral N-Methyl-D-aspartic acid receptor (NMDAR) modulator that acts as a functional glycine-site partial agonist. NYX-2925 is currently in Phase 1 studies and is indicated for treatment of pain associated with painful diabetic neuropathy. The present studies examine the effect of NYX-2925 in rat models of neuropathic pain when administered over a wide dose range as a single dose or two weeks of daily dosing.

Methods:

The analgesic effect of NYX-2925 was evaluated in the rat Bennett (chronic constriction injury, CCI) model, the streptozotocin (STZ) model of diabetic neuropathy, and the formalin model of persistent pain.

Results:

A single oral dose of NYX-2925 produced a rapid-acting (1 hr post-dosing) and long-lasting (24 hrs and 1 week post-dosing) analgesia in the CCI and STZ models with statistically significant efficacy over vehicle seen between 1-30 mg/kg in the CCI model and 1-10 mg/kg in the STZ model. In contrast, the gabapentin (150 mg/kg PO) only produced analgesic effect 1 hr post-dosing in the both models. A single oral dose of 1-10 mg/kg NYX-2925 also reduced flinching in the late phase of the formalin test 1 hr post dosing to a similar degree as gabapentin (150 mg/kg PO). Daily oral administrations of NYX-2925 over 14 days results in significant efficacy over vehicle that was sustained throughout the dosing period.

Conclusions:

These data show that NYX-2925 has therapeutic potential as a daily administered neuropathic pain compound with both rapid acting and long-lasting therapeutic effect.
Background and Aims:

Several types of analgesics are currently used in treatment for neuropathic pain (NP). These analgesics, however, often fail to achieve beneficial therapeutic outcome due to increased incidence of the CNS adverse effects such as dizziness and somnolence, resulting in poorer adherence and treatment discontinuation. We had explored and found a series of compounds that show analgesic but not sedative effects at all. Here, we describe an orally-available and central-penetrating analgesic, TRK-700, now being developed.

Methods:

We evaluated the anti-allodynic effects in various animal models of NP. In addition, we recorded electrophysiological response of wide dynamic range (WDR) neurons in spinal cord during mechanical stimulation with a von Frey filament, and examined the effect by spinal superfusion in the rat spinal nerve ligation model to reveal the site of action.

Results:

TRK-700 had anti-allodynic effects with a similar potency to that of pregabalin in the mouse partial sciatic nerve ligation model. Meanwhile, TRK-700 showed no effect on motor functions at supra-effective doses. TRK-700, additionally had anti-allodynic effects in rat models of diabetic neuropathy and fibromyalgia, demonstrating that TRK-700 is an orally-available and broad-spectrum analgesic without affecting CNS. In electrophysiological studies, spinal superfusion of TRK-700 suppressed the frequency of firing in WDR neurons evoked by mechanical stimulation, suggesting that TRK-700 acts to at least partly spinal cord and suppresses the activation of WDR neurons.

Conclusions:

TRK-700 could be a new type of analgesic for the treatment of chronic pain such as diabetic neuropathy and fibromyalgia.
IMPACT OF ETIOLOGY AND DURATION OF PAIN ON PHARMACOLOGICAL TREATMENT EFFECTS IN PAINFUL POLYNEUROPATHY

S. Sindrup¹, J. Holbech¹, D. Demant¹, N. Finnerup², F. Bach³, T. Jensen²
¹Faculty of Health Sciences - Clinical Research, Odense, Denmark
²Aarhus University Hospital, Danish Pain Research Center, Aarhus, Denmark
³Aarhus University Hospital, Neurology, Aarhus, Denmark

Background and Aims:

The pharmacological treatments for painful polyneuropathy have not changed much for more than a decade, and less than half of the patients obtain adequate pain relief with first line treatments. Therefore, patient-specific factors which could predict drug response are searched for.

Methods:

We analysed data from 4 published, randomized, controlled trials of drugs in painful polyneuropathy to see if diabetic etiology of polyneuropathy and duration of neuropathic pain had an impact on drug efficacy. The studies had a cross-over design, and had nearly similar outcome recordings as well as a thorough baseline registration of symptoms, signs and quantitative sensory testing. 244 patient records of drug effect distributed over treatments with 3 antidepressants and 2 anticonvulsants were analysed.

Results:

Diabetes as etiology of polyneuropathy had no impact on the effect of antidepressants (imipramine, venlafaxine, escitalopram), but there was a significant interaction with treatment effect on anticonvulsants with better effects in diabetics (0.86 NRS points, p = 0.021) with most pronounced interaction for oxcarbazepine (1.47 NRS points, p = 0.032). There was an interaction between duration of neuropathic pain and treatment with antidepressants with better effect with duration less than 3 years (0.62 NRS points, p = 0.036), whereas anticonvulsants tended to work best with duration of pain for more than 3 years.

Conclusions:

It is suggested that diabetic etiology of polyneuropathy may impact on the efficacy of anticonvulsants, and that duration of neuropathic pain may impact on the efficacy of antidepressants.
CLINICAL: PHARMACOTHERAPY OF NP - PART 2

NEUP7-0359
STABILITY STUDY OF ORIGINAL AND GENERIC PREGABALIN PRODUCTS AVAILABLE IN THAILAND
T. Suansanae¹, J. Suksinworaphong¹, J. Leanpolchareanchai¹, N. Nuchtavorn²
¹Faculty of Pharmacy- Mahidol University, Pharmacy, Bangkok, Thailand
²Faculty of Pharmacy- Mahidol University, Pharmaceutical Chemistry, Bangkok, Thailand

Background and Aims:
According to pregabalin structure, it has a chiral molecule which leading to contain both S-and R-isomers. S-isomer has more potent pharmacological effects than R-isomer approximately 10 times. Therefore, limiting the amount of R-isomers in raw materials of pregabalin is recommended by the European Pharmacopoeia. After manufacturing, S-pregabalin can be degraded to R-pregabalin by multiple environment factors which might affect clinical efficacy and safety. Thus, this study was aimed to determine the stability of pregabalin products which were available in Thailand.

Methods:
Stability of pregabalin was assessed by determination the amounts of S-pregabalin and R-pregabalin in each product at the marketed time which were stored at the hospital. The quantification of the isomer was performed by a derivertisation method and analyzed by using high-performance liquid chromatographic method.

Results:
The original product of pregabalin (Lyrica®) and 2 generics (X and Y) were tested. S-pregabalin was found 107.9%, 106.0% and 106.3%, respectively. They were in the accepted range of labeled amount (90.0-110.0%). R-pregabalin was not detected in all products.

Conclusions:
This study confirmed that generic pregabalin (Brand X and Y) contained S-pregabalin within the same range of the original pregabalin product. However, further stability test should be conducted to examine products which were dispensed and stored by patients because there were different environmental conditions from within hospital.
Background and Aims:

Transcutaneous spinal direct current stimulation (tsDCS) is a neuromodulatory tool which could be in managing refractory chronic neuropathic pain (NP). As tsDCS-induced aftereffects depend on the electrodes montages, we evaluate the potential of tsDCS with unusual electrode montage to relieve chronic NP in individuals of chronic spinal cord injury (SCI)

Methods:

A single-blind crossover design was used to investigate the effects of single sessions of both anodal and sham tsDCS (2 mA, 20 min) on NP in a group of 8 chronic complete motor cervical SCI volunteers. In this study, the active electrode was over the spinal process of the tenth thoracic vertebra and the reference was placed over top of the head. Pre- to post-tsDCS intervention changes in pain intensity (numeric rating scale, 0-10) were assessed before and after tsDCS session (immediately post-stimulation, and at 1 and 2 hours after stimulation).

Results:

Mean pain intensity at baseline, immediately post-stimulation, at 1 and 2 hours after stimulation were respectively 5.9±1.3, 4.6±2.3, 4.8±2.0 and 4.6±2.1 for anodal tsDCS versus 5.6±1.4, 4.8±2.0, 4.4±2.5 and 4.8±2.6 for sham tsDCS. There was no significant pain intensity reduction from baseline to post-treatment assessment under both anodal tsDCS group (p=.47) and sham tsDCS group (p=.47). Furthermore, no significant difference was found between treatment groups at each assessment (p=.71, .96, .62 and .59, respectively).

Conclusions:

This study result suggests that anodal tsDCS with the montage used in this study is not more effective than sham tsDCS for reducing pain intensity in individuals with chronic cervical SCI.
Background and Aims:

Persistent postoperative pain is acknowledged as an adverse effect of a number of thoracic surgeries. However, only few studies have assessed the procedure-specific postoperative pain pattern after cosmetic surgical procedures in the thoracic region. In this study, we quantitatively assessed the characteristics of persistent postoperative pain and associated sensory changes following surgical repair of pectus carinatum.

Methods:

Using a prospective observational design, 28 patients were assessed before, 6 weeks and 6 months after corrective surgery for pectus carinatum. Postoperative pain was assessed using the Short Form McGill Pain Questionnaire. Bedside sensory testing was conducted to detect pinprick hyperalgesia and brush-evoked allodynia. In addition, generic and disease-specific health-related quality of life was assessed using the Short Form-36 Health Survey and the Nuss Questionnaire modified for Adults.

Results:

Six weeks after surgery, 10 patients reported mild or discomforting pain. Six months after surgery, 4 patients reported only mild pain. Pinprick hyperalgesia was detected in 8 patients 6 weeks after surgery and in 6 patients 6 months after surgery (p=0.51). Brush-evoked allodynia was detected in 2 patients at 6 weeks and 6 months after surgery. Generic health-related quality of life was significantly improved over time (<0.0001).

Conclusions:

Overall, surgical repair of pectus carinatum significantly improved health-related quality of life with a limited risk of clinically relevant persistent postoperative pain and sensory disturbances. Future studies may include a longer follow-up period to determine if these positive results are sustained.
Background and Aims:

Neuropathic mechanisms are involved in burning mouth syndrome (BMS), and variation of dopamine D2 receptor (DRD2) gene may contribute to pain perception. We investigated whether the neurophysiologic findings differ in BMS patients compared with healthy controls, and whether 957C>T polymorphism of the DRD2 gene influences perception or interference of pain in daily life in BMS.

Methods:

45 BMS patients (43 women, mean age 62.5 years) and 32 healthy controls (30 women, mean age 64.8 years) participated. Patients estimated pain intensity, suffering, quality of life (QoL) and sleep with NRS. Blink reflex (BR) of the supraorbital (SON), mental (MN) and lingual (LN) nerves and thermal quantitative sensory testing were done. The results were analysed with RM ANOVA. DRD2 gene 957C>T polymorphism was determined in 31 patients and its effects on neurophysiologic and clinical variables analysed.

Results:

Cool (p=0.0090) and warm detection thresholds (p=0.0229) were higher in BMS patients than controls. The stimulation threshold for SON BR was higher in patients than in controls (p = 0.0056). The latencies of R2 component were longer in BMS patients than in controls (p=0.0055) at the SON distribution. Habituation of SON BR did not differ between the groups. The heat pain thresholds were highest (p=0.0312) in patients with 957TT genotype, and they also reported the lowest QoL, more suffering and sleep disturbances (p=0.0254-0.0352).

Conclusions:

The patients showed thermal hypoesthesia within LN distribution compatible with small fibre neuropathy in BMS. The DRD2 957 C>T genotype may influence perception and interference of pain in BMS.
Background and Aims:

Oxaliplatin-induced peripheral neuropathy (OXIPN) is common among colorectal cancer (CRC) patients. OXIPN is known to be difficult to relieve once established and affect functional status and quality of life subsequently. Thus, efforts to identify patients with high risk of OXIPN are necessary for prevention and early symptom management. However, the consensus on clinical risk factors to predict OXIPN has not been made yet. This study aims to examine the prevalence of OXIPN and its association with obesity among CRC patients.

Methods:

The medical records of 1,047 CRC patients received chemotherapy with OXL at a university-affiliated hospital in Seoul, Korea between 2010 and 2014 were reviewed and a total of 478 patients were included in this study. Cox proportional hazard regression was used to identify risk factors for OXIPN.

Results:

OXIPN was occurred in 264 patients (55.2%) under chemotherapy. No significant difference was found in gender or age between patients who do have OXIPN and who do not. However, patients with higher BMI (≥ 23) and higher cholesterol (≥ 200) were 2.3 times more likely to have OXIPN than patients with normal BMI and cholesterol (CI: 1.044-5.214, p=.04).

Conclusions:

The result of this study showed higher BMI and higher cholesterol level could associated with OXIPN. Further studies to determine the roles and mechanism of obesity as a risk factor for OXIPN are needed. The work of this research was supported by grants from National Research Foundation of Korea, 2015. (NRF-2014R1A1A305386)
Background and Aims:

The Multidisciplinary High Risk Foot Protection Clinic was designed to offer support to both patients and family physicians related to neuropathy pressure wound prevention and management of associated symptoms. The clinic offers early screening, diagnosis, and multidisciplinary intervention pertaining to lower limb neuropathy by providing education, resources, and appropriate supports with the goal of enhancing the patients’ knowledge and self-management skills.

A medical director provides direct clinical and consultative support. The clinic liaises with the identified community partners to provide the appropriate resources and supports. Following the assessment, the patients are provided with written resources including a comprehensive activity/intervention plan identifying the relevant areas discussed; follow up visits in the clinic or home visits are options for additional support.

Methods:

Each patient seen in the Multidisciplinary High Risk Foot Protection Clinic is screened prior to scheduling an appointment and must meet 2 of the following criteria including: evidence of loss of protective sensation, history of a neuropathic wound, foot deformity, neuropathy/pain, and edema. Each patient was provided with the EQ-5D-5L (EuroQol survey) prior to being assessed by the Nurse Practitioner and Occupational Therapist.

Results:

A mail out follow up survey will determine whether patients found the educational resources regarding neuropathy beneficial, and if the clinic was helpful in enhancing their knowledge and self-management skills.

Conclusions:

Anecdotal evidence is promising, as patients have subjectively indicated that the information provided throughout the assessment process was helpful and exceeded their expectations.
Background and Aims:

Chronic pain in spinal cord injury (SCI) often engenders negative emotions which can exacerbate pain and negatively impact psychological, social and physical functions. Positive emotions have been found to be analgesic. This study evaluates the efficacy of a tailored positive psychology intervention on well-being and pain in individuals with SCI.

Methods:

This was a community-based, single-blind, randomized-controlled, parallel-group trial including individuals with traumatic/non-traumatic SCI and chronic pain (i.e., pain intensity of $\geq 4$ [0-10] at least half of the days in the past month). Participants in the intervention group were asked to practice 4 personalized positive psychology exercises for 8 weeks. Participants in the control group were asked to be mindful and write about their life. At baseline, mid-treatment, post-treatment, and 3 months follow-up, participants completed measures of well-being and pain-related outcomes.

Results:

108 participants completed follow-up (35% attrition). Relative to pre-treatment participants in the intervention group reported significantly less pain; endorsed more pain control and positive emotions, and reported lower pain interference and pain catastrophizing. Between-group differences at post-treatment were statistically significant for pain intensity. Participants in the intervention condition continued maintaining their improved levels of well-being and perceived pain control 3 months after treatment.

Conclusions:

Positive psychology exercises can be easily incorporated into the lives of individuals with SCI and chronic pain, and appear to have many benefits, including a reduction in pain intensity. More research is needed to replicate findings. If so, dissemination to make these exercises more accessible to individuals with SCI would be warranted.