Summer School

EFIC- European Pain School 2008

University of Siena, Italy

Hyperexcitable Neurons as Pain Generators

Venue: University of Siena, Toscany, Italy
Certosa di Pontignano (Historical Site in Chianti)

Date: June 15 to 22, 2008

Web: www.unisi.it/pain-school/

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Mission of the European Pain School

Pain causes an enormous amount of suffering and disability in 19% of the adult population in Europe. The resulting direct medical and indirect social costs in European countries amount to an estimated 500 Million € per 1 Million of population annually.

Pain may become progressively severe and often is unrelated to the course of an underlying organic disease. Therefore, chronic pain has recently been recognized a disease entity by its own.

A number of somatic, psychosocial and genetic risk factors have been identified which facilitate chronicity, e.g. in low back pain, neuropathies, fibromyalgia and headache. Acute pain not adequately treated constitutes a particular case of high risk to result in long lasting or chronic pain.

Basic and clinical research provide some understanding of progressive pain chronicity. The mechanisms involve long term nervous system plasticity that results in the sensitization of the pain system under repeated or prolonged pain conditions. Thus, both basic and clinical research predict that early preventive measures have a major potential against pain chronicity.

The European Pain School is providing interdisciplinary training to younger scientists, in order to stimulate pain research and improve the concepts for the prevention and treatment of chronic pain in European health systems. Since 2006 the European Pain School cooperates with EFIC, the European Federation of IASP Chapters, and is partially funded by this organization.

European Pain School at Siena: a short history and perspective

The idea of founding an European Pain School was first conceived in 1994, when Anna Maria Aloisi, Giancarlo Carli and Manfred Zimmermann met for a Symposium at the Certosa di Pontignano, a most atmospheric 16th century monastery in the Chianti region, now a Conference center of the University of Siena. In 2002 we could convince FENS, the Federation of European Neuroscience Societies, to give our project the status of a FENS School, including financial resources for our start up in 2003. In our announcement we aimed at giving young scholars a place of transnational scientific interaction in the field of pain science, a branch of increasing recent interest in the plethora of biomedicine.

Finally 40 Scholars were elected, with nearly half of them from Eastern Europe, and 15 Faculty. All of us were faced with the novel experience of spending 24 hours together, Scholars and Faculty, from early morning exercise in the cloisters to midnight talks and drinks in the sala Focolare. In between we had a rich day of listening and talking to the experts, with discussion extending to the large round tables were we enjoyed Lunches and Dinners in the traditions of Toscana, including local wines from vineyards owned by the University of Siena. During all of the week we were living somehow in the tradition of the medieval monastery, changing our habits of academic communication: for a short while we abandoned the spatiotemporal distance of pulpit and classroom schedule and went into the structure of a scientific family, with affective components fostering the transfer of concepts and facts in science, in pain science.

Manfred Zimmermann

International Faculty

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EFIC – European Pain School, Siena 2008 Programme

June 15, Sunday

15:00 – 17:00 Registration in the Certosa di Pontignano

15:00-17:00 Sala Veranda, Chronic Pelvic Pain

18:00 - 19:00, Session I Opening Ceremony

Welcome Addresses

- **Dean or Rector of Siena University**
- **AISD, the Italian IASP Chapter**
- **EFIC – European Pain School**
- **The Certosa di Pontignano**

Students' Self-introduction, - to be continued informally at the subsequent wine reception

June 16, Monday

9:15 – 12:45, Session II

Lecture 1: Introduction to Pain: History, Suffering, Science
Manfred Zimmermann, Heidelberg, Germany

Lecture 2: Neuronal Hyperexcitability: Cellular Mechanisms and Genetic Determinants
Marshall Devor, Jerusalem, Israel

Scholar 1: Li Lili, Stockholm. The adenosin A_{2A} receptor is involved in carrageenan-induced inflammatory hyperalgesia in mice.

Scholar 2: Rony-Reuven Nir, Haifa. Brain activity correlates with subjective pain perception in humans: A study of pain-evoked potentials and electromagnetic tomography

Monday, 15:30 – 19:00, Session III

Lecture 3: Neuronal Activitiy and Psychophysics of Pain – Correlations and Discrepances Hermann Handwerker, Erlangen, Germany

Lecture 4: Neuropathic Pain: The good, the Ugly and the Bad
Jordi Serra, Barcelona, Spain

Scholar 3: Maxim Sokolov, Moscow. Role of beta subunits in functional properties of recombinant human $Na_v1.7$ channels expressed in HEK293 cells.

Scholar 4: Kestutis Petrikonis, Kaunas (Lithuania). Neuropathic pain profile and quantitative sensory testing (QST) in diabetic polyneuropathy

Scholar 5: Devi Sagar, Nottingham. Inhibition of fatty acid amide hydrolase produces peroxisome proliferator activated receptor-α mediated analgesia in a rat model of inflammatory pain

June 17, Tuesday

9:15 – 12:45, Session IV

Lecture 5: Trigeminal Pain Mechanisms
Giorgio Cruccu, Rome, Italy

Lecture 6: Theories of Tic douloureux
Marshall Devor, Jerusalem, Israel & Manfred Zimmermann, Heidelberg, Germany

Scholar 6: Alexander Dimitrov, Sofia (Bulgaria). A mechanism of axonal hyper-excitability predicted by computer simulation

Scholar 7: Riccardo Storchi, Modena. Stable discharge patterns in multiunit recordings of S1 ongoing activity in neuropathic rats

Scholar 8: Stefano Cobianchi, Rome. Botulinum neurotoxin A reduces allodynia and improves functional recovery in a mouse model of neuropathic pain

Tuesday, 15:30 – 19:00, Session V

Lecture 7: How does the Brain Control Neuropathic Hypersensitivity?
Antii Pertovaara, Helsinki, Finland

Lecture 8: Neurotrophic Factors and Pain
Stephen McMahon, London, UK

Scholar 9: Isabel Martins, Porto. Reversal of allodynia and hyperalgesia in a rat model of neuropathic pain by a recombinant herpes vector targeting a supraspinal pronociceptive area

Scholar 10: Claudia Nohn, Hamburg. Gamma oscillations in anterior cingulate cortex are amplified by selective attention in human subjects

Scholar 11: Hanna Viisanen, Helsinki: Antinociception by motor cortex stimulation in neuropathic rats: role of rostroventromedial medullary neurons

June 18, Wednesday

9:15 – 12:45, Session VI

Lecture 9: Pain Studies on Humans – Neuroimaging of Neurotransmitter Receptors
Antii Pertovaara, Helsinki, Finland

Lecture 10: The Clinical Encounters with Neuropathic Pain – why should the clinician guide the basic scientist?
Jordi Serra, Barcelona, Spain

Scholar 12: Leonor Goncalves, Minho and Helsinki. Response properties of amygdala nociceptive neurons to peripherally-evoked stimulation and cortical influence in the neuropathic rat

Scholar 13: Lydia Puljak, Split: Lidocaine injection into the Sprague-Dawley rat dorsal root ganglion causes neuroinflammation and pain-related behavior

Scholar 14: Christian Kamp Nielsen, Copenhagen. Involvement of the $P2X_7$ receptor in development of neuropathic pain in BALBc/j wildtype and knock-out mice

Wednesday, 15:30 – 19:00, Session VII

Lecture 11: Peripheral and central sources of hyperexcitability: pain and itching
Hermann Handwerker, Erlangen, Germany

Lecture 12: Hyperalgesia and Allodynia – Clinical Signs and the Mechanisms behind
Didier Bouhassira, Boulogne, France

Scholar 15: Michael Böttger, Jena. TNF-α inhibition attenuates pain-related behaviour and sensory fiber sensitization in antigen-induced arthritis of the rat.

Scholar 16: Andrew Grant, London. Agonists of protease-activated receptor 2 (PAR_2) sensitize transient receptor potential vanilloid 4 (TRPV4) to induce mechanical hyperalgesia in mice

Scholar 17: Lydia Staniaszek, Nottingham. A role for endocannabinoids in NSAID-mediated antinociception? A study in naïve rats.

June 19, Thursday

9:15 – 12:45, Session VIII

Lecture 13: Sensitization of Pain Signalling Systems in Man and Animals
Stephen McMahon, London, UK

Lecture 14: New Treatment Approaches for Neuropathic Pain
Didier Bouhassira, Boulogne, France

Scholar 18: Miriam Galova, Martin (Slowakia). Phantom limb pain prevention with the application of ketamine

Scholar 19: Katherine Roberts, London. Contact heat evoked potentials (CHEPS) following topical capsaicin in human subjects: a model of "neuropathic pain".

Scholar 20: Kiran Bali, Heidelberg. Experimental pain in mice contributes to regulation of the kinase mTOR and the small GTPase Rac1 in the spinal cord

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Excursion to Siena Old Town

15:00 – 16:30 Guided tour to historical Siena

16:30 – 18:00 Free time in Siena

20:00 Dinner in the Certosa di Pontignano

Film on the Siena Palio

June 20, Friday

9:15 - 12:45, Session X

Lecture 15: Plasticity of the first pain synapse
Marzia Malcangio, London, UK

Lecture 16: Steroids, the 'new' Neurotransmitters and their Role in Pain
Anna Maria Aloisi, Siena, Italy

Scholar 21: Christian Brenneis, Frankfurt. Altered eicosanoid patterns in mPGES-1-deficient mice and its consequence for nociceptive processing

Scholar 22: Robby Jaken, Maastricht. Early immunomodulation following experimental spinal cord injury using nuclear hormone receptor agonists

Scholar 23: Ewelina Rojewska, Krakow. Early immunomodulation following experimental spinal cord injury using nuclear hormone receptor agonists

Friday, 15:30 - 19:00, Session XI

Lecture 17: Channelopathies in Pain
John Wood, London, UK

Lecture 18: Glial Cells – New Functions in Chronic Pain
Marzia Malcangio, London, UK

Scholar 24: Anna Folkesson, Malmö. Effect of co-administered gabapentin and venlafaxine in the rat spared nerve injury model of neuropathic pain

Scholar 25: Olga Kiskira, Greece. Preemptive effect of gabapentin on postoperative pain and morphine consumption after lumbar fusion surgery

Scholar 26: Mohammad Yosry, Cairo. Fluoroscopy guided Selective Nerve root injection for unilateral lumbar radicular pain, is it more effective than blind paramedian translaminar epidural injection after one year?

June 21, Saturday

9:15 - 12:45, Session XII

Lecture 19: Chronic Widespread Pain - The Fibromyalgia Syndrome
Giancarlo Carli, Siena, Italy

Lecture 20: Genetic Approaches to Pain Mechanisms and Treatment.
John Wood, London, UK

Scholar 27: Ruth Moont, Haifa. Interaction between Diffuse Noxious Inhibitory Controls (DNIC) and Distraction in Human Subjects

Scholar 28: Joana Maria Fereira-Gomes Porto. Increased CGRP immunoreactivity in rat primary afferent neurons innervating osteoarthritic knee joints

Saturday, 15:00 – 17:00, Session XIII

Evaluations of the European Pain School by Scholars and Faculty

Discussions on the European Pain School – the strong and week sides

How should we improve the Format of the future Pain School?

17:30 transfer in Rapolano Terme and Farewell Dinner and

Party in the "Terme Antica Querciolaia", Rapolano Terme (SI)

June 22, Sunday, Departures

Experimental pain in mice contributes to regulation of the kinase mTOR and the small GTPase Rac1 in the spinal cord

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Background and Aims. Functional and structural plasticity of central synapses in pain pathways has been claimed to be responsible for allodynia, hyperalgesia and pain memory. However, molecular mechanisms underlying this experience dependent plasticity are not yet clearly understood. mTOR (mammalian Target of Rapamycin) is an evolutionarily conserved serine-threonine kinase and regulates translation of mRNA via its different down stream targets. It is shown to play a significant role in the LTP, LTD and in local translation of plasticity relevant mRNAs. Rac1 is a small GTPase and is shown to regulate spine enlargement, activity-dependent dendrite growth, and synaptic clustering of AMPA receptors during synapse maturation in neurons *in vitro*.

Our goals include analysis of potential regulation of mTOR and Rac in different pain states, viral mediated down- and up-regulation in spinal dorsal horn using AAV (Adeno Associated Virus) and/or Lenti viral system, analysis of their role in pain behaviour and further dissection of underlying molecular pathways.

Methods. Male C57BL6 mice were briefly anaesthetised using ether and 1% formalin or CFA was injected into hind paws bilaterally. L4 and L5 segments of spinal cord were collected after 15, 30, 45 and 60 minutes of formalin injection and 2&6 hours after CFA (complete Freund´s adjuvant) injection, and lysed. Level of expression was detected by immunoblotting using antibodies against total mTOR and total Rac1. Signal intensity is quantified and difference in expression is expressed as percentage change when compared to controls. Levels of active mTOR and active Rac were estimated using an ELISA based method. All procedures were performed in accordance with ethical guidelines laid down by local governing body (Regierungspräsidium Karlsruhe, Germany)

Results. After 15 and 30 minutes of Formalin injection, expression of total and active forms of mTOR and Rac1 showed significant reduction and after 45 and 60 minutes, no significant difference in the level of expression was observed when compared to control. After CFA injection, expression of total mTOR and total Rac1 showed no differences at 2 and 6 hours time points but active mTOR showed significant reduction at both time points.

Conclusions. Our results suggest that there is indeed regulation of proteins involved in cytoskeletal modifications (Rac) and local protein synthesis (mTOR) in pain states. These results also point out differential regulation of these proteins in acute and chronic pain states. In ongoing and future experiments, we plan to address functional roles of these proteins in acute and chronic pain.

Supported by Landesstiftung RNAi grant from Landesstiftung, Baden-Württemberg

TNF-α inhibition attenuates pain-related behaviour and sensory fiber sensitization in antigen-induced arthritis of the rat.

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Background. Compounds inhibiting tumor necrosis factor (TNF-α) have shown beneficial effects in the treatment of rheumatoid arthritis, leading to improved function, decelerated destruction and attenuated pain in the respective joints. The latter might result from an attenuation of the inflammatory process *per se* and/or from neutralization of endogenous inflammatory mediators which act directly on nociceptive neurons.

Aims. Here, we addressed the question whether analgesic effects of the neutralization of TNF-α are due to an attenuation of inflammation or whether direct neuronal effects significantly contribute to pain relief in the course of therapy.

Methods. Locomotor and pain-related behaviour and histology were assessed in rats with chronic antigen-induced arthritis (AIA) in the knee joint, and rats were either treated with systemic saline or etanercept or infliximab. The expression of TNF receptors in dorsal root ganglia was assessed using immunohistochemistry and PCR. Action potentials were recorded from afferent Aδ- and C-fibers of the medial knee joint nerve and etanercept and infliximab were injected intra-articularly into normal or inflamed knee joints (AIA or kaolin/carrageenan-induced).

Results. In AIA rats both etanercept and infliximab significantly decreased inflammation-induced locomotor and pain-related behaviour while joint swelling was only weakly attenuated and histomorphology still revealed pronounced inflammation. In particular, the mechanical threshold at the inflamed knee was significantly increased and gait abnormalities normalized earlier in the TNF-inhibitor treated groups. A large proportion of dorsal root ganglion neurons showed TNFR1- and TNFR2-like immunoreactivity, which did not change during the course of AIA, thus reflecting a stable target for TNF-α. Responses of joint C-fiber afferents to noxious outward rotation of the acutely inflamed knee joint but not of the normal joint were significantly reduced starting from 30 min after intraarticular injection of etanercept, whereas no change was observed after application of infliximab. Furthermore, neither treatment reduced the responses in Aδ-fibers.

Conclusions. Overall, these data show pronounced antinociceptive effects of TNF-α neutralization and suggest that the reduction of TNF-α effects on pain fibers themselves significantly contributes to pain relief.

Supported by the Interdisciplinary Centre for Clinical Research (IZKF), Jena

Reference

Boettger MK et al., Antinociceptive effects of TNF-α neutralization in a rat model of antigen-induced arthritis. Evidence for a neuronal target. Arthritis Rheum., *in press*

Altered eicosanoid patter in mPGES-1-deficient mice and its consequence for nociceptive processing

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Background: Previous studies have shown that Cyclooxygenase-2 (COX-2) dependent prostaglandin (PG) E_2 synthesis in the spinal cord plays a major role in the development of hyperalgesia and allodynia. Microsomal PGE_2 synthase-1 (mPGES-1) isomerizes COX-2-derived PGH_2 to PGE_2.

Aim: Here we aimed to evaluate the effect of mPGES-1-deficiency on spinal prostanoid synthesis and the nociceptive behaviour in various pain models.

Methods: Primary spinal cord neurons and spinal tissue from wildtype and mPGES-1 deficient mice were stimulated with LPS and TNFα. Then we compared the expression levels of COX-2, mPGES-1, mPGES-2 and cPGES by westernblot analysis and the prostanoid levels by LC-MS/MS. The following prostanoids were monitored: $PGF_{2\alpha}$, PGE_2, PGD_2, TxB_2, 11-dh-TxB_2, 6-keto-$PGF_{1\alpha}$, Δ12-PGJ_2, 15d-PGJ_2, 13, 14-dh-15-keto-PGD_2, 13, 14-dh-15-keto-PGE_2, PGA_2, PGB_2, 15d-PGD_2 and LTB_4.

To investigate the effect of mPGES-1 deficiency for nociceptive behaviour we compared the mPGES-$^{-/-}$ mice with their littermates in models for acute pain (hot-plate-test, tail-flick-test) for visceral pain (writhing-test), COX-1 dependent hyperalgesia (formalin-test) and COX-2 dependent hyperalgesia (zymosan-test).

Results: As described previously mPGES-1 deficient mice exhibited in the writhing test (visceral pain) reduced nociceptive behavior. However, in the hot plate test, in the tail flick test, in the formalin test and surprisingly in the zymosan-induced mechanical allodynia, the nociceptive behaviour was not altered in knockout mice as compared to wild type animals despite a marked decrease of the spinal PGE_2 synthesis. Importantly, primary embryonic spinal cord neurons as well as LPS stimulated spinal tissue from mPGES-1 deficient mice showed a redirection of the PG-synthesis from PGE_2 to PGD_2, PGF_2 and 6-keto-PGF_1 (stable metabolite of prostacyclin). For all these prostanoids pronociceptive effect had been described at the level of the spinal cord.

Conclusions: The fact that the administration of the COX-2-selective inhibitor etoricoxib but not the mPGES-1 deletion decreased the nociceptive behaviour in the zymosan test, suggests that in mPGES-1 deficient mice the shift in prostanoid synthesis to other pronociceptive prostanoids neutralizes the antinociceptive effect due to the reduced PGE_2 synthesis.

The work was supported by the DFG grant GE695/2-2.

Reference:

C Brenneis, O Coste, R Schmidt, C Angioni, L Popp, RM Nusing, K Scholich, G Geisslinger (2008) Consequences of altered eicosanoid patterns for nociceptive processing in mPGES-1-deficient mice"; J Cell Mol Med. 12(2):639-648

Botulinum neurotoxin A reduces allodynia and improves functional recovery in a mouse model of neuropathic pain

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Background and Aims. Neurotoxins affecting neuroexocytosis can represent an innovative pharmacological approach in the comprehension of neural mechanisms of pain as well as therapeutic tool in the field of pain control. Recent data support *botulinum* neurotoxins (BoNTs) utilized as new therapeutic agents in pain relief in clinical practice. Recently, it has been demonstrated that botulinum neurotoxin serotype-A is able to induce analgesia in inflammatory pain conditions. The goal of this research was to investigate the effects of two different serotypes, A and B (BoNT/A and BoNT/B), in the development and recovering from neuropathic pain in mice subjected to the sciatic nerve ligation (CCI model).

Methods. Mice where subcutaneously injected into the plantar suface of both hindpaws either with BoNT/A (two doses: 7.5 or 15 pg/paw), BoNT/B (3.75 pg/paw) or saline, on different days before and after CCI.

The temporal trend of neuropathy over a long time interval (80 days) was analyzed measuring the mechanical allodynic response to the Dynamic Aesthesiometer Test. Functional recovery of the injured paw was followed examining the mice walking pattern and measured by the Sciatic Static Index (SSI), as well as by the weight bearing (Incapacitance test).

The research plan was approved by the Ethical Committee of the Italian Health Ministry in accordance with the National law (DL116/92, application of the European Communities Council Directive 86/609/EEC) on care and handling of the animals.

Results. Remarkably, a single administration of BoNT/A, but not BoNT/B, was sufficient to induce antiallodynic effects starting from the day after the injection. The effect was dose-dependent and lasted for at least 3 weeks. The administration of BoNT/A before the CCI was ineffective.

Furthermore, BoNT/A injection accelerated the functional recovery, enhancing the SSI scores and restoring the normal weight bearing.

Conclusions. This result is particularly relevant since neuropathic pain is poorly treated by current drug therapies. Parallel to the reduction of pain, signs of functional recovery appeared in mice as demonstrated by the weight balance between hindpaws and by the walking pattern. The different modulatory action of the two botulinum serotypes is an important and useful result to take into account for both the study and the comprehension of the mechanisms involved in their action and for the use of the BoNTs in the clinical practice.

References

Luvisetto S, Marinelli S, Cobianchi S and Pavone F, 2007. Anti-allodynic efficacy of Botulinum neurotoxin A in a model of neuropathic pain. Neurosci 145:1-4.

Supported by research grants FISR-CNR Neurobiotecnologia 2003 and FILAS Regione Lazio (Italy).

A mechanism of axonal hyperexcitability predicted by computer simulation

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Background & Aims Pain is related to repetitive neuronal firing whose source is usually sought outside the myelinated axon (MA). A series of medicines used for treatment of pain acts as openers of K^+channels. In MA, the K^+channels are located in internodes. It is not trivial to study the processes in a very thin periaxonal space (about 5 nm) experimentally because of few electric charges there and of more than two orders thicker microelectrode diameter. Therefore, the most adequate way to study internodal processes is a proper computer modelling. Our previous model study (Dimitrov 2005) predicted generation of full-size internodal action potentials (APs) in response to an AP propagating saltatorially along the MA. The activation existed irrespective of the fact that density of internodal Na^+channels was only 3-6% of the nodal one. The very thin periaxonal space beneath a normal myelin made the activation possible. We aim to study the effect of deficit of internodal K^+channels on hyperexcitability of MA.

Methods Original multi-cable mathematical model of myelinated axon with 21 nodes was used. It is based on the Hodgkin-Huxley approach. To simulate internodal processes, each internode was represented by 338 segments. K^+channels were blocked in some or all internodes. The initial concentration of Na^+ ions in the periaxonal space was varied. The model takes into account accumulation of ions. A single short stimulus (50μs) was applied to the 1st node.

Results The absence of internodal K^+conductance causes a pronounced and prolonged depolarization of internodal axolemma at the portions without K^+channels. This forms a transition zone between normally charged membrane adjacent to the node and the depolarized portion(s) of the internode(s). The currents produced by the transition zone induce repetitive activation of the MA internodes whenever periaxonal concentration of Na^+ions is above the threshold one. The internodal transition zone steadily moves towards midinternode. Any internodal activation returns the internodal transition zone to its initial position. Even if the periaxonal concentration of Na^+ions becomes subthreshold, the transition zone continues to move along the internodal membrane and supports it in an excitable state. If the periaxonal concentration of Na^+ions is increased, the MA firing starts again. Duration of the excitable state defines the maximal interpulse interval in the low-frequency firing of MA.

Conclusions Internodes of MA can be a source of MA firing upon deficit of K^+conductance.

Acknowledgements This work was supported by Bulgarian National Science Fund grant MU-L-1508/05.

Reference

Dimitrov AG (2005) Internodal sodium channels ensure active processes under myelin manifesting in depolarizing afterpotentials. J Theor Biol, 235(4):451-462

Increased CGRP immunoreactivity in rat primary afferent neurons innervating osteoarthritic knee joints

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Background & Aims: Pain is a prominent feature of osteoarthritis (OA). To contribute to a better understanding of the pain mechanisms in this pathology, we studied the neurochemical markers CGRP, IB4 and NF200 in neurons innervating the knee joint in the mono-iodoacetate (MIA) model of OA.

Methods: OA was induced by injection of 2 mg of MIA, in the left knee joint of adult male Wistar rats. Control animals were injected with saline. Identification of the cell bodies of joint afferents was performed by backlabelling with Fluorogold (FG). Total number of cells backlabelled for FG was determined by stereological methods. In adjacent sections, immunofluorescence reactions for CGRP, IB4 and NF200 were performed, and double labelled cells (FG+marker) were quantified. Double labelling for CGRP and NF200 was also performed. The areas of the cells analysed were measured. In order to quantify the total DRG cell number, slices of DGR were stained with Nissl and analysed through stereological methods. This work was approved by the Ethical Committee for Health of the Hospital S. João, Porto, Portugal.

Results: The OA animals showed a 40% decrease in the total FG cell number ($P<0.01$). This decrease was more pronounced in the small cells, although also observed in the medium size cells. The total DRG cell number was similar in the control and OA animals. However, the distribution of these cells per area showed some differences, with a decrease of the total number of the small cells and an increase in the medium-large size cells.

The results of CGRP immunofluorescence showed an increase in the percentage of positive backlabelled cells in OA animals. The percentage of these cells was increased in the small and in the larger ones. No change was observed in the percentage of backlabelled cells positive for IB4 or NF-200. In the CGRP and NF200 double staining no change was observed in the total percentage between the two groups of animals. Nevertheless, there was a decrease in the percentage of small cells and an increase in the larger cells in the OA animals.

Conclusion: The results suggest an increase in the expression of CGRP in this model of OA with occurrence of changes in the pattern of cell size. This may be a phenotypic switch or a result of small cell hypertrophy. The fact that the total number of L3, L4 and L5 DRG cells was not altered in OA rats, but a slight increase in the total number of medium-large size cells was observed leads to the possibility that the decrease in DRG backlabelled cells might not be due to a selective loss of cells innervating the OA knee, but to the occurrence of cellular hypertrophy in the DRGs of OA animals.

Supported by FCT and POCI 2010 and co-financed by FEDER

Effect of co-administered gabapentin and venlafaxine in the rat spared nerve injury model of neuropathic pain

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Background and aim Two thirds of neuropathic pain patients experience insufficient pain relief and the pharmacological treatment is often associated with intolerable adverse effects. Thus, combination therapy of drugs with complementary mechanisms of action may be a rational strategy to obtain improved results at lower doses and with fewer side effects. In this study, the antiallodynic effect of co-administered gabapentin (GBP) and venlafaxine (VFN) was investigated in the rat spared nerve injury (SNI) of neuropathic pain (Rode et al, 2006). Given that GBP and VFN act at different sites within the nociceptive transmission pathway, it was assumed that a combination may provide superior pain relief than either drug alone.

Method Male Brown Norwegian rats were used in the study. The method of SNI surgery involves a ligation and dissection of two branches of the sciatic nerve and has been described previously (Decosterd and Woolf, 2000). A set of von Frey filaments (0,008 – 60 g) was used to test the withdrawal threshold of the injured hind paw, as an indication of mechanical allodynia. The filaments were applied to the lateral plantar surface with increasing force, and the force in g able to cause 3 withdrawals after 5 consecutive stimulations was taken as the withdrawal threshold. The experiments were performed with permission from the Danish Committee for Experiments on Animals.

Results GBP (50 and 100 mg/kg s.c.) produced a dose-dependent reversal of mechanical allodynia in the SNI rats. VFN (10 and 50 mg/kg s.c.) had no effect on the withdrawal threshold in response to von Frey stimulation. A combination of GBP (50 mg/kg) and VFN (50 mg/kg) failed to reverse the withdrawal threshold. Furthermore, when a higher dose of GBP (100 mg/kg) was administered with VFN (50 mg/kg) no reversal in the withdrawal threshold was observed. However, when the dose of VFN was reduced to 10 mg/kg, the antiallodynic effect of GBP (100 mg/kg) was restored.

Conclusion and future directions This study revealed that a high dose of VFN severely compromises the antiallodynic actions of GBP in the rat SNI model. Future studies in rats will investigate whether the observed interaction has a pharmacokinetic origin, and if so, to find the mechanism(s) behind. In parallel to animal studies, the potential human implications of the observation will be evaluated in a clinical intervention study.

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Phantom limb pain prevention with the application of ketamine.

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Background. Phantom pain has still a high incidence (35-80%) and mostly occurs after limb amputation. There is some evidence about preemptive analgesia as a prevention of phantom pain (Bach, 1988), where the author used lumbar epidural blockade for 72 hours prior to the operation. The positive results of this study were not confirmed by others. Therefore, we started with our study in the field of phantom limb pain prevention.

Aim. The purpose of our study is to determine the necessity and consumption of analgesics during the first week after lower limb amputation, and the incidence of phantom limb pain three months after surgery.

Materials and methods. It is a blind, prospective, placebo-controlled pilot study. Patients with diabetic leg condition, who have undergone lower limb amputation surgery in general anesthesia were included (n=20). They were divided into 3 groups. After administration of a general anesthetic, a 0.5 mg/kg bolus of i.v. ketamine was given two minutes before the operation (Group 1, n=5; Group 2, n=10). Those with different medical conditions who were unable to receive ketamine were placed in Group 3 (n=5). Right after surgery, participants received a 48 hour post-operative intravenous infusion of 0.1 mg/kg/h (Group 1) or 0.05 mg/kg/h (Group 2) ketamine. The third, control group received a 48 hour post-operative intravenous infusion of 1.68 g/24h magnesium. The patients' conditions were rechecked on day 2, 7 and at 3 months following surgery.

Results. The necessity of analgesic treatment in the first week after lower limb amputation was higher in the placebo controlled group. The incidence of phantom pain 3 months after surgery was 0% in Group 1, 10% in Group 2 and as much as 60% in Group 3 (placebo-controlled).

Conclusions. Administration of a continuous post-operative 48 hour intravenous ketamine infusion for diabetic patients undergoing lower limb amputation significantly reduced the incidence of phantom pain. On the basis of such promising preliminary results, we decided to carry on with the project until more patients are recruited in order to increase statistical power.

Response properties of amygdala nociceptive neurons to peripherally-evoked stimulation and cortical influence in the neuropathic rat

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Background and Aim. Literature has shown that the amygdala (AMY) has a central role in emotional responses to pain and results in neuroplastic changes following neuropathic pain. It receives nociceptive inputs from the periphery and central modulatory influence from limbic sites, including the anterior cingulate cortex (ACC). However, the influence of neuropathic pain on the response of AMY neurons and their central modulation are poorly known. The aim of this study was to analyse the alterations in the electrophysiological activity of AMY neurons resulting from 1 or 8 weeks (1W or 8W) peripheral neuropathy and the influence resulting from local glutamate stimulation or NMDA inhibition of the ACC.

Methods. We determined the response AMY neurons to peripheral noxious stimulation and their modulation by ACC injection of a NMDA agonist and an antagonist in animals submitted to 1W or 8W weeks of a spared nerve injury (SNI) neuropathy. In order to determine behavioural correlates for neuronal findings, the aversive quality of noxious stimulation was evaluated by the behavioural test paradigm (LaBuda and Fuchs, 2000). Experiments were conducted in accordance with local regulations, European Union Directive 86/609/EEC, NIH guidelines and IASP ethical guidelines.

Results. After 1W SNI, ACC inhibition with the NMDA antagonist decreased the aversive behaviour resulting from noxious stimulation of the lesioned hindpaw. The spontaneous activity of nociceptive neurons from amygdalar nuclei increased in both nuclei of SNI animals, following 1W and 8W neuropathy; after 1W SNI, the peripherally-evoked activity of ipsilateral BLA neurons increased and that of CeA neurons decreased following all types of noxious stimuli, whereas in contralateral BLA and CeA neurons occurred exactly the opposite; after 8W SNI, glutamate stimulation of the ACC increased the neuronal activity of ipsilateral BLA neurons, an effect that was reverted by blocking the ACC; the opposite effects on neuronal activity were obtained if contralateral BLA neurons were recorded following both glutamate stimulation or inhibition of the ACC.

Conclusions. Results indicate that the installation of a neuropathy results in alterations in the activity of BLA and CeA neurons from induction to sustained neuropathy and in opposite neuronal changes on the activity of nociceptive ipsilateral and contralateral AMY neurons.

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Agonists of protease-activated receptor 2 (PAR_2) sensitize transient receptor potential vanilloid 4 (TRPV4) to induce mechanical hyperalgesia in mice

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Background: Proteases that are generated during injury and inflammation, such as trypsins and tryptase, cleave PAR_2 on primary sensory neurons to induce thermal and mechanical hyperalgesia. Thermal hyperalgesia depends on protein kinase A (PKA) and PKCε-mediated sensitization of the ion channel TRPV1, but the mechanisms of PAR_2-induced mechanical hyperalgesia are unknown.

Aims: We hypothesized that PAR_2 induces mechanical hyperalgesia by sensitizing TRPV4, which mediates pain responses to mechanical and osmotic stimuli. This hypothesis was tested by a variety of methodologies: Ca^{2+} fluorimetry, neuropeptide release from spinal cord slices and *in vivo* behavioural assays.

Methods & Results: Sensitization of TRPV4-dependent changes in $[Ca^{2+}]_i$ were examined in human bronchial epithelial cells (HBE), which naturally express PAR_2 and TRPV4, and HEK cells, which express PAR_2 and were transiently transfected with human TRPV4. The TRPV4 agonist 4α-phorbol 12,13-didecanoate (4αPDD) and hypotonic stimuli increased $[Ca^{2+}]_i$ in HBE and HEK-TRPV4 cells, but not in untransfected HEK cells, suggesting activation of TRPV4. Preincubation with PAR_2 activating peptide (AP, SLIGRL-NH_2) enhanced the effect of 4αPDD and hypotonic stimulus in HBE and in HEK-TRPV4 cells, indicating TRPV4 sensitization. Inhibitors of PKA (H89) and PKC isozymes (GF109203X and Gö6976) inhibited sensitization by >50%. The inactive reverse peptide sequence (PAR_2-RP), and PAR1-AP (TFLLRN-NH_2), which do not cause hyperalgesia, had no effect, confirming specificity. Superfusion of slices of rat spinal cord with 4αPDD or hypotonic solution stimulated the release of the nociceptive peptides substance P by >4-fold and calcitonin gene-related peptide by >9-fold. Preincubation with PAR_2AP enhanced the effects of 4αPDD on release of SP and CGRP by 3-fold and 2-fold, and enhanced the effects of hypotonic solution of release of both SP and CGRP by 1.5-fold, compared to PAR_2-RP. In wild type mice, intraplantar injection of PAR_2-AP increased the frequency of paw withdrawal to mechanical stimulation with a von Frey hair (0.174 mN), and enhanced the response to 4αPDD or hypotonic stimulus, indicative of sensitization. These effects were absent from $TRPV4^{-/-}$ mice.

Conclusion: Activation of PAR2 sensitizes TRPV4 by PKA and PKC-dependent mechanisms to potentiate the release of nociceptive peptides from primary spinal afferent neurons and cause hyperalgesia to mechanical stimulation.

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Early immunomodulation in the rat following experimental spinal cord injury using nuclear hormone receptor agonists

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Background. Chronic central pain (CCP) and functional impairments following spinal cord injury (SCI) have a major impact on the patient's life. Almost thirty percent of these patients develop CCP. The mechanisms underlying CCP are not well understood. Consequently, effective treatments are still lacking. Increasing data suggest that both functional outcome and CCP are strongly dependent on early activation of the immune system.

Aim. Our aim was to study whether early inflammation can be suppressed by acute treatment with two different nuclear hormone receptor agonists following spinal cord injury in the rat. As a result of such reductions in early inflammation, we hypothesize that functional impairments and CCP development are also decreased.

Methods. Male Wistar rats were subjected to a moderate spinal cord contusion injury. Following impact, animals (n=4/group) were treated with either a LXR-agonist (T09), a PPAR-γ agonist (pioglitazone) or vehicle via intraperitoneal injection. T09 was administered at 5 minutes following injury in a concentration of 10, 20, 30 or 50 mg/kg. Pioglitazone was injected twice, at 5 minutes and 3 hours respectively, in a concetration of 1,5 mg/kg. Animals subjected to laminectomy without impact to the cord served as controls. Six hours after injury, rats were perfused with saline, two pieces of spinal cord tissue (i.e. one containing the epicenter and the other just rostral to the epicenter) were dissected out and tissues were quick-frozen in liquid nitrogen. Next, mRNA was extracted from both spinal cord tissues and analyzed for expression of tumor necrosis factor-α (TNF- α), interleukin-1ß (IL-1 ß) and interleukin-6 (IL-6) using quantitative PCR (Q-PCR).

Results. At six hours after moderate contusion injury, mRNA levels of IL-1ß and IL-6 were significantly increased at the epicenter as compared to control animals. Rostral to the epicenter, significant increases were only found for TNF-α mRNA. None of the treatments with agonists for nuclear hormone receptors were found to alter the cytokine expression levels at six hours after moderate contusion injury.

Conclusions. These preliminary results show that several important pro-inflammatory cytokines are highly upregulated following moderate spinal cord contusion injury. However, acute intraperitoneal injections of the nuclear hormone receptor agonists T09 and pioglitazone fail to decrease these cytokine mRNA levels at six hours after injury.

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Preemptive effect of gabapentin on postoperative pain and morphine consumption after lumbar fusion surgery

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Background & Aims It has been suggested that central neuronal sensitization may play an important role in postoperative pain [1]. Gabapentin(G), was developed as a structural GABA analog. Gabapentin does not have direct GABAergic action nor does it affect GABA uptake or metabolism [3] . Gabapentin has been shown to have central and peripheral antinociceptive activity in neuropathic and inflammatory pains which develop after surgical incision [2] . The use of gabapentin in the perioperative setting has been evaluated in several studies. These studies report promising reduction in postoperative analgesic consumption [4,5] The aim of this study was to investigate the impact on postoperative morphine consumption and pain levels when G was used for preemptive analgesia in patients undergoing lumbar fusion surgery.

Methods. With approval of the local ethics committee and written informed consent, 40 patients ASA I–II physical status, aged 24–70, gave informed consent and were recruited in this randomized double-blind study. All patients underwent elective lumbar fusion surgery. The evening before surgery, patients were instructed in the use of the 10-cm visual analog scale (VAS).One hour (h) prior the operation they received 1200 mg G or placebo (P) orally. Analgesia in the PACU was initially provided by titrating morphine (M) in increments of 1 mg until the VAS pain score was <3 cm. Patients were given access to a Patient Controlled Analgesia (PCA) device set to deliver 0.5 mg boluses of intravenous morphine (M) and a constant flow of 0.3 mg per hour after they were discharged from PACU. Total M consumption was recorded from 0 to 24 h postoperatively. Pain was evaluated on a VAS; (0–10 cm; 0=no pain and 10=worst possible pain) at rest at 1st, 2nd, 4th, 8th, 16th, 24th h after the termination of the operation.

Results. Each group included twenty (20) patients. Final VAS score (24 h postoperatively) was 2.55 $\pm$ 0.83 for the G group and 2.6$\pm$ 0.99 for the P group. The p-value for the comparison of VAS score was 0.0113 and 0.0007 for the comparison of the rate of the change of VAS score alterations between the two groups.

Gabapentin reduced total morphine consumption (12.31 $\pm$ 5.55 mg for the G group, 16.54 $\pm$ 8.46 mg for the P group, p=0.0284). One patient included in the G group experienced dysarthria.

Conclusion. Premedication with Gabapentin decreased the severity of postoperative pain occurrence and diminished the analgesic requirements.

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The adenosin A_{2A} receptor is involved in carrageenan-induced inflammatory hyperalgesia in mice

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Backgrounds. It is well established that adenosine, adenosine analogues and inhibitors of adenosine metabolism affect nociception in rodents and humans. But relatively little is known about the physiological importance of adenosine and its receptors in pain.

Aim. In the present study, we have studied the potential involvement of peripheral adenosine A_{2A} receptors in hyperalgesia produced by localized inflammation in mice.

Methods. Genetically modified mice lacking the A_{2A} receptor or a double knock-out of both A_{2A} and A_1 receptors and wild-type controls were used. Hind paw inflammation was produced by unilateral s.c. Administration of carrageenan (2% in 20 μl) and mechanical hyperalgesia was examined using calibrated von Frey hairs. The effect of s.c. CGS 21480, a selective A_{2A} receptor agonist, was also examined.

Results. Carrageenan-induced inflammation produced significant mechanical hyperalgesia 24 h after administration in the ipsilteral hind paw in wild-type mice. In homozygote mice lacking the A_{2A} receptor, carrageenan-induced hyperalgesia was significantly reduced, but not abolished. The reduction in inflammatory hyperalgesia seen in A_{2A} knock-out mice was not associated with changes in paw edema. Furthermore, s.c. CGS 21480 at 1 nmol also produced mechanical hyperalgesia in wild-type mice, en effect that was markedly reduced in the A_{2A} knock-out mice. Interestingly, although heterozygote mice of double knock-outs of A_{2A} and A_1 receptors also showed reduced response to CGS 21480, the inflammatory hyperalgesia was unchanged in these mice.

Conclusions. Peripheral adenosine A_{2A} receptors may be involved in inflammatory hyperalgesia independent of the extent of inflammation. The A_1 receptor, on the other hand, may play an inhibitory role during inflammation.

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Reversal of allodynia and hyperalgesia in a rat model of neuropathic pain by a recombinant herpes vector targeting the dorsal reticular nucleus

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Background & aims. We sought to attenuate neuropathic pain in the spared nerve injury (SNI) model by targeting a supraspinal facilitatory area, the dorsal reticular nucleus (DRt), with a herpes simplex virus type 1 (HSV-1) vector. First, we established the migration dynamics of a HSV-1 vector expressing *lac*Z (DPZ), coding β-galactosidase (β-gal) from the DRt and studied the co-localization of β-gal with tyrosine hydroxylase (TH). This study showed transduction of numerous noradrenergic DRt afferents. Then, we determined the effect of noradrenergic input to the DRt. Based on the outcome of these studies we evaluated the effect of decreasing the noradrenergic input to the DRt by a HSV-1 vector with the TH promoter driving transcription of the TH cDNA in reverse orientation (THa vector).

Methods. Experiments were performed in accordance with the European Community Council Directive 86/609/EEC. *DPZ migration dynamics:* Male Wistar rats were injected with DPZ into the DRt (n=3) and perfused 7 days after. β-gal was detected by immunocytochemistry and its co-localization with the TH was performed by double-immunodetection. *Effect of α-adrenoreceptor (AR) agonists:* Two weeks after SNI surgery, a cannula was placed into the DRt; 10µg of phenylephrine (α1-AR agonist), clonidine (α2-AR agonist) or saline were injected (n=6/drug) and their effect evaluated by von Frey hair filaments, pin-prick and acetone tests assessing respectively, mechanical allodynia, mechanical hyperalgesia and cold allodynia. *Effect of the THa vector:* Two weeks after SNI, rats injected into the DRt with THa or the control vector (THZ), in which *lac*Z replaces the TH cDNA, were split in 3 groups. In group 1 pain behavior was assessed before and for up to 2 months after injection of THa (n=6) or THZ (n=6). In group 2 & 3 instantly after vector injections, a cannula was also placed into the DRt. In group 2, THa injected rats were administered 10µg of phenylephrine (n=6) or saline (n=5) and pain behavior was assessed. In group 3, a microdialysis probe was inserted in THa (n=5) or THZ (n=6) injected rats and noradrenalin was quantified by HPLC.

Results. After DPZ injection in the DRt retrograde migration was detected in several brain areas. A strong co-localization of β-gal with TH was found in the A5 noradrenergic cell group and *locus coeruleus*. Administration of phenylephrine into the DRt increased cold allodynia and mechanical hyperalgesia whereas clonidine induced the opposite. THa injection into the DRt showed: i) slight attenuation of mechanical allodynia, sustained attenuation of cold allodynia for 50 days and mechanical hyperalgesia for 22 days which were reversed by the administration of phenylephrine and ii) a significant reduction of noradrenalin.

Conclusion. These results show that α1 and α2-AR mediate opposite actions in the DRt. Blocking α1-AR by selectively decreasing TH expression into DRt noradrenergic afferents is a useful strategy to attenuate pain-related behaviors in the SNI model.

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Interaction between Diffuse Noxious Inhibitory Controls (DNIC) and Distraction in Human Subjects

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Background and Aims. Diffuse Noxious Inhibitory Controls (DNIC) is a form of supraspinal descending endogenous analgesia. We aim to determine whether distraction and tonic heat pain attenuate pain through the same mechanism or via different mechanisms. This will be achieved by examining whether such attenuation by each of tonic pain and distraction occurs to a similar extent or if there is an additive effect on further pain attenuation.

Methods. Young healthy right-handed students were recruited after approval from the local Helsinki Ethics Committee. Tonic conditioning heat pain was induced by placing the right hand in a hot water bath (46.5^{o}C / 80secs). A contact Peltier heat thermode at a temperature tailored to the individual subject (using a step-wise procedure to reach a stable 60/100 level within the 49–53^{o}C range), was used to induce 'test-pain' of pulses along 1 min at the left volar forearm. Distraction was provided by 3 different difficulty levels of continuous cognitive visual tasks. Experimental blocks consisted of: (1) test-pain alone - 'B', (2) test-pain + conditioning pain (DNIC) - 'T', (3) test-pain + distraction 'D', and (4) test-pain + conditioning pain + distraction - 'DT'. These four different conditions were randomized and repeated 3 times, each time with a different level of distraction task. Pain intensity and unpleasantness of the test stimuli were recorded using a numerical pain scale (0-100).

Results. So far 30 subjects (24 ± 4.1 years, 18 male, 12 female) have been recruited. Four factor mixed-model ANCOVAs (condition, distraction difficulty, gender and combinations, with score at baseline 'B' as covariate), showed an overall effect of condition on the last stimulus pain intensity scores ($F(2, 220) = 5.22$, $P = 0.0061$), calculated as the delta between pain scores under conditions 'T', 'D' or 'DT' relative to baseline 'B' test pain scores. Tukey tests revealed this to be due to a significant reduction in pain intensity ratings under 'DT' (-23.1±2.5; mean±SE) compared to 'T' alone (-17.0±2.5) or 'D' alone (-18.4±2.5). There was also an overall effect of distraction on both intensity and unpleasantness pain scores. Tukey tests showed the moderate-level distraction task to be the most effective in reducing pain perception in males only.

Conclusion. These early results indicate an additive effect of distraction and tonic heat pain on attenuating pain further than either of them alone, suggesting DNIC may be independent of distraction. In order to gain further understanding of the neural basis, we plan to record neurophysiological pain evoked potentials and use Low Resolution Brain Electromagnetic Tomography (LORETA) to estimate the corresponding cortical source locations activated.

Involvement of the $P2X_7$ receptor in development of neuropathic pain in BALBc/j wildtype and knock-out mice

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Background and Aims. Studies by Chessel and co-workers [1] showed that disruption of the murine ATP receptor $P2X_7$ inhibited development of neuropathic pain induced by partial ligation of the sciatic nerve. Based on immunochemical staining procedures the $P2X_7$ receptor ($P2X_7R$) has been shown to be expressed on astrocytes, microglial and neuronal cells although some of the antibodies were shown to recognise the presence of the $P2X_7R$ in cells from $P2X_7R$ knock-out mice[2]. Pursuing investigation of the cellular events behind development of neuropathic pain caused by direct injury to the nervous tissue or cancer the role of this receptor is under further investigation.

Methods. All experiments were conducted on $P2X_7R$ knock-out mice backcrossed into BALBc/j mice for more than 5 generations and the corresponding wild type mice. Neuropathic pain is introduced by the spared nerve injury (SNI) operation in which the tibial and common peroneal nerves are ligated with non-absorbable suture and cut distal to the ligation. The sural nerve is left unharmed. The wild type mice were divided into two groups of which one group underwent SNI operation (n=9) and one a sham operation (n=5). Equally the $P2X_7R$ knock-out mice were divided into two groups, undergoing operations by SNI (n=7) or sham (n=5). Development of mechanical allodynia was evaluated by von Frey filaments two days prior to the operation and up to three weeks after (days 1, 2, 3, 6, 9, 15, 21).

Location of the $P2X_7R$ in cells of the dorsal root ganglia (DRG) was investigated by ATP and BzATP induced calcium imaging on primary cell cultures isolated from DRG (L4-6) of $P2X_7R$ knock-out or wild type animals. The cells were grown on poly D-lysine coated coverslips and loaded with 4 µM fura-2, AM for 30 min after which the pharmacological stimulation were performed. All experiments involving animals were performed with permission from the Danish Animal Experiments Inspectorate.

Results. We found that wild type mice became allodynic already two days after the SNI operation and stayed allodynic for up to three weeks after the operation. In contrast neither wild type sham operated nor $P2X_7R$ knock-out mice developed SNI induced hypersensitivity during the same time period. Our imaging results shows that the $P2X_7R$ is located on dorsal root ganglia satellite cells and not on neurons.

Conclusion. The $P2X_7R$ is involved in development of neuropathic pain in mice and the related cellular and molecular events are under investigation.

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Brain activity correlates with subjective pain perception in humans: A study of pain-evoked potentials and electromagnetic tomography

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Background and Aims. Multiple studies support the usefulness of standardized low-resolution brain electromagnetic tomography (sLORETA) in localizing generators of scalp-recorded potentials. The current study implemented sLORETA on pain-evoked potentials (EPs), primarily aiming at validating this technique for pain research by identifying well-known pain-related regions of interest (ROIs). Subsequently, since the literature of pain intensity coding offers inconsistent findings, we pointed at revisiting this still controversial topic, focusing on the relative impact of relevant ROIs on subjective pain perception.

Methods. EPs were recorded from 32 electrodes in response to application of brief contact-heat stimuli to the volar forearm of healthy subjects (N=17). 5 contact-heat stimulation blocks were applied. Each block, comprised of 60 stimuli of the same intensity, was divided into two sets of 30 trials. From a baseline temperature of 35^{o}C, the applied blocks' intensities increased to 35 (control), 41, 46, 49, and 52^{o}C. Numerical pain scores (NPSs) were obtained after each stimulus using a 0-10 scale, 0 denoting "no sensation", 4 - "pain sensation threshold", and 10 - "worst imaginable pain". Current density values of a spatial local maximum of each ROI were measured at 500 Hz using sLORETA, yielding high-resolution temporal curves. Areas under these curves (AUCs) were calculated and correlated with NPSs. Rambam Health Care Campus Ethical Committee approved the study protocol.

Results. Compared to 35^{o}C, 52^{o}C stimuli induced significant activations of the bilateral S1 and ACC, and of the contralateral operculoinsular (OIC) and dorsolateral prefrontal (DLPFC) cortices (P<0.05 for each). Positive correlation between average NPSs and AUCs were observed at the bilateral S1 and ACC, and contralateral OIC (P<0.05 for each). Multiple regression analysis (r=0.80; P=0.024) revealed only the contralateral S1 significantly contributed to subjective pain perception (P=0.020). A reduced regression model (r=0.75; P=0.008), employing the contralateral S1 alone as a predictor of pain perception, yielded a linear relationship (P=0.005).

Conclusions. Based on (i) the correspondence of the pain ROIs identified by sLORETA with the acknowledged imaging-based pain-network, and (ii) S1 proving to be the most contributing region in pain intensity coding, which is in accordance with the classical clinical thinking relating S1 lesions to diminished pain perception, we find sLORETA an appropriate tool for relevant pain research, and further substantiate the role of S1 in pain perception.

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Gamma oscillations in anterior cingulate cortex are amplified by selective attention in human subjects

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Background. The role of attention for the modulation of pain has been shown for the clinical setting and by physiological experiments involving functional imaging and evoked potentials. However, induced oscillations, i.e. rhythmic fluctuations in the postsynaptic potentials that are time-locked but not phase-locked to a stimulus, have not been studied using EEG to clarify attentional effects on pain processing.

Aim. Here we investigate electrical brain activity in the time-frequency domain following attended and ignored laser stimuli of two intensities that were delivered to the left index and ring finger.

Methods. The study was approved by the local ethics review board. We recorded electrical brain activity using 128 channel EEG from 21 volunteers during painful Thulium YAG laser stimulation (1 ms duration, 5 mm beam diameter, 2 µm wavelength), while spatial attention and stimulus intensity (1.5 and 2fold individual pain threshold) were systematically varied. The subjects' task was to evaluate the pain intensity at the attended finger, while ignoring laser stimuli delivered to the other finger. A computer-based analysis was conducted using the matlab toolbox fieldtrip. Time-frequency analysis was done to extract the time and frequency information from the data simultaneously. In short, window functions of different frequencies are multiplied by the different data samples. Source localization was performed by means of linear beamforming: for each anatomical voxel a spatial filter is calculated that passes activity from that location with unit gain and maximally suppresses activity from other locations.

Results. Time-frequency transformations of the EEG data revealed that spatial attention and high intensity significantly increased delta (2-6 Hz) and gamma oscillations (60-84 Hz). Furthermore, a decrease in pain-induced oscillations in the alpha-band (6-16 Hz) was found. Generators of pain-induced oscillations were localized in somatosensory and prefrontal areas. Interestingly, gamma oscillations could be localized for the first time in areas including the anterior cingulate cortex.

Conclusions. We suggest that the attentional modulation of the time-frequency pattern is a manifestation of lowered pain perception. In particular, the gamma-component located in the anterior cingulate gyrus possibly serves the integration of the multi-factorial pain experience that leads to the subjective perception of the pain stimulus and can be modulated by cognitive and affective factors.

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Neuropathic pain profile and quantitative sensory testing (QST) in diabetic polyneuropathy

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Background. Pain is a common problem in diabetic neuropathy, but relatively little information has been published regarding the nature and scope of this pain condition.

Aim of investigation. To assess neuropathic pain profile and their association with quantitative sensory tests in painful diabetic polyneuropathy (DPN).

Methods. This pilot study was conducted in all consecutive diabetic patients who had symmetric neuropathic complaints during admission in Endocrinology Department of Kaunas University of Medicine Hospital between 1st of February and 30th of April 2007. The patients were younger than 70 years, haven't other conditions or using drugs potentially inducing neuropathy. The protocol was approved by the local Ethics Committee. Data were collected and examination made by trained neurologists. The patients were (1) interviewed for their complaints and (2) clinical neurological examination was carried out by single neurologist; (3) patients were asked to fill out the Neuropathic Pain Scale-NPS (Galer & Jensen 1997); (4) quantitative sensory testing (QST) was performed using NervScan2000. The neuropathy group was made of patients who revealed at least 2 symmetric neuropathic impairments.

Results. 61 patients (41 male, 20 female) were examined. Mean age was 48.2±12.8 years, mean disease duration was 13.07±9.54 years. 27 patients had type 1 diabetes mellitus (DM), 34 had type 2 DM. Mean HbA1c was 9.32±2.3 %. 49 (80.3%) patients were clinically determined to have DPN. 19 (31.1%) patients complained of pain in the lower extremities on clinical examination, 36 (50.9%) of the patients' NPS10 score was more than 0. Mean (N=36) NPS Intensity 2.1±2.7, sharp pain 3.5±3.6, hot pain 2.7±3.1, dull pain 3.1±2.6, cold pain 1.1±2.1, skin sensitivity 2.3±2.9, itch 2.1±2.8, unpleasant pain 5.9±2.2, deep pain 4.4±3.3, superficial pain 2.9±2.6. Only skin sensitivity score from NPS correlated significantly with QST results for 2000 Hz frequency (Aβ fibers), superficial pain NPS score had significant correlations or tended to correlate with QST results for all frequencies. According to NPS results, patients who experienced purely deep (n=11) and purely superficial (n=10) pain were separated out. Although there was no significant difference in pain intensity score (in NPS) between the groups, the deep pain group's unpleasant pain scores (mean 6.27±2.37) in NPS were significantly higher than the superficial pain group's scores (mean 4.30±1.42) p=0.034))

Conclusions. These data suggests that deep and unpleasant neuropathic pain qualities have major influence onto general pain sensation in DPN patients but conventional QST correlates significantly with disorder of superficial pain sensation.

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Lidocaine injection into the Sprague-Dawley rat dorsal root ganglion causes neuroinflammation and pain-related behavior

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Background & Aims: Injury of a spinal nerve or dorsal root ganglion (DRG) during selective spinal nerve blocks (SSNB) is a potentially serious complication that has not been adequately investigated. Our hypothesis was that local anesthetic injection into these structures may result in an inflammatory response and hyperalgesia.

Methods: All experimental protocols were approved by the Ethics Committee of the University of Split, School of Medicine. A total of 56 Sprague-Dawley rats weighing 150-200 g were used. Rats underwent minimal laminectomy and injection of 4 uL lidocaine into the L5 spinal nerve or L5 DRG. The control group received injection of 0.9% NaCl. The injections were made using a 29-gauge needle with a slightly bent beveled tip. We performed extensive methodological research regarding targeted delivery of substances into the DRGs because of the various methods reported in the literature. The DRG injection following minimal laminectomy proved to be the most successful approach in terms of precision.

Before and after surgery, behavioral testing was performed to determine the response to nociceptive mechanical stimulation of the rat paw. DRGs were harvested, stained, and rings of immunoreactive satellite cells around neurons were counted. We did not try to superfuse the DRGs with non-invasive methods.

Results: Animals demonstrated hyperalgesia on the ipsilateral paw up to 4 days after lidocaine injection into the DRG but not after injection into the spinal nerve. The number of GFAP immunopositive glial cell rings, which represent an activation of satellite cells, significantly increased in DRGs after injection of lidocaine into either the DRG or the spinal nerve. Sporadic OX-42 immunopositive cells, which represent activated microglia, were also seen in lidocaine-injected DRGs. Testing for Pan-T expression, which labels activated T lymphocytes, showed no positive cells.

Conclusions: Lidocaine injection into the DRG is producing hyperalgesia, possibly as a consequence of an early neurinflammation that will later become evident through activation of resident satellite glial cells. In addition, we showed that the optimal method for targeted DRG delivery in the rat was injection after minimal laminectomy, which may be useful for further animal studies. The clinical implications are that an effort should be made to minimize risk of neural injuries while performing SSNBs.

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Contact heat evoked potentials (CHEPS) following topical capsaicin in human subjects: a model of "neuropathic pain".

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Background. The contact heat evoked potential stimulator (CHEPS) rapidly stimulates cutaneous small nerve fibres, and resulting Aδ evoked potentials can be recorded from the scalp. We recently reported in patients with small fibre neuropathy that Aδ evoked potential amplitudes were reduced, and significantly correlated with a reduction of intra-epidermal nerve fibres expressing PGP 9.5 (a structural marker), and TRPV1 (the heat and capsaicin receptor) in skin biopsies[1].

Aims. Here we have studied the sensory effects of capsaicin application using quantitative sensory testing, flare response, contact heat evoked potentials, and reported pain scores.

Methods. Fourteen volunteers (7M, 7F, average age 28y) were exposed to three ascending concentrations of capsaicin (0.075%, 1%, 3%) and a control cream (Unguentum M) applied to four separate areas of volar forearm skin. Thermal perception thresholds, area of flare, contact heat evoked potentials (10 x 51°C stimuli, 7 seconds apart), and evoked pain scores were recorded before and 15 minutes after application.

Results. Capsaicin caused a significant reduction in heat pain threshold at all three concentrations, but no change in warm perception threshold, suggesting a purely nociceptive effect. Topical capsaicin also caused a decrease in evoked potential amplitude; however this trend was not statistically significant. A significant negative correlation between evoked potential amplitude and evoked pain scores was observed following topical capsaicin application, similar to that observed in some neuropathic patients. In contrast, without capsaicin, there was an expected significant positive correlation between evoked potential amplitude and evoked pain scores.

Conclusion. CHEPS following topical capsaicin application may provide a "neuropathic pain" model which could be used in efficacy studies of novel analgesics, including TRPV1 antagonists.

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Streptozotocin-induced diabetic conditions are associated with neuropathic pain symptoms in mice.

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Background and Aims. Painful neuropathy is one of the most common complications of diabetes. Emerging evidence indicates that activated glia in the spinal cord play a crucial role in allodynia and hyperalgesia following nerve injury. However, it remains unknown whether spinal microglia or astroglia are activated under diabetic conditions and whether they contribute to diabetes-induced neuropathic pain symptoms. The aim of the present study was to evaluate the neuropathic pain symptoms in streptozotocin-induced diabetic mouse model and to investigate involvement of spinal glia in diabetic conditions.

Methods. All experiments were performed on Albino Swiss mice injected with streptozotocin (STZ; 200 mg/kg; i.p.) as was suggested by Kanki et al. 1999. Two behavioral tests were conducted in order to evaluate tactile allodynia and cold hyperalgesia, e.g. von Frey test and cold plate tests, respectively. To confirm that the intraperitoneal injection of STZ caused hyperglycemia in mice, the blood glucose concentration and body weight were measured. To study a potential role of glia we studied in the spinal cords from control and STZ mice the C1q (microglia marker) and GFAP (astroglia marker) mRNA levels using competitive RT-PCR. The experiments were carried out according to the Institute`s Animal Research Bioethics Committee and in accordance with IASP rules (Zimmermann, 1983).

Results. After 14 days of STZ injection diabetic mice exhibited a significant hyperalgesia (control 2.8 s vs. STZ mice 14.4 s) and allodynia (2.9 g vs. 5.5 g) along with an increased plasma glucose (147 mg/dl vs. 440 mg/dl) and decreased body weights (35 g vs. 42 g) as compared with control mice. The changes were noted already three days after STZ injections and were observed till 30th day. Additionally preliminary RT-PCR results will be present at the coming European Pain School in Siena concerning the activation of glia cells (microglia and astroglia) in control and streptozotocin-induced diabetic mouse model.

Conclusions. Summing up, STZ injection caused strong hyperglycemia in mice, which resulted in development of neuropathic pain symptoms like allodynia and hyperalgesia, possibly related to glia activation at the spinal cord level.

Supported by statutory funds of the Institute of Pharmacology PAN and Polish MNSW Scientific Network fund

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Inhibition of fatty acid amide hydrolase produces peroxisome proliferator activated receptor-α mediated analgesia in a rat model of inflammatory pain

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Introduction. Inflammatory pain behaviour can be attenuated following a peripheral pre-administration of the fatty acid amide hydrolase (FAAH) inhibitor URB597, an effect which is accompanied by increased levels of endocannabinoids in the inflamed paw. In addition to the clear role of cannabinoid receptors, there is increasing evidence for a role of peroxisome proliferator-activated receptors (PPARs) in the inhibitory effects of endocannabinoids and endocannabinoid-related compounds.

Aim. Here we investigate the neuronal mechanisms underlying the analgesic effects of URB597 by determining the effects of URB597 on receptive field expansion of spinal neurones in a model of inflammatory pain and to characterise the receptors mediating these effects.

Methods. Extracellular recordings of wide dynamic range dorsal horn (WDR) neurones (laminae V and VI) were made in anaesthetised (1.5% isoflurane in 66% N_2O/33% O_2) male Sprague Dawley rats (250-300g). Peripheral receptive fields of WDR neurones on the hind-paw were mapped using innocuous (8g) and noxious (26g) mechanical stimuli (von Frey monofilaments) before and after intraplantar injection of carrageenan (100µl 2% in saline). Effects of intraplantar pre-administration (30 min prior) of URB597 (25µg/50µl) or vehicle (50µl 3% Tween80 in saline) on carrageenan (2%) -induced expansion of peripheral receptive fields were determined. The contributions of cannabinoid CB_1 receptors and PPAR-α receptors to the effects of URB597 were investigated using the CB_1 receptor selective antagonist AM251 (30µg/50µl) and PPAR-α selective antagonist GW6471 (30µg/50µl) respectively, both of which were co-administered with URB597.

Results. Intraplantar administration of carrageenan produced a robust expansion of peripheral receptive fields of WDR neurones to 8g and 26g stimuli in vehicle treated rats, respectively. This inflammation-evoked expansion of peripheral receptive fields of WDR neurones was significantly attenuated following pre-administration of URB597. Inhibitory effects of URB597 were significantly blocked following co-administration of URB597 with GW6471, but not AM251.

Conclusions. These data suggest that the analgesic effects of URB597 in models of inflammation arise as a result of inhibition of peripheral and central sensitisation of WDR neurones. Previously we have shown that intraplantar URB597 increases levels of 2-arachidonoyl glycerol and anandamide in the carrageenan-inflamed hind paw. Since effects of URB597 were blocked by a PPAR-α antagonist and not a CB_1 receptor antagonist, our data suggest a role for PPAR-α in mediating analgesic effects of FAAH inhibition on receptive field expansion.

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Role of beta subunits in functional properties of recombinant human $Na_v1.7$ channels expressed in HEK293 cells.

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Background. Voltage-gated sodium channels (Na_v) play a crucial role in cellular excitability. To date 10 α subunits, which constitutes the conduction pathway of functional Na_v channels have been identified. Na_v channel function is further regulated by one of four different auxiliary β subunits encoded by a separate gene. In man the $Na_v1.7$ subtype channel is expressed mainly in the peripheral nervous system including nociceptors. Recent studies have identified mutations in the Na_v gene causing a truncated $Na_v1.7$ protein and loss of functionality. This mutation has been linked to the congenital insensitivity to pain (CIP) condition, which only affects pain perception leaving other types of sensation intact. These observations makes $Na_v1.7$ a very promising pharmacological target for pain treatment.

Aims. We studied the possible role of β2 and β3 subunits in the regulation of functional properties of the $Na_v1.7$ channel.

Methods. Three different cell lines were generated and used for the functional characterisation of $Na_v1.7$ currents. First stable expression of the α subunit of $Na_v1.7$ was established in HEK293 cells. Next the α+β2 and α+β3 heteromeric expression was generated based on the α expressing cell line. To investigate the interaction of α and β subunits in channel composition on the plasma membrane, native electrophoresis, immunoprecipitation and surface biotinylation experiments were performed and analyzed on Western blots. Conventional and automated patch-clamp systems were used for current recordings. Basic biophysical parameters like voltage dependence of activation and inactivation were determined for each cell line.

Results. Analysis of co-expression and interaction demonstrated that only a small fraction of the $Na_v1.7$ α subunits formed complexes with β2 while most of the $Na_v1.7$ α subunits were in complex with the β3 subunits. Preliminary data show only minimal differences in the biophysical properties between all three channel expression combinations. Surprisingly, co-expression of the $Na_v1.7$ α subunit with the β3 subunit showed a 2-3 fold increase in current amplitude without changing the kinetics of the channel based on I-V curves and activation/inactivation parameters.

Conclusion. In our recombinant expression systems β2 and β3 subunits did not significantly change the biophysical properties of the $Na_v1.7$ channel. This however does not preclude their regulatory role in vivo, which could depend on yet unidentified associated proteins. The tendency of the β3 subunit to increase current amplitude suggests an increased number of functional channels on the plasma membrane.

A role for endocannabinoids in NSAID-mediated antinociception? A study in naïve rats.

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Background and Aims: Analgesic effects of endocannabinoids (EC) are mediated by cannabinoid (CB) receptors and limited by metabolism by enzymes such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Other enzymes such as cyclooxygenase-2 (COX-2) may also metabolise ECs. The aim was to investigate effects of the COX-2 inhibitor nimesulide on evoked responses of spinal neurones and determine the contribution of the CB_1 receptor to these effects.

Methods: Extracellular recordings from laminae V and VI dorsal horn (DH) neurones were made in anaesthetised (1.5% isoflurane in 66% N_20 / 33% O_2) male Sprague Dawley rats (210-260g, Elmes *et al*., 2004). Effects of spinal administration of nimesulide (1-100µg/50µl) or vehicle (3% Tween80 in saline) on mechanically-evoked responses of DH neurones were studied. The ability of pre-administration of the CB_1 receptor antagonist AM251 (1 µg/50µl) to modulate the effects of nimesulide (25 µg) was studied. Experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and IASP guidelines.

Results: Nimesulide showed a tendency to attenuate mechanically evoked responses of DH neurones in naïve rats (n=6 neurones in 6 rats, Fig 1), however, significance was not reached. The effects of nimesulide were blocked by pre-administration of AM251.

Conclusions: These data support evidence (Ghilardi *et al.,* 2004) that COX-2 is constitutively active in the spinal cord. The ability of AM251 to block the inhibitory effects of nimesulide suggests that these effects are mediated at least in part, by ECs acting via CB_1 receptors. Future studies will further investigate the role of COX-2 metabolism of ECs in the spinal cord under these conditions.

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Stable discharge patterns in multiunit recordings of S1 ongoing activity in neuropathic rats

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Background and Aims Few or no exhaustive studies in literature characterize the ongoing neuronal activity in chronic pain in terms of spike patterns, as structures distributed on the simultaneously recorded traces (or parallel concurrent patterns, PCP). Former works in normal animals showed small stereotyped spatiotemporal patterns of discharge during both ongoing and evoked activity (1,2). On these premises, we extracted essential classes of PCPs (EPCPs) in S1 cortex and found that they were more stable in neuropathic than in a normal animals.

Methods Normal and neuropathic (Seltzer model, 3) Sprague-Dawley rats weighting 350-400 gm were used. The rats underwent barbiturate anesthesia for the surgical experimental preparation and Isoflurane 0.6-0,8 l/min and O_2 0.15-0.2 l/min gaseous anesthesia for the recording sessions (sleep related EEG spectral frequencies of 0,5-15 Hz). Gallamine triethiodide (20 mg/kg/h) was delivered throughout the entire experiments to ensure muscle relaxation. Matrices of extracellular multiple electrodes framed in 3x3 arrays were used in the SSI somatotopical cortical projection areas. The protocol was approved by the Ethical Committee of the National Research Council. For the statistics, PCPs were sampled on multiunit recordings by keeping constant the overall number of spikes. PCPs were then clustered in classes and the most frequently occurring classes were marked as EPCPs. We tested the hypothesis that, after onset, EPCPs tended to be maintened longer than expected by chance. In positive case we identified the PCP associated to EPCPs as stable patterns.

Results We analyzed a wide range of ongoing activity regimes. Short moderatly stable PCP were typically detected in almost all control animals. In neuropathic animals, exhibiting significantly higher spiking frequencies than control rats, PCP of different length were significanlty more stable than in control rats. This property is completely independent to the spiking frequency an has never been observed before.

Conclusions We demonstrated in S1 cortex of Seltzer rats that, when hyperactivity is really observable, PCP of different length are more stable than in control animals. An altered organization of PCP embedded in ongoing activity of small S1 neuronal networks could represent a general substrate for the altered sensory responses typical of chronic pain.

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Antinociception by motor cortex stimulation in neuropathic rats: role of rostroventromedial medullary neurons

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Background and aim: Motor cortex stimulation produces antinociception in both rats and humans. However the roles of various subcortical relay mechanisms in the antinociceptive effect of motor cortex stimulation are not yet fully understood. We studied whether stimulation of the primary motor cortex (M1) produces antinociception in rats with experimental neuropathy and whether M1 stimulation induces a corresponding change in the discharge rates of putative pain-modulatory neurons in the rostroventromedial medulla (RVM), a major relay for descending pain regulation.

Methods: Single unit recordings and withdrawal reflex assessments were performed in spinal nerve-ligated and sham-operated rats under light pentobarbital anesthesia 4 weeks following operation. RVM neurons were classified as presumably pronociceptive ON-cells and antinociceptive OFF-cells giving an excitatory or inhibitory response to noxious heat, respectively. Spontaneous activity of RVM neurons was studied after microinjection of saline or glutamate (2.5 nmol and 25 nmol) into M1. Moreover, electrical stimulation was applied to M1 to assess descending antinociceptive effect on the heat-induced hind limb withdrawal latency. The experiments were performed according to the Guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Institutional Ethics Committee.

Results: Withdrawal responses indicated that descending antinociception induced by electrical stimulation of M1 was significant both in nerve-injured and sham-operated animals. Muscimol-induced block of RVM or block of spinal 5-HT_{1A} receptors by WAY-100635 failed to reverse antinociception induced by M1 stimulation. Recordings of RVM cells indicated that a low dose of glutamate in M1 increased spontaneous activity of ON-cells in neuropathic but not sham-operated animals, while glutamate at a high dose failed to affect discharge rates of ON-cells in both experimental groups. Glutamate failed to influence discharge rates of OFF-cells, independent of the dose or the experimental group. Moreover, RVM cells not responding to heat (NEUTRAL-cells) had decreased discharge rates after a high dose of glutamate in the neuropathic but not sham-operated group.

Conclusions: Stimulation of M1 produces significant antinociception in neuropathic and sham animals. Discharge properties of ON- or OFF-cells in the RVM may not explain the descending antinociceptive effect by M1 stimulation. In neuropathy, if anything, M1 stimulation-induced ON-cell activity might attenuate antinociception. Descending pathways not relaying in RVM may play a major role in M1-induced descending antinociception.

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Fluoroscopy guided Selective Nerve root injection for unilateral lumbar radicular pain, is it more effective than blind paramedian translaminar epidural injection after one year?

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Background and Aims. The results of previous studies support nerve root injection over epidural injection for managing lumbar sciatic pain. We reported the 1-year results of a randomized controlled trial evaluating the efficacy of a single injection of depot steroid by both means for lumbar sciatica.

Methods. After approval of the study protocol by our ethics committee, the study was performed on 80 patients with unilateral lumbar sciatic pain with a single level disc herniation who were allocated to a single injection of 40 mg methylprednisolone diluted in lidocaine and hypertonic saline 2.7% either by blind paramedian translaminar epidural technique (E-group) or fluoroscopic guided nerve root injection (N-group). Patients with neural entrapment detected by straight leg raising-SLR=60º, in addition to a disc herniation in MRI, were included in the study. Differences in the clinical examination parameters, reported symptoms, sick leaves, number of discectomies, adverse effects, and the course of high-sensitive C-reactive protein (hsCRP) were assessed in relation to the course of pain and clinical function over the 1-year follow-up.

Results. No significant differences in baseline variables existed. 85% of patients in N-group reported no pain by the end of the year compared with 75% in the E-group ($P = 0.73$). Similar efficacy was observed between treatment groups for other outcomes. At the beginning of the study, there were no statistically significant differences in mean hsCRP levels in N-group (1.5mg/L) compared to the levels obtained in E-group (1.3mg/L). In N-group, hsCRP declined significantly during the initial period of 3 months with a corresponding decrease in pain and improvement in SLR, whereas after this period, the course of the hsCRP did not correspond with the changes in pain and SLR. In patients in E-group, hsCRP remained approximately constant throughout the whole period with no correlation with pain or function. 10 patients required discectomy (4 in N-group and 6 in E-group). No adverse reactions were encountered in any of the groups.

Conclusions. The long-term results of this randomized trial do not support the use of fluoroscopic guided nerve root injection (N-group) over paramedian blind translaminar epidural injection (E-group) for lumbar radicular pain in patients with disc herniation-induced sciatica. Therefore, according to this study levels of hsCRP do not have a major clinical relevance when evaluating the long-term course of patients with disc herniation-induced sciatica and therefore should not be taken into primary consideration when decisions on therapy are made.

	Name **Age**	**Country** **City** **Abstract**	**Degree** **Level or Posi-tion**	**Department** **Institution** **University**	**Eng-lish self declar**	**Mentors or References**	**Fields of Study**	**A: Abstract** **R: Research Topic** **PhD: Doctoral Thesis**	**Publi-cations** **Abstr/ Congr**	**Sabbatical abroad, where?** **Special Award**
1.	Böttger Michael 32 m Michael.Boettger@mti.uni-jena.de	Germany Jena	MD Neurology Univ Würzburg	Univ Würzburg Physiol Inst	fluent		Animal and hu-man pain research	R: Ca-activated K-channel SK1 in injured human sensory nerves, regulation by neurotrophic factors	21 0	London NeurPath Hammersmith German Pain Award
2.	Brenneis Chris-tian, 30 m c.brenneis@med.uni-	Germany Frankfurt	PhD Pharmacol MSc Biology	Univ Frankfurt Clin Pharmacol PostDoc Fellow		Geisslinger G	Animal pain pharmacology	R: Role of spinal microsomal Prostaglandin E synthase (mPGES-1) in nociceptive processing (and sensitization)	7 5	IBRO 07 Travel Award Melbourn
3.	Galova, Miriam 36 f miriamgalova@yahoo.com	Slovakia Martin	PhD student MD Anesthesiol	Jessenius Fac Med, Martin Dept Anesthesio	me-dium	D Mistuna M Kuli-chova	Clin Pain Re-search	PhD: Phantom limb pain prevention with the appli-cation of ketamine		
4.	Goncalves Leonor, 29 f leonorg@ecsaude.uminho.pt	Portugal Porto	PhD student Degree Biology	Minho Univ		A Almeida A Per-tovaara	Biology and Neurophysiology of Pain	R: Nociceptive Neurons in Amygdala	3 4	Portland USA Neurosurgery
5.	Grant Andrew 30 m andrew.2.grant@kcl.ac.uk	UK London	PhD Pharmacol	King's College London	native	N Bunnett S Brain McMahon	Neuropeptides NeurogenInflamm Pain Vanilloid	R: Protease signalling to nervous and vascular system A: Agonists of PAR_2 sensitize TRPV4 to induce mechanical hyperalgesia in mice	15 5	Wellcome Res Fellowship 4 yrs
6.	Jaken, Robby 24 m r.jaken@np.unimaas.nl	Netherlands Maastricht	PhD Student MSc? Mol Life Sci, Neurosci	Maastricht Univ Dept Anesthesia			Cell Biol & Behav Pharmacol Neurol Diseases	R: Combinatorial therapy of enriched environment and rolipram/thalidomide in rat spinal cord injury	6 7	Germany Aachen Univ Neurology Science
7.	Lilli Li, 28 f lili.li@ki.se	Sweden Stockholm	PhD Student MS Phcarmacol China	Karolinska Inst Clin Neurosci	excel-lent	Xiaojun Xu Wiesenfeld	Neuropath Pain	A: The adenosin A2A receptor is involved in carra-geenan-induced inflammatory hyperalgesia in mice R: Genetic and sex differences in neuropathic pain	0 1	
8.	Moont Ruth, 32 f ruth.jalfon@gmail.com	Israel Haifa	PhD Student MSc Med Sci in London	Technion Inst Technology		S Hocher-man D Yar-nitzky	Neurophysiology, Neuropsychology	PhD: Pain inhibits Pain Mechanisms R: Intensity Coding in Somat Cortex of heat-evoked potentials with Electromagn Tomography	1 5	MSc study in London UK

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9.	Nielsen Christian Kamp, 39 m CKN@FARMA.KU.DK	Denmark Copenha-gen	PostDoc PhD Exp Pathol MSc Biochem	Univ Copenha-gen Dept Phar-macol		O Bjerrum	Mol & Cell Pa-thology Biochem-istry	PhD: Regulat & Function of Human Leukotriene D4 receptor CysLT1 in Epithel Cells & Colon Cancer R: Sortilin in Spinal Nerve Injury (SNI) induced Neuropathic Pain in Mice	9 5	
10.	Nir Rony-Reuven 28 m ronynir@tx.technion.ac.il	Israel Haifa	PhD Student BSc Biomed Engineering	Technion Inst Technology	Excel-lent	Yarnitzky	Biotech lin Neu-rophysiol Pain	PhD: Pain Processing and Modulation in Man	3 6	Germany TH Karlsruhe Biomed Eng
11.	Nohn Claudia 26 f c.nohn@uke.de	Germany Hamburg	PhD Student MA Psychology	Uni Hamburg Dept Neuro-physiol	fluent	Andreas Engel	EEG/MEG in human; Neuro-psych tests	PhD: Psychological influences on pain-induced (neuronal) oscillations (in the EEG)	2 2	Netherlands Univ Nijmegen
12.	Pascual –Rubio Vincenc, 31 m vpascual@nsc-tec.com	Spain Barcelona **WITH-DRAWN**	MD Neurology Barcelona Univ	MC Mutual Barcelona	Ad-vanced level	J Serra	Clin Neurophysiol Microneurography Humans Animals	R: Abnormal Activity in pathol human nerve C-fibres	0 19	IASP Member
13.	Petrikonis Kestutis, 36 m kestutispetrikonis@yahoo.com	Lithuania Kaunas	PhD Medicine MD Neurology	Univ Kaunas Lecturer Med Neurology	good	Arunas Sciupokas	Clinical Pain Research Neurol-ogy	PhD: Lumbosacral Radicular Syndrome: Influence of Pain Parameters to Funct Pain Status & Qual of Life	2 7	Prague Univ Rehab Med
14.	Roberts Kathe-rine, 24 f k.roberts05@imperial.ac.uk	UK London	PhD Student BSc Physiol Pharmacol	Imperial Coll London		P Anand RJ Evans S Nahorski	Human & Animal Pain research Biochemistry	R: Molecular and Pathophysiol Mechanisms of Topical Agents in Chronic Pain and Models	3 6	
15.	Rojewska Eweli-na, 23 f ewelinarojewska@tlen.pl	Poland Krakow	PhD Student MSc Zoology	Polish Acad Sci Krakow Inst Pharmacol		B Plytycz, B Przew-locka	Animal Pain Behaviour	R: Comparison of Mouse Model of Neuropathic Pain and its Therapy; A: The role of glia in neuropathic pain symptoms in streptozotocin-induced diabetic mice.	0 3	
16.	Sagar Devi Rana 28 f Devi.sagar@nottingham.ac.uk	UK Nottingham	PhD Neurosci BSc Physiol Pharmacol	Univ Notting-ham Inst Neurosci		D Kendall V Chapman	In vivo Electrophy Animal Behav	R: Cannabinoids in Somatosens Processing in Rat Models of Inflam & Neuropath Pain	8 4	

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17.	Sokolov Maxim 37 m Maxim.Sokolov@astrazeneca.c	Russia Moscow	PhD MD Med & Dentistry	Research Astra Zeneca Sweden	fluent	L Voronin	Neuroscience	R: Na voltage gated channels: role of ß subunits in Physiol & Pharmacol	12	10 yrs PostDoc Dublin Glasgow & Magdeburg
18.	Yosry Mohammad 39 m m_yosry_m@yahoo.com	Egypt Giza	MD Anesthesiol	Assoc Prof Anesthesiol Pain Managem	English School Educat		Cardiac Anesth Intensive Care Pain Manag	R: Ketorolac in earle extubation after open heart surgery; oxygen flux and hemodynamics	16 4	Turkey Istanbul Pain Unit Erdine
19.	Viisanen Hanna, 25 f hanna.viisanen@helsinki.fi	Finland Helsinki	PhD Student MSc Neurobiol	Univ Helsinki Inst Biomedicin	Very good	Pertovaara	Electrophysiology animal studies	PhD: Descending Pain Modulation pathways in Neuropathic Pain	1 3	
20.	Ferreira Gomes Joana Maria, 30 f jogomes@med.up.pt	Portugal Porto	PhD Student MSc Microbiol Univ London	Univ Porto Med Faculty, Inst Histol, Neurosci	Was living in UK	Josè Castro Lopes	Biochemistry, Animal behav	PhD: Neurobiological mechanisms of pain in os-teoarthritis R: Metabotropic receptors in the thalamic process-ing of chronic pain	4 8	UK, London Imperial College Grünenthal Award 2005
21.	Kiskira Olga, 35 okiskira@yahoo.com	Greece Athens	MD Anesthes	Gen Hospital Evangelismos Dept Anesth Athens	yes		Pain Treatment, Pain Specialist in Greece	**Clin:** Epidural & intrathecal analgesia, Postop & Cancer Patients, Intrathecal Baclofen for Spasticity, Periop Analg in Arthroscopy, Gabapentin, Pare-coxib, Trigger Points	1 33	
22.	Martins, Isabel 31 f isabmart@med.up.pt	Portugal Porto	PhD student MSc Paris Biology Studies	Univ Porto, Med Fac, Dept Histol Embryol	1 yr USA	I Tavares D Lima	Biology & Genetic of Pain	PhD: Gene therapy for chronic pain control by manipulation of the supraspinal pain control system MSc: Migration dynamics of a recomb HSV-1 vector from caudal medulla oblongata …	2 18	France, Paris 3 yrs USA Univ South Carolina 1 yr Zaldiar Prize 08
23.	Bali, Kiran 26 m kiran.bali@pharma.uni-heidelberg.de	Germany Heidelberg	PhD Student BSc Biology & Chemistry, Biotech, India	Univ.Heidelberg Dept Pharmacol	Yes TOEFL	R Kuner	Mol Neuroscience Proteomics of Pain Brain development Animal behavior	PhD: Application of RNAi in chronic pain R: CDP-choline supplement in early life induces stable increase in dendritic complexity in neurons in somatosensory cortex	1 4	
24.	Folkesson, Anna 27 f af@farma.ku.dk	Sweden Malmö	PhD Student MSc Pharmacy	Univ Copenhag Dept Pharmacol		OJ Bjerrum	Animal pain behav Clin analgesic studies	PhD, A: Pharmacokinetic & -dynamic studies of Gabapentin in treatment of neuropath pain	2 2	

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25.	Staniaszek, Lydia 26 f mbxles@nottingham.ac.uk	UK, Not-tingham	PhD Student BSc Pharmacol Univ Bristol	Univ Nottingha School Biomed Sci	Teache r in English	V Chapman	Animal behav electrophysiol Molec Biol	PhD: Effects of Endocannabinoid system in the MIA model of osteoarthritis	3	IASP Member
26.	Dimitrov, Ale-xander, 35 m Alexander.Dimit-	Bulgaria Sofia	MSc Physics, Sofia Univ	Bulg Acad Sci Ctr Biomed Eng	fluent	GV Dimi-trov	Biophysics Nerve & Muscle	R: Spontaneous repetitive activation of myelinated axon, e.g. by lack of K+ channels	8 5	
27.	Puljak, Livia 30 f livia.puljak@gmail.com	Croatia Split	MD Univ Split 2002	Univ Split Med School, Acad Research Inst	Excel-lent		Cell Cult, Patch Clamp, Animal Pain Techniques Health Polit Ethics	R: Targeted delivery of pharmacol agents into dorsal root ganglion R: Basic diabetes research	6+6 8	NL Univ Nijmeg, CAN Univ Ottaw, USA Univ Tex, Colorado, >3yrs
28.	Storchi, Riccardo 26 m riccardo.storchi@gmail.com	Italy Modena	MSc Biomed Eng Zurich CH & Bioing Poli-tec Milan	PhD Student Univ Modena Dept Biomed Sci	TOEFL test OK	DBD Rubin G Baselli C Porro G Biella	Mol Physiol & Bioimaging of Pain	R: Human cortical neuronal recording during neuro-surgery, tactile & noxious; Neurophysiopathol of Pain Lab at Segrate with G Biella MSc: Neuronal models of learning		Univ Manchester Fac Life Sci 2007 Univ Zurich CH MSc 2005
29.	Cobianchi Stefa-no, 28 m s.cobianchi@ipsifar.rm.cnr.it	Italy Rome	PhD Student MSc Psychol	Univ Rome CNR Inst Neu-rosci	fluent		Animal pain models	R: New pharmacological research strategies on inflammatory and neuropathic pain	2	Scholar EPS 2006 NeuPsic Berlin