European Pain School 2010

Translating Pain Science into Pain Medicine

30 May – 6 June 2010
University of Siena
Certosa di Pontignano, Siena, ITALY

Programme & Abstracts
European Pain School 2010

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Acknowledgments

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European Neuroscience Schools, a joint venture of IBRO, the International Brain Research Organization, and FENS, the Federation of European Neuroscience Societies

Prof. Roberto Caminiti, Univ. of Rome, Chairman of FENS/IBRO School Committee

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Mission of the European Pain School

Pain causes an enormous amount of suffering and disability, with those directly affected estimated at 19% of the adult population in Europe. The resulting medical and social costs in European countries amount to an estimated 500 million € per 1 million of population annually.

Pain may become progressively more severe over time, with intensity often unrelated to the course of the precipitating organic condition. Therefore, chronic pain has recently been recognized as a disease entity in its own right.

A number of somatic, psychosocial and genetic risk factors have been identified which determine facilitate chronicity, e.g. in low back pain, neuropathies, fibromyalgia and headache. Acute pain not successfully treated carries a particularly high risk of persisting and becoming chronic pain.

Basic and clinical research provides some understanding of 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 predicts that early preventive measures may have a major potential against pain chronicity.

The European Pain School provides interdisciplinary training to younger scientists with the aim of stimulating pain research and ultimately improving prevention and treatment of chronic pain.

European Pain School at Siena: a short history and perspective

The idea of founding a European Pain School was first conceived in 1994, when Anna Maria Aloisi, Giancarlo Carli and Manfred Zimmermann met at a symposium at the Certosa di Pontignano. The Certosa is a most atmospheric 16th century monastery in the Chianti region, now a Conference Center of the University of Siena. In 2002 we convinced 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 venue for transnational scientific interaction in the field of pain science, a branch of increasing recent interest in the arbour of biomedicine.

Finally 40 scholars were selected to attend the School, nearly half of them from Eastern Europe, and 15 faculty. All of us were thrilled by the novel experience of spending many hours together, scholars and faculty, from early morning exercises in the Cloisters to midnight talks over 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 in the Cloisters where we enjoyed fine lunches and dinners in the tradition of Toscana, including local wines from vineyards owned by the University of Siena.

For a week we were living 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 adopted a mode of a scientific family. The transition fostered in a wonderful way the transfer of concepts and facts in pain science, and led to the formation of many new friendships.

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Programme
Saturday, 30 May

11:00- 16:00  Arrival of Scholars and Faculty at the Certosa di Pontignano

14:00- 16:00  Registration  
Refreshments: coffee and cakes in the Loggia

16:30-18:00  Session I  
[Sala Bracci]
Opening Ceremony - Welcome Addresses

Welcome and Introduction to the European Pain School 2010
PROF. ANNA MARIA ALOISI  School Director  
Univ. of Siena, Italy

Welcome by the President of Medical School, University of Siena

IASP, the World Strategy Against Pain
PROF. GERALD F. GEBHART  IASP President  
Univ. of Pittsburgh, USA

The FENS/IBRO Neurosciences School Program
PROF. ROBERTO CAMINITI  Chair of the School Committee  
Univ. of Rome La Sapienzia, Italy

Research against pain
GABY E RKENS  Scientific Relations Management Grunenthal GmbH  
Aachen, Germany

Pain a field of science
PROF. MARSHALL DEVOR  
The Hebrew University, Jerusalem, Israel

The Certosa di Pontignano
PROF. GIANCARLO CARLI  
Univ. of Siena, Italy

Monasteries, the medieval forerunners of Universities and Hospitals
PROF. MANFRED ZIMMERMANN  
Univ. of Heidelberg, Germany
18:00  **Concert & Wine in the Cloister of the Certosa**  
Scholars and Faculty of the European Pain School are cordially invited to attend an Event organized in the Cloisters by **Chianti Classico**, the regional winemakers’ association, with wine and classical chamber music. In particular you will experience a variety of the Vin Santo, a white dessert wine from the Chianti region.

20:00  **Dinner**  
Joining Scholars and Faculty, and get some announcements for the following day

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**Monday, 31 May**

9:00-12:45  **Session II**  

[Lecture 1.](#) **Introduction to pain: history, suffering and ethical issues**  
**Manfred Zimmermann** (Heidelberg, Germany)

[Lecture 2.](#) **Neuronal excitability and hyperexcitability: cellular mechanisms and genetic determinants**  
**Marshall Devor** (Jerusalem, Israel)

**Oral Presentations by Scholars:**

ATP induces release of cathepsin S from primary microglia  
**Francesca Guida** (Naples, Italy)

Spontaneous activity and multiple spikes in C-nociceptors in rat induced by intraplantar injection of Nerve Growth Factor (NGF)  
**Barbara Cokic** (Barcelona, Spain)

Nociceptive behaviour in mutant mouse models related to psychosis: focus on neuregulin-1 and catechol-O-methyltransferase  
**Jeremy Walsh** (Dublin, Ireland)
15:00-19:00  **Session III**  

[Lecture 3. Pain from inside - Translational research on visceral pain](#)  
**Gerald F. Gebhart (Pittsburgh, USA)**

[Lecture 4. Strategies for the pharmacotherapy of pain - Does the understanding of pathomechanisms help?](#)  
**Eija Kalso (Helsinki, Finland)**

**Oral Presentations by Scholars:**

Intrathecal blockade of Trk receptors and neurotrophin sequestration reduces referred pain in an animal model of chronic cystitis  
**Barbara Frias (Porto, Portugal)**

Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans  
**Roi Treister (Haifa, Israel)**

Neuropathic rats have an impaired performance in the reversal steps of an attentional set-shifting task  
**Hugo Leite-Almeida (Braga, Portugal)**

**Poster viewing on demand and by appointment, see Wednesday and Friday for details**

21:30-22:00  **Evening session**  

Behavioral Tests & Tools in Pain Research  
A hands-on presentation of equipment for experimental pain research  
Lead by **Federico Montechiaro, PhD**  
**Ugo Basile Biological Research Apparatus, Comerio, Italy**
Tuesday, 1 June

9:00 - 12:45  **Session IV**  

**Lecture 5.** Insights into the use of opioids and cannabinoids - Benefits and harms in the management of chronic pain
Eija Kalso (*Helsinki, Finland*)

**Lecture 6.** Specificity in pain processing: from the nociceptor to the cerebral cortex
Allan I. Basbaum (*San Francisco, CA, USA*)

**Oral Presentations by Scholars:**

- Role of corticotropin-releasing factor and urocortin 1 systems in pain-induced maladaptation and comorbid diseases
  Tom Rouwette (*Nijmegen, The Netherlands*)

- The wound healing mediator EGF abolishes PGE2 induced mechanical hyperalgesia
  Christine Andres (*Berlin, Germany*)

- High-dose bupivacaine remotely loaded into multivesicular liposomes demonstrates slow drug release without systemic toxic plasma concentrations after subcutaneous administration in humans
  Simon Haroutianian (*Jerusalem, Israel*)

**Poster viewing on demand and by appointment**

15:00 - 19:00  **Session V**  

**Lecture 7.** Genetic approaches to pain mechanisms and treatment
Michel Pohl (*Paris, France*)

**Lecture 8.** Pain studies on humans: insights from functional imaging with MRI and PET
Vania Apkarian (*Chicago, IL, USA*)
Oral Presentations by Scholars:

Susceptibility to chronic pain following nerve injury is genetically controlled by CACNG2
JONATHAN NISSENBAUM (Jerusalem, Israel)

Belief modulates remifentanil effects on pain-evoked responses: a phfMRI study of hidden versus open opioid administration
LAUREN ATLAS (New York, NY, USA)

Analgesic and antipyretic properties effects of ethanolic extract of tapinanthus dodoneifolius leaves in rats
BAMIDELE OWOYELE (Ilorin, Nigeria)

21:30-24:00 Evening session
Group sessions to prepare “debates” on Thursday morning

Group 1 [Sala Palio]
Is pain the exclusive business of afferent A-delta- and C-fibers?

Group 2 [Sala Veranda]
Does the cerebral cortex play a central role in pain processing?

Wednesday, 2 June

9:00-12:45 Session VI [Sala Palio]

Lecture 9. Molecular biology of neuropathic pain
ALLAN I. BASBAUM (San Francisco, CA, USA)

Lecture 10. Drugs for pain - Understanding the molecular biopharmacology
THOMAS HERDEGEN (Kiel, Germany)

Special Interactive Workshop of Scholars and Faculty
Ethical motivations and limitations of pain research and treatment - do we need guidelines?
Introduction: MANFRED ZIMMERMANN (Heidelberg, Germany)
Poster viewing on demand and by appointment

15:00–19:00 Session VII

Lecture 11. Targeting pain at the molecular level by imaging of the human brain
VANIA APKARIAN (Chicago, IL, USA)

Lecture 12. Network processes of pain control during general anesthesia
MARSHALL DEVOR (Jerusalem, Israel)

Poster Presentations 1–6

1. The effect of protein kinase C inhibition on NMDA receptor phosphorylation during diabetes in the rat
HARUTYUN ALAVERDYAN (Yerevan, Armenia)

2. Clinical evaluation of molecular mechanisms involved in degenerative disc related pain
PABLO ANDRADE (Maastricht, The Netherlands)

3. Bioinformatic and biochemical studies on the phylogenetic variability of proenkephalin-derived octapeptides
ENGIN BOJNIK (Szeged, Hungary)

4. Mechanisms involved in chronic neuropathic pain after spinal root avulsion injury
DANIEL CHEW (London, United Kingdom)

5. A role for the scaffolding molecule PSD-95, which assembles signalling complexes with glutamate receptors, in chronic pain
ADA DELANEY (Edinburgh, United Kingdom)

6. Botulinum toxin A in a rat model of migraine and/or trigeminal neuropathy
BORIS FILIPOVIC (Zagreb, Croatia)
Thursday, 3 June

9:00-12:45  Session VIII

Lecture 13. Somatosensory examination of the clinical pain patient
TROELS JENSEN (Aarhus, Denmark)

Scholars debate
Moderator MARSHALL DEVOR (Jerusalem, Israel)

Group 1
Is pain the exclusive business of afferent A-delta- and C-fibers?
Yes!: 5 scholars
No!: 5 scholars

Group 2
Does the cerebral cortex play a central role in pain processing?
Yes!: 5 scholars
No!: 5 scholars

Lecture 14. Strategies for analgesic drug development - the biotechnology approach
THOMAS HERDEGEN (Kiel, Germany)

14:30-24:00  Excursion to Siena old town

14:30  Bus departs from the Certosa

15:00-18:00  Guided tour to historical Siena, visit of Cathedral and City Hall

18:00-24:00  Free time in Siena
Friday, 4 June

9:00- 12:45  Session IX  [Sala Palio]

**Lecture 15.** Progressive pain following spinal cord injury - a cascade of spreading pathobiology in the CNS?
**Manfred Zimmermann (Heidelberg, Germany)**

**Lecture 16.** Opioids: Molecules controlling pain and mediating euphoria, a Janus face?
**Ryszard Przewlocki (Krakow, Poland)**

**Oral Presentations by Scholars:**

- Synthesis, transport and neuronal sorting of SDF-1 / CXCR4 at the spinal level: implications in nociception
  **Annabelle Reaux Le Goazigo (Paris, France)**

- Feedback from peripheral musculature to central pattern generator in the neurogenic heart of the crab callinectes sapidus: role of mechanosensitive dendrites
  **Keyla Garcia Crescioni (Old San Juan, Puerto Rico)**

- Signalling pathways activated by Sphingosine-1-phosphate in nociceptors in culture
  **Maria Camprubi Robles (Innsbruck, Austria)**

**Poster viewing on demand and by appointment**

15:00- 19:00  Session X  [Sala Palio]

**Lecture 17.** Migraine - New molecular mechanisms of an old plague
**Daniela Pietrobon (Padua, Italy)**

**Lecture 18.** Pain in transgendered people
**Anna Maria Aloisi (Siena, Italy)**
Poster Presentations 7–12

7. Sigma-1 receptors are involved in the visceral pain induced by intracolonic administration of capsaicin in mice
Rafa Gonzales (Granada, Spain)

8. Effect of sex hormones on mechanical and cold allodynia following sciatic nerve ligation in rats
Andrey Malyshkin (St. Petersburg, Russia)

9. Phenomics and QTL mapping of sensitivity to noxious heat and mechanical stimuli in naïve A/J, C57BL/6J and their 23 AXB-BXA descendant recombinant inbred (RI) mouse lines
Mariam Mashregi (Toronto, ON, Canada)

10. Novel behavioral models for the assessment of the emotional component of pain and for spontaneous pain in rats
Kris Rutten (Achen, Germany)

11. 5-HT7 receptor-mediated modulation of mechanical nociception in naïve versus neuropathic rats – Implication of Cl- transport dynamics
Florent Viguier (Paris, France)

12. Nucleotides excite sensory neurons via two P2Y receptors and a dual signaling cascade
Arsalan Yousuf (Vienna, Austria)

Saturday, 5 June

9:00- 12:45  Session XI  [Sala Palio]

Lecture 19. The enigma of chronic widespread pain - The fibromyalgia syndrome
Giancarlo Carli (Siena, Italy)

Lecture 20. How can the plethora of new mechanisms help in neuropathic pain treatment?
Troels S. Jensen (Aarhus, Denmark)
Oral Presentations by Scholars:

Evaluation of the effects of a new analgesic on ventilation in healthy subjects
**Felicitas Thom (Aachen, Germany)**

The influence of vitamins B on immunological system and pain in patients with low back pain
**Nataliya Yavorska (Lviv, Ukraine)**

Central mechanisms and neurochemistry involved in placebo analgesia and nocebo hyperalgesia
**Nathalie Wrobel (Hamburg, Germany)**

Evaluations of the European Pain School by Scholars and Faculty – the FENS/IBRO questionnaire

16:00-24:00  **Farewell Session**

16:00  Bus Transfer to Rapolano Terme, a hot mineral water Spa in Tuscany

17:00-20:00  Stay and relax at “Terme Giovanni”, Rapolano Terme, bathing in the waters of a hot mineral spring used by the ancient Romans

20:30-24:00  Farewell dinner and party in restaurant “Locanda da Annita” in Rapolano Terme

24:00  Bus return to the Certosa di Pontignano

**Sunday, 6 June**

**Departures**
Oral presentations
The wound healing mediator EGF abolishes PGE2 induced mechanical hyperalgesia

C. Andres¹,², O.A. Dina³, J.D. Levine³, T. Hucho¹
¹Max Planck Institut für molekulare Genetik, Berlin, Germany; ²Freie Universität Berlin, Berlin, Germany; ³University of California, San Francisco, USA

Background and Aims. Nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF) is involved in regeneration and sensitization of nociceptors subgroups. Another growth factor is central for tissue regeneration, epidermal growth factor (EGF). If EGF influences also nociceptor function is so far unknown. NGF, GDNF and EGF activation of the MAP-kinase ERK 1/2 have been reported. In PC12 cells stimulation with NGF or EGF lead to opposing phenotypes, differentiation versus proliferation, depending on the respective ERK 1/2 activation kinetic. We now asked, if EGF can act on nociceptors, if there are differences in NGF and EGF induced ERK 1/2 kinetics, and what behavioral phenotype results from EGF treatment.

Methods. To observe ERK 1/2 activation kinetics in single neurons we introduced an automatized quantitative microscopy approach to cultures of dorsal root ganglia (DRG) from adult Sprague-Dawley rats. NGF, GDNF or EGF treated cells were imaged with an automatic microscope and fluorescence intensities derived from phosphospecific Erk 1/2 antibodies were quantified on single neuron base (n(total)>75000). Effects of EGF injection on mechanical pain sensitisation were tested using the Randall-Selitto paw pressure test.

Results. We detected expression of the EGF receptor in all DRG-neurons. We detected differential ERK 1/2 activation in response to NGF, GDNF and EGF treatment. We provided thereby prove of principle for a quantitative microscopy approach to subgroup specific kinetic data collection of endogenous signaling components. NGF and GDNF stimulation led to strong ERK 1/2 activation in differential subsets of neurons marked by the lectin IB4. In contrast, EGF appeared not to have any effect on ERK 1/2 phosphorylation in sensory neurons. Corroborating our cellular results, intradermal EGF injection induced no mechanical pain sensitisation. Considering the opposing phenotype of NGF and EGF treatment on PC12-cells, we tested if EGF might inhibit mechanical pain sensitisation. Indeed, pretreatment with EGF abolishes PGE2 induced mechanical hyperalgesia.

Conclusions. Using automatized quantitative microscopy we are able to follow endogenous activation of signaling pathways in single neurons. NGF and EGF stimulation of DRG-neurons showed differential ERK 1/2 activation responses, which we correlated with behavioral phenotypes. Our results indicate that EGF can abolish PGE2 induced mechanical hyperalgesia and can act as modulator of pain sensitization.
Belief modulates remifentanil effects on pain-evoked responses: a phfMRI study of hidden versus open opioid administration

L.Y. Atlas\textsuperscript{1,}, R.A. Whittington\textsuperscript{2,}, M.A. Lindquist\textsuperscript{3,}, N. Sonty\textsuperscript{2,4,}, T.D. Wager\textsuperscript{1,5}

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Background and Aims. Though common brain regions are influenced by placebo and opioid treatments [1], it is unknown whether knowledge of drug delivery influences pain-related brain responses during drug treatment. We compared the effects of overt and covert remifentanil administration during noxious thermal stimulation to determine how pharmacological treatments are affected by awareness and belief. We were particularly interested in whether knowledge potentiates drug-induced decreases in pain-evoked responses in the pain-processing network (PPN).

Methods. Subjects (n=21) went through two runs of fMRI scanning during thermal stimulation. In one run (“Open”), subjects were told that they would be receiving remifentanil, while in the other run (“Hidden”), subjects were told they would receive no drug. In fact, infusion proceeded identically in both runs, allowing us to compare pain-evoked responses under Open versus Hidden administration. Thermal pain was delivered via a thermode, and subjects rated pain evoked by high and low intensity stimulation. Analyses were conducted using SPM5 and custom software. Parametric regressors modeling drug concentration were constructed based on a pharmacodynamic model of predicted drug concentration [2].

Results. Pain was reduced during remifentanil administration in both Open and Hidden runs, and overt administration potentiated analgesia (p<.05). A number of PPN regions showed greater drug-induced decreases in response to noxious thermal stimulation under Open administration, including right SI, right anterior insula, and rostrodorsal anterior cingulate. This expectancy-based enhancement of remifentanil modulation was distinct from decreases in pain-evoked responses due to context (Open>Hidden overall), specific expectancies (infusion period Open>Hidden), and pure drug-related effects (Hidden Drug).

Conclusion. Effects of standard open pharmacological therapies are composed of not only pure drug effects, but also specific enhancement due to expectancy and awareness. Our results suggest that studies of opiates and other pharmacological agents should consider how expectations might interact with drug effects, and clinicians should consider patient cognitive factors as important contributors to pharmacological treatment outcomes.

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Signalling pathways activated by Sphingosine-1-phosphate in nociceptors in culture

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¹Division of Physiology, Department of Physiology and Medical Physics, Innsbruck Medical University, Innsbruck, Austria; ²Instituto de Biología Molecular y Celular, Universitas Miguel Hernández, Elche, Spain

Background and Aims. Sphingosine-1-phosphate (S1P) is an important immune modulator which has been associated with inflammatory conditions including rheumatoid arthritis. S1P is known to have a key role during activation and subsequent development of the inflammatory response and is also part of the inflammatory process. Extracellularly, S1P activates five G-protein-coupled S1P receptors (S1P1 to S1P5) but also intracellularly modulates cellular activity through unknown pathways. Therefore, we investigated whether S1P has a role in nociceptor sensitization. The vanilloid receptor-1 (TRPV1) is a pain-integrator ion channel mainly expressed in nociceptive neurons and after inflammation is sensitized by different pro-algesic factors released by the damaged tissue including several growth factors and cytokines.

Methods. To address whether S1P induces sensitization of TRPV1 channels in nociceptive neurons we performed calcium (Ca^{2+}) imaging measurements in primary cultures obtained from mouse dorsal root ganglion (DRG) to study the effect of S1P on TRPV1 activation by capsaicin (Caps).

Results. We found that the TRPV1 activity was tremendously increased in presence of the sphingolipid which produced a sensitizing effect in about 60% of Caps-sensitive neurons. Similarly, SEW2871, a specific agonist of S1P1 receptor considerably augmented the Cap-activated currents. Unlike SEW2871, S1P induced a transient rises in the intracellular Ca^{2+} concentration in some Caps-responsive neurons. In the absence of extracellular Ca^{2+} S1P-mediated Ca^{2+} transients were not detectable. S1P-induced Ca^{2+} increase was also blocked by high concentrations of SKF96365, an inhibitor of receptor-mediated and voltage-gated Ca^{2+} entry.

Conclusion. These results demonstrate that S1P induces sensitization of TRPV1. Secondly, S1P-evoked Ca^{2+} transients may produce an increase in nociceptor response through regulation of TRPV1. Studying the signalling pathways involved in the sensitization of TRPV1 evoked by S1P-activated intracellular cascades can provide effective drug targets for the treatment of pathological inflammation.

Funded by Austria science funds (FWF).
Spontaneous activity and multiple spikes in C-nociceptors in rat induced by intraplantar injection of Nerve Growth Factor (NGF)

B. Cokic, P. Penza, R. Solà, J. Serra
Neuroscience Technologies, Barcelona Science Park, Barcelona, Spain

Background and Aims. NGF is a neuronal survival factor and a key pain mediator. Intradermal NGF administration in humans induces symptoms similar to painful neuropathy. Our aim was to define a new surrogate model of spontaneous pain and hyperalgesia in rats induced by intraplantar administration of NGF. We characterized the effect of NGF on electrical activity of the C-nociceptors, behavioral measures of evoked pain and epidermal innervation.

Methods. Action potentials from C-fibers were recorded from the sciatic nerve using microneurography in rats (Sprague-Dawley, 250 g) after intraplantar injection of NGF. Animals were anesthetized with ketamine (60 mg/kg) and xilaxine (6 mg/kg), and the right sciatic nerve was exposed at the mid-thigh level. Recordings were performed at different time intervals (30 min, 120 min, 24 h, 48 h) following NGF injection, and at different doses (2 and 5 µg). Responses to electrical stimuli were recorded as raster plots of latencies. Assignment of axonal type was based on the patterns of activity-dependent slowing (Serra et al., 1999). Mechanical nociceptive thresholds, expressed in grams, were measured using a Dynamic Plantar Aesthesiometer. To assess thermal hyperalgesia a paw withdrawal test was applied, in which paw withdrawal latency to a noxious thermal stimuli was measured. Skin samples were obtained with a 3 mm punch, sectioned at 30 µm, and stained for anti PGP 9.5 using conventional immunohistochemical procedures. Intraepidermal nerve fiber (IENF) densities were measured according to published standards (number of single nerves crossing the dermal-epidermal junction per mm). Experiments were carried out according to a protocol approved by the local animal ethics committee.

Results. 538 C-fibers were recorded from the sciatic nerve. The ongoing spontaneous activity was detected exclusively in Type 1B (mechano-insensitive) C-nociceptors. The percentage of nociceptors displaying spontaneous activity was 33%. Also, 20% of Type 1B mechano-insensitive C-nociceptors had double or triple spikes in response to single electrical pulses. Strikingly, none of the Type 1A mechano-sensitive C-nociceptors had these abnormal behaviors. NGF-treated rats showed both mechanical and thermal hypersensitivity. We found no difference in the IENF density between NGF-treated and control rats, suggesting that the total number of epidermal fibers is not affected by the NGF treatment. However, we did observe some morphological abnormalities in the skin biopsies from the NGF-treated rats, such as dermal sprouting and increased complexity of the subepidermal plexus.

Conclusions. Microneurographic recordings showed ongoing spontaneous activity and multiple spikes in identified subtypes of C-nociceptors. These findings are relevant for understanding the origin of the symptoms induced by NGF and represent a new surrogate model of spontaneous pain and hyperalgesia in animals.

Intrathecal blockade of Trk receptors and neurotrophin sequestration reduces referred pain in an animal model of chronic cystitis

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**Background and Aims.** Interstitial cystitis/bladder pain syndrome (IC) is a common and highly debilitating disease. IC is more prevalent in women and it is characterized by referred pain and bladder overactivity, which may result in incontinence. Because the true causes of IC remain to be fully characterized, the treatment of symptoms is not always effective. In IC patients levels of neurotrophins, such as nerve growth factor (NGF) and brain-derived nerve factor (BDNF), were shown to be elevated. These neurotrophins may contribute to visceral pain by sensitizing bladder sensory afferents and regulating neuroplasticity events. Thus, the main goal was to clarify the effects of NGF and BDNF in visceral pain, using an animal IC model.

**Methods.** Female Wistar rats (n=4/group) were anaesthetized for surgical placement of a chronic intrathecal silicone catheter. Four days later, cystitis was induced by a single intraperitoneal injection of cyclophosphamide (CYP, 200 mg/kg). Animals were divided into 4 groups. Each group was injected intrathecally with saline, k252a (tyrosine kinase receptor antagonist), TrkA-Ig2 and TrkB-Ig2 (recombinant proteins that specifically sequester NGF and BDNF, respectively). Referred pain was evaluated by assessing the presence of mechanical hypersensitivity of the lower abdomen and right hindpaw. The mechanical thresholds (MT) of the lower abdomen and hindpaw were established after intrathecal injections using the von Frey filaments before and at 4h, 24h and 48h post-CYP injection.

**Results.** Our results demonstrate that chronic cystitis induced by CYP results in mechanical allodynia in the lower abdomen and right hindpaw, the basal mechanical threshold being 26.0±0.0 on both sites. In CYP-inflamed animals receiving intrathecal saline, the MT was significantly reduced to 8.5±2.6g (p<0.05) on the abdomen and to 8.2g±2.0g (p<0.001) at the right hindpaw at 4h and 24h post-CYP injection, respectively, and remained similarly low at all time points of the experiment. In contrast, in CYP-inflamed animals treated intrathecally with k252a, TrkA-Ig2 or TrkB-Ig2 significantly improved MT of the abdominal region and right hindpaw at all time points in comparison with intrathecal saline.

**Conclusion.** These results support a role for NGF and BDNF for the development of referred pain during chronic cystitis. It is possible that neurotrophin sequestration may be a future useful treatment for IC.
Feedback from peripheral musculature to central pattern generator in the neurogenic heart of the crab *Callinectes sapidus*: role of mechanosensitive dendrites

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**Background and Aims.** The neurogenic heart of decapod crustaceans is a very simple, self-contained, model central pattern generator (CPG)-effector system. The CPG, the nine-neuron cardiac ganglion (CG), is embedded in the myocardium itself; it generates bursts of spikes that are transmitted by the CG’s five motor neurons to the periphery of the system, the myocardium, to produce its contractions. Considerable evidence suggests that a CPG-peripheral loop is completed by a return, feedback pathway through which the contractions modify, in turn, the CG motor pattern. One likely pathway is provided by dendrites, presumably mechanosensitive, that the CG neurons project into the adjacent myocardial muscle. Here we have tested the role of this pathway in the heart of the blue crab, *Callinectes sapidus*.

**Methods.** We performed “de-efferentation” experiments, in which we cut the motor neuron axons to the myocardium, and “de-afferentation” experiments, in which we cut or ligated the dendrites (approved by Institutional Animal Care & Use Committee “IACUC”)

**Results.** In the isolated CG (ICG), these manipulations had no effect on the CG motor pattern. When the CG remained embedded in the myocardium, however, these manipulations, interrupting either the efferent or afferent limb of the CPG-peripheral loop, decreased contraction amplitude, increased the frequency of the CG motor neuron spike bursts, and decreased the number of spikes per burst and burst duration.

**Conclusion.** We conclude that feedback through the dendrites indeed operates in this system, and suggest that it completes a loop through which the system self-regulates its activity.

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ATP induces release of cathepsin S from primary microglia

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Background and Aim. Microglia play important roles in pathophysiological situations of the CNS including neuropathic pain. Following peripheral nerve injury, damaged neurons release nucleotides which lead to microglial cells stimulation through P2X receptors thereby contributing to the development of neuropathic pain. In this study we evaluated the release from cultured microglia of the lysosomal cysteine protease Cathepsin S (CatS), which has been showed to exert a pro-nociceptive effect via shedding of neuronal fractalkine in the dorsal horn of the spinal cord.

Methods. Mixed glial cultures were prepared from spinal cords of Wistar rat pups (P3-7) and microglial cells were harvested by shaking after 12-14 days in culture. Cells were plated for 48 hrs, stimulated and extracellular media as well as cell lysates were collected to measure release of enzymatically active CatS and protein expression by Western blotting. Enzymatic activity was assessed by measuring the fluorescence produced following sample incubation with the substrate Z-Val-Val-Arg-MCA.

Results. We found that 3hrs after LPS priming (10 ?g/ml), incubation with ATP (1 mM but not 50 ?M) resulted in significant release of CatS enzymatic activity which was completely prevented by the presence of the CatS inhibitor LHVS (10 ?M). Moreover, western blotting analysis revealed that the combination of LPS and ATP, but not LPS or ATP alone, resulted in appearance of mature CatS protein in the cell media. We observed that CatS release induced by ATP required extracellular calcium but also recruitment of intracellular calcium stores. The prevention of LPS+ATP-induced release of CatS by the selective antagonist A438079 strongly suggests the involvement of the low affinity receptor P2X7 in mediating the effects of ATP. The observation that the p38 MAPK inhibitor, SB203580 and phospholipase A2 blocker AACOCF3, both prevented ATP-induced release of CatS activity supports the suggestion that CatS release occurs from PLA2-dependent lysosomal pools via p38 MAPK signalling.

Conclusions. These data indicate that the release of active CatS from spinal microglia, which plays a critical role in neuropathic pain states, is likely to be triggered by the neuro/glial transmitter ATP via P2X7 receptor activation.
High-dose bupivacaine remotely loaded into multivesicular liposomes demonstrates slow drug release without systemic toxic plasma concentrations after subcutaneous administration in humans

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Background and Aims. Depot formulations prolong the analgesic effect of local anesthetics and reduce peak plasma drug concentration. This allows for safer administration of larger doses of local anesthetics, which further prolongs the duration of analgesic effect. We previously reported the development of large multivesicular vesicles (LMVVs) remotely loaded with bupivacaine (LMVV liposomal bupivacaine) and demonstrated a 5-fold prolongation of analgesic effect in animals and humans. The aim of the current study was to investigate the pharmacokinetics of LMVV liposomal bupivacaine in humans.

Methods. Healthy volunteers received subcutaneous injections of 20 mL plain 0.5% bupivacaine and, 1 week later, 20 mL of 2% LMVV liposomal bupivacaine in a prospective, open-label, crossover, controlled study. The study was approved by Hadassah Medical Center Helsinki Committee.

Results: Eight subjects were studied. No subjective side effects of local anesthetics were observed. The maximal plasma concentration and the time to achieve maximal plasma concentration were assessed by modeling plasma concentration–time profiles. Maximal plasma concentration was not significantly different between groups (0.87±0.45 g/mL and 0.83±0.34 g/mL for plain and liposomal bupivacaine, respectively; P=0.83). These values are well below the putative bupivacaine toxic plasma concentration (2 to 4 g/mL). Time to achieve maximal plasma concentration was 7-fold greater for the liposomal preparation (262±149 minutes vs 37.5±16 minutes, P<0.01).

Conclusion: Peak plasma bupivacaine concentrations were not different between the 2 groups, despite a 4-fold increase in total bupivacaine dose administered in the novel liposomal preparation. The delayed elimination and prolonged redistribution of liposomal bupivacaine to plasma is compatible with the depot-related slow-release effect leading to the prolonged pharmacodynamic effect previously reported.
Neuropathic rats have an impaired performance in the reversal steps of an attentional set-shifting task

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Background and Aims. Chronic pain is frequently accompanied by mood disorders and cognitive impairments. The neural substrates underlying these altered behaviours are not fully understood but recent research indicates that the prefrontal cortex is a central player in the phenomena as structural and functional alterations have been described in this area following chronic pain. To test this hypothesis, we have tested the performance of neuropathic rats in an attentional set-shifting task (ASST), the gold standard behavioral test for PFC performance.

Methods. An experimental neuropathy was induced in one group of rats. Under deep anaesthesia, two sciatic branches were tightly ligated and distally sectioned sparing the sural. Similar procedures were repeated in controls except that all nerves were left intact. All animals developed allodynia, a hallmark of ongoing neuropathy. 1 month later animals were tested in the ASST in which the animal has to attend to one of the dimensions of odour/texture pairs to discriminate between baited and unbaited pots, in a series of 8 tasks. In the first four tasks odour is the relevant dimension, the relevant stimulus being reversed from the first to the second (A relevant/B irrelevant - B relevant/A irrelevant); the other 2 being similar but with a pair of different odours. The last 4 tasks are similar, but with texture as the relevant dimension. The criteria for a successful task consisted in a series of six consecutive correct trials.

Results. When a new pair of stimuli was introduced but the relevant dimension was kept the same (intradimensional shift), controls and SNI animals performed similarly. The same occurred when the relevant dimension shifted from odour to texture (extradimensional shift), although both groups increased the number of trials needed to fulfil the criteria. Importantly, differences between groups were only apparent in the 4 reversal tasks, SNI systematically performing worse than controls.

Conclusions. This selective impact of the neuropathy on the reversal component of the ASST test confirms a deficit of PFC function after nerve lesion.
Susceptibility to chronic pain following nerve injury is genetically controlled by CACNG2

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Background. Previous studies have mapped on mouse chromosome 15 the pain1 QTL affecting predisposition to neuropathic pain. We here present the identification path of the pain1 underlying gene.

Methods. 1. The pain1 mapping interval was refined using the Recombinant Progeny Testing and Recombinant Inbred Segregation Test strategies. By RPT, the pain1 was located relative to individual recombination points obtained by backcrossing C58/JxC3H/HeN (low vs. high autotomy mouse strains). By RIST its location was determined relative to recombination points defined in the BXA7 and BXA8 RI, strains. 2. Fully co-segregating pain susceptibility/tolerance SNPs were selected in-silico using sequence information for 7 inbred strains with established pain phenotype. 3. Candidate genes in the pain1 region were identified by whole-genome gene expression analysis of the L5 DRG: gene expression levels were compared in 5 different strains for nerve injured (L5 spinal nerve transection) versus sham operated and for high vs. low autotomy strains in nerve injured mice. 4. The elected gene Cacng2 was tested using its natural KO (stargazer) by pain behavior segregation analysis and electrophysiological analysis of spontaneous ectopic discharge generated in afferent neurons axotomized by nerve injury. 6. Finally, an association analysis of human CACNG2 polymorphisms with chronic pain was conducted in a 549 post mastectomy cohort using 12 SNPs covering exons and regulatory regions.

Experiments were approved by the Institutional Animal Care and Use Committee.

Results. By RPT and RIST we mapped pain1 the 76.5 - 78.9 Mb region. In this interval only 23/78 genes showed full co-segregation with the pain phenotype. In the mRNA profiling, 17 genes showed a transcript fold change >1.5 (p<0.001) when comparing nerve injured vs. sham operated mice, and 4 in the high vs. low strains comparison. Only Cacng2 met all our screening criteria. The phenotype split between stargazer genotypes (stg/stg and either stg/+ or +/+3) was highly significant (p≤0.00022) and the electrophysiology measurements demonstrated almost double ectopic discharge in the stg/stg compared to the stg/+ mice, 12.2% vs. 6.9%, respectively (p=0.05).

In humans, the ACC haplotype at the rs4820242, rs2284015 and rs2284017 SNPs significantly increased susceptibility to pain (OR=1.65; p=0.001).

Conclusions. Identification of Cacng2 as a pain gene provides additional support to a genetic control of susceptibility to chronic pain and it provides a solid basis for understanding pain physiology.

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Analgesic and antipyretic properties effects of ethanolic extract of tapinanthis dodoneifolius leaves in rats

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Background and Aim: *Tapinanthis dodoneifolius* is a parasitic plant that is used frequently for the treatment of several ailments such as stomach ache, diarrhoea, wounds and pain (Gill, 1992). Therefore the present study was carried out to validate the analgesic and antipyretic activities of the ethanolic extract of the leaves of the plant

Methods: The Hot plate latency assay and Formalin-induced paw licking models were used to evaluate analgesic activities (Owoyele et al., 2007) while Brewer’s yeast model was used to induced pyrexia. Animals were divided into groups comprising of five rats each. There were control (administered saline) and reference (administered indomethacin) groups. Also there were three extract groups administered 25, 50, 100 or 200 mg/Kg body weight of extracts. Different sets of rats were used for the inflammatory studies although animal grouping for extract administration was as in analgesic studies.

Results: The result show that all the extract of *T. dodoneifolius* significantly (P<0.05) inhibited the pain induced by pre heated hot plate and formalin injection. Likewise the results show that the extract produced significant antipyretic activity in the Brewer’s yeast test.

Conclusion: In conclusion the findings in the present study have confirmed the analgesic and anti-pyretic effects of *T. dodoneifolius*.

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References
Synthesis, transport and neuronal sorting of SDF-1 / CXCR4 at the spinal level: implications in nociception

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Background and Aims. The chemokine CXCL12, formerly named Stromal cell Derived Factor-1 (SDF-1) and its cognate receptor, CXCR4, have been postulated to play a role in pain modulation. However the expression, transport and axonal sorting of neuronal SDF-1 and CXCR4 in nociceptive structures as well as their participation in the modulation of nociception remains unknown.

Methods. Adult male Sprague Dawley rats were used. The cellular and subcellular distribution of SDF-1, CXCR4 and calcitonin gene-related peptide (CGRP) at the spinal level was studied in free floating sections stained with polyclonal antibodies. Fluorescence and electron microscopic observations were realized. For behavioral studies, animals received an intrathecal injection of saline, SDF-1 or SDF-1 plus a CXCR4 antagonist (AMD3100). The mechanical hypersensitivity was measured with dynamic Von Frey device. The molecular changes induced in dorsal root ganglia (DRG) and spinal cord induced after the injection of SDF-1 were measured by RT-PCR and western blot.

Results. Immunohistochemistry study revealed a highly correlated distribution of SDF-1 and CXCR4 with CGRP in DRG neurons and their central and peripheral terminals. We provided ultrastructural evidence that SDF-1 is sorted into synaptic vesicles, is neurosecreted by sensory nerve endings and that CXCR4 may serve autoreceptor functions in the dorsal spinal cord. Interestingly, double immunogold labeling revealed a partial colocalization of SDF-1 and CGRP in axonal terminals in the dorsal spinal cord. Behavioral studies revealed that single intrathecal administration of SDF-1 in naïve animals induced a significant mechanical hypersensitivity partially antagonized by AMD 3100. In vivo, exogenous SDF-1 activated the mitogen-activated protein kinase Erk and induced expression of an inflammatory cytokine interleukine 6 in both the rat spinal cord and DRG. These SDF-1-induced effects were further observed in vitro in DRG primary cell cultures.

Conclusions. These morphological and physiological data evidence a trafficking of SDF-1 and CXCR4 in nociceptive fibers, a neurosecretion of SDF-1 in the spinal cord and comfort a participation of SDF-1/CXCR4 in the modulation of nociceptive signaling.
Role of corticotropin-releasing factor and urocortin 1 systems in pain-induced maladaptation and comorbid diseases

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Background and Aims. Neuropathic pain patients have a high incidence of depression and anxiety and although the precise etiology of these pain-related mood alterations is not known, there is evidence for an involvement of the corticotropin-releasing factor (CRF) system. We hypothesize that neuropathic pain disrupts the homeostatic functioning of the CRF system of the hypothalamic paraventricular nucleus (PVN), the bed nucleus of the stria terminalis (BST), the central amygdala (CeA), and the urocortin 1 (Ucn1) system of the non-preganglionic Edinger-Westphal nucleus (npEW), and consequently will trigger stress-related symptoms of depression and anxiety. We furthermore explore the response of these brain regions to acute pain stress (APS) to elucidate the signaling pathways activated by this pain stressor.

Methods. Neuropathic pain is induced in rats by chronic constriction injury (CCI) of the sciatic nerve. Rats are tested for hyperalgesia/allodynia and anxiety/depression. For studying APS, rats are injected with 50 µl formalin into the hind paw. Brains are analyzed by quantitative immunohistochemistry, in situ hybridization and Western blotting.

This work is approved by the Radboud University Nijmegen Animal Care Ethics Committee.

Results. CCI leads to marked cold allodynia 24 days post-surgery and increased CRF mRNA in the CeA and BST and higher CRF-immunoreactivity in the CeA. There are no changes in CRF and CRF mRNA in the PVN. Also, the npEW does not differ in Ucn1 and Ucn1 mRNA.

Upon APS, corticosterone rises and both the PVN and the npEW are activated, as shown by an increase in cFos-positive cells. In the npEW, Ucn1 and Ucn1 mRNA contents peak 1-2 h after APS initiation. CRF and CRF mRNA contents in the PVN, BST and CeA are currently being evaluated. DCLK-short and -long splice variants are present in both npEW and PVN neurons, but only DCLK-long is phosphorylated. APS increases this phosphorylation in the npEW but not in the PVN.

Conclusion. Our results indicate that neuropathic pain-like behavior in rats leads to a habituated stress response with persistent increases in CRF in the CeA and BST, possibly leading to symptoms of depression and anxiety. APS, however, activates the PVN and npEW, but to different degrees and via different signaling pathways, with DCLK being involved in intracellular signaling in the npEW. Taken together, our data show that acute and chronic pain comprise separate signaling cascades in different, stress-sensitive brain nuclei.
Evaluation of the effects of a new analgesic on ventilation in healthy subjects

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Background and Aims. The effect of opioids has been studied in a model developed at Leiden University Medical Center (LUMC) to detect respiratory depression. Using this model of respiratory depression, the clinical Phase I trial described here assesses the safety profile of a newly developed analgesic compared to fentanyl in terms of its effect on ventilation. In addition to discussing the experimental setup and initial results obtained, the aim of the presentation is to highlight the challenges of a clinical Phase I trial in this setting, and to develop strategies to overcome these challenges.

Methods. A randomized, open-label, single center Phase I trial using healthy human subjects is being conducted to evaluate the effect of a new analgesic based on a respiratory depression model (2-way crossover; N=12; treatment: new analgesic; fentanyl). Data from a respiratory depression model will be compared with pain thresholds and tolerance levels evaluated by means of an electrical pain test and pupillometry in order to compare the effect of the applied substances. Different Clinical Research Organisations are being utilized. The Central Committee on Research Involving Human subjects and the Ethics Committee of the LUMC approved the trial.

Results. The trial is being conducted in accordance with laws and regulations, and in compliance with the requirements of Clinical Trials Directive by EC 2001/20/EC. In addition to the documents routinely used in clinical trials, several tools such as technical and operational manuals were created in cooperation with all parties involved to conduct, manage and monitor the trial. The trial was launched in March 2010 and initial results will be discussed. Data obtained on the basis of the respiratory depression model will be compared with pain thresholds and tolerance levels in order to characterize the pharmacodynamic parameters of the new analgesic.

Conclusions. In addition to the safety and tolerability profile for newly developed opioid analgesic drugs, the characterization of potential advantages in terms of their pharmacodynamic or safety parameters is essential. Grünenthal GmbH will be able to gain valuable insight about this new substance with respect to the undesired respiratory depression side-effect through their collaboration with an academic institution specializing in this area of expertise.

Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans

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Background and Aims. Although evidence shows that several dopamine neurotransmission pathway genes are associated with specific clinical pain syndromes, such as fibromyalgia, chronic headache, and postoperative pain, the exact role of dopamine in pain processing is not fully understood. The aim of this study was to explore the relationship between functional polymorphisms in dopaminergic candidate genes and sensitivity to pain in healthy subjects.

Methods. Healthy subjects (n = 192; 105 F, 87 M) were exposed to experimental tonic cold pain (1° C) and phasic heat pain (47° C) stimuli. DNA samples were obtained from both participants and their parents. The relationships between pain response (intensity in response to heat and cold; threshold and tolerance in response to cold only) and the functional Variable Number of Tandem Repeat (VNTR) polymorphisms of three dopamine-related genes were investigated using a Transmission Disequilibrium Test (TDT).

Results. Specifically, 30-bp repeat in the promoter region of the monoamine oxidase-A gene (MAO-A), 40-bp repeat in the 30-untranslated region of the dopamine transporter gene (DAT-1), and 48-bp repeat in the exon 3 of the dopamine receptor 4 gene (DRD4) were examined. Significant associations between cold pain tolerance and DAT-1 ($p = 0.008$) and MAO-A ($p = 0.024$) polymorphisms were found. Specifically, tolerance was shorter for carriers of allele 10 and the rarer allele 11, as compared to homozygous for allele 9, and for carriers of allele 4 as compared to homozygous for allele 3, respectively.

Conclusions. These results, together with the known function of the investigated candidate gene polymorphisms, suggest that low dopaminergic activity can be associated with high pain sensitivity and vice versa.
Nociceptive behaviour in mutant mouse models related to psychosis: focus on neuregulin-1 and catechol-O-methyltransferase

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Background and Aims. Experimental studies have reported altered pain sensitivity in patients with schizophrenia; a recent review found that 16 of 19 studies reported a significant abnormality in pain or thermal perception in patients with schizophrenia [1]. The catechol-o-methyltransferase (COMT) and neuregulin [1] (NRG1) genes have been implicated as risk factors for schizophrenia and may also play a role in pain modulation. The aims of this study were to (a) systematically assess and compare thermal pain sensitivity in mice with full or partial knockout of the COMT and NRG1 genes, respectively, and (b) examine the anti-nociceptive effects of acute systemic Δ⁹-tetrahydrocannabinol (THC) in both mutant models.

Methods. Pain sensitivity was measured for each mutant line using the tail-flick and hot-plate tests. To determine the effect of an acute dose of THC on pain sensitivity in these mutant models, pain sensitivity was measured 20 minutes following the subcutaneous administration of THC (8mg/kg) or vehicle.

Results. Mice with loss of COMT activity displayed increased thermal pain sensitivity on the tail flick test but this was not apparent on the hot plate test. In contrast, heterozygous deletion of NRG1 [homozygous KO being lethal] was associated with reduced pain sensitivity on both tail-flick and hot-plate tests. THC had an anti-nociceptive effect on both tests across both mutant lines.

Conclusion. Reduced reactivity to pain in schizophrenia has been proposed to represent an important clinical endophenotype. COMT and NRG1 genes have both been implicated in schizophrenia, as well as playing a role in pain modulation. In this study, a putative relationship between reduced NRG1 activity and reduced pain sensitivity may have been identified and the findings indicate a differential contribution of COMT and NRG1 genes to the processing of nociceptive stimuli.

Central mechanisms and neurochemistry involved in placebo analgesia and nocebo hyperalgesia

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Background. Pain perception and the processing of nociceptive input is subject to substantial pro- and antinociceptive modulation. A powerful clinical example of the cognitively triggered modulation of pain perception is the placebo analgesia (PA). Therefore, PA has been increasingly studied in the past years and our knowledge of their underlying neurobiology has significantly advanced in conjunction with the advent of brain imaging tools. However, there are open questions that still need to be answered. Although PA has been shown to rely on the endogenous opioid system in some cases, pharmacological studies have convincingly identified opioid independent components of PA. Accordingly, the contribution of other neurotransmitters known to be involved in pain modulation, such as serotonin and dopamine, needs to be ventured. In contrast to PA, nocebo hyperalgesia (NH) which represents the aggravation of pain by negative beliefs or expectations regarding a treatment, is much less studied. Clinical studies suggest that nocebo effects contribute substantially to the development of adverse effects in clinical trials and medical care, however, the underlying brain mechanisms are largely unclear. One pioneering fMRI study of NH points to the predominant involvement of affective-cognitive pathways and the hippocampus in its mechanism (Kong et al., 2008). This pattern is in line with the concept that anxiety plays a major role in nocebo responses.

Aims. During my PhD studies I will perform a series of pharmacological and Neuroimaging studies to elucidate the underlying neurobiology of PA and NH. Specifically, I will perform three different experiments:
Study 1: The first study will investigate the underlying brain mechanisms of NH and the specific contribution of anxiety to its mechanisms. Therefore we will apply a local NH model and experimentally modulate the factor anticipatory anxiety and examine brain mechanisms using fMRI. (Ethics committee approval is at hand)
On the neural level, we expect the involvement of the descending pain modulatory system that will be tuned by emotional regions such as the amygdala and the hippocampus.
In studies 2 and 3, we will use pharmacological modulations to elucidate the role of the endogenous dopaminergic system for PA and NH. We will use an established (and published) local placebo or nocebo model and apply the D2-antagonist haloperidol in 50% of the participants using a double blind design. (Ethics committee approval is awaited)
The influence of vitamins B on immunological system and pain in patients with low back pain

N.P. Yavorska
Lviv National Medical University named after Danylo Halytsky, Assistant Professor

Background and Aims: to determine the immune reactions and pain dynamics in patients with low back pain due to degenerative disk disease (DDD).

Methods: general and neurological examination, McGill Pain Questionnaire, lateral and straight X-ray examination of lumbar part of spine, CT and MRI of lumbar part of spine, investigation of immune system status (CD3, CD4, CD8, CD19, CD16, CD95, IgA, IgM, IgG). 40 patients (age 31 to 52 years) with low back pain were examined before and after (on the 7th day) standard conservative treatment (analgetics, nonsteroidal anti-inflammatory drugs, muscle relaxants) and standard conservative treatment with vitamins (B1, B6, B12) per os additionally. 30 healthy volunteers were in the control group. The study received ethics approval from the medical ethics committee of Lviv medical university, Ukraine.

Results: The following figures were significant (?<0,05) low before treatment: CD3- 1,00±0,05 G/l, CD4-0,58±0,03 G/l, another ones were high (?<0,01)- IgM- 1,94±0,14 gr/l, IgG- 17,95±0,95 gr/l. Other data was not significantly different.

After standard conservative treatment (20 patients) the low levels of CD3 (0,92±0,08 G/l), CD4 (0,50±0,04 G/l) and CD8 (0,38±0,02 G/l) were revealed. The quantity of IgM was still high (1,66±0,20 gr/l). The following figures were not significantly different. The figures of McGill Pain Questionnaire were decreased: rank index of pain from 25,85±1,90 to 19,00±1,93 (?<0,05), number of descriptors from 11,80±0,96 to 9,40±0,88.

After conservative treatment with vitamins (20 patients) almost all parameters normalized and were not significantly different from control group. Although the quantities of IgM and IgG were also still high (1,69±0,14 gr/l and 17,89±1,24 gr/l accordingly, ?<0,01). The figures of McGill Pain Questionnaire were decreased: rank index of pain from 26,25±1,43 to 17,50±2,46 (?<0,01), number of descriptors from 11,25±0,77 to 9,05±0,91.

Conclusions: The presence of significant immunologic changes in patients with low back pain due to DDD in acute period suggests their participation in pathogenesis of DDD. The vitamins B has a immunocorrective effect in these patients and are favorable to pain decrease, so they can be helpful for treatment of this disease.
Posters
The effect of protein kinase C inhibition on NMDA receptor phosphorylation during diabetes in the rat

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Background and Aims. Chronic pain is one of devastating syndromes typical for diseases accompanied by chronic inflammation and neuropathy of different origins. Although in most cases chronic pain is originated by peripheral damage, the following central sensitization plays no less decisive role. Therefore, the prevention of central sensitization might be beneficial effect on pain intensity.

The high level of phosphorylated NMDA receptor in spinal cord is referring to state of central sensitization. Rate of phosphorylation is possibly molecular substrate of pain intensity. Prevention of NMDA receptor phosphorylation by therapeutical agents might be the goal of therapeutical intervention. The aim of this study is to investigate whether protein kinase C inhibition can decrease the level of phosphorylation of NMDA receptors during diabetic neuropathy.

Methods. NMDA receptor has different sites of phosphorylation and for our study we selected Serine 896 of NR1 subunit, which has a protein kinase C dependent phosphorylation. The level of phosphorylated Serine 896 NR1 of NMDA receptor was measured by western-blotting.

All experiments were conducted on male rats 3-5 months aged. Diabetes was induced by single intraperitoneal injection of streptozotocin with dosage 55 mg/kg.

The phosphoprotein level was measured in L4-L5 spinal cord segments of diabetic and healthy rats after sham and formalin intraplantar injection. As a protein kinase C inhibitor is used chelerythrine hydrochloride.

Results. The basal level of phosphorylated NMDA receptor during diabetes was increased compared with norma by 60%. The formalin injection induced elevation of phosphorylated NMDA by 115% and 62% in spinal cord of healthy and diabetic rats consequently, whereas the increase induced by injection of formalin after chelerythrine pretreatment was 79% and 31% consequently.

Conclusion. The NMDA receptor phosphorylation at protein kinase C dependent site in spinal cord is increased after noxious stimulation and during diabetes. This increase might be partially prevented by protein kinase C inhibitor treatment. So inhibition of protein kinase C might be the goal of therapeutical intervention in order to alleviate central sensitization.
Clinical evaluation of molecular mechanisms involved in degenerative disc related pain

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**Background and Aims.** Chronic neuropathic pain (CNP) is a complex and extensive condition secondary to tissue damage. Previous studies in animals have shown that TNF-\(\alpha\) is a key player to develop inflammation and pain in the dorsal root ganglion and dorsal horn [1]. Our objectives are to clarify the participation of peripheral inflammation induced by TNF-\(\alpha\) in disc hernia patients with CNP; to elucidate the role of this agent in apoptosis in the dorsal horn; and to propose novel analgesic therapeutics for this condition.

**Methods.** Patients diagnosed with lumbar disc hernia (n=10) and control patients that underwent back surgery without inflammatory disease and no history of pain (n=5) were included in this study. Different tissues including muscle, ligamentum flavum, annulus fibrosus and nucleus pulposus were collected in order to perform immunoblotting and qPCR to search for TNF-\(\alpha\). Clinical pain assessment with the visual analogue scale (VAS) was applied before surgery and 6 weeks after the procedure to correlate it with laboratory findings.

**Results.** Preliminary data show no differences between disc hernia patients and controls for immunoblotting in muscle, ligamentum flavum and annulus fibrosus. However, higher densities in nucleus pulposus were detected on the patients with higher VAS scores at 6 weeks follow-up. No correlation of TNF-\(\alpha\) levels was found between black disc disease (n=3) and sequester type of hernias (n=4) with clinical outcome. At present, qPCR techniques are being applied to validate blotting results.

**Conclusion.** The presence of nucleus pulposus with higher levels of TNF-\(\alpha\) in a chronic exposition probably determines the clinical outcome independently of the surgical performance. Inflammation could determine not only the presence of pain but also the outcome even when proper treatment is applied. Supported by VENI-Laureaat NOW grant.

**References.**
Bioinformatic and biochemical studies on the phylogenetic variability of proenkephalin-derived octapeptides

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Leu- and Met-enkephalins are proenkephalin-derived endogenous pentapeptides with opioid (morphine) activity. Among the seven enkephalin units found in the human proenkephalin (PENK), the fourth copy being an octapeptide: Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu (HsYGGFMRLG). Bioinformatic analysis of the available PENK sequences revealed the presence of other octapeptide orthologues in these precursor polypeptides.

Four types of the elongated Met-enkephalins we identified by searching protein databases, XlYGGFMRGY (three frog species and platypus), GgYGGFMRSV (chicken and one fish species), HpYGGFMNGF (shark) and MmYGGFMRSLS (mouse and two lungfish species) were chemically synthesized and studied in receptor binding and G-protein activation assays performed on rat brain membranes. All peptides have also been prepared containing oxidized methionine (M(O)). The overall binding and signalling profile of the novel octapeptides revealed moderate opioid agonist activities and a rank order of potencies for the mu = delta >> kappa receptor binding sites. Peptides with the oxidized M(O) residue were found to be less potent in both receptor binding and G-protein stimulation studies. Phylogenetic neuropeptide libraries, defined here as a collection of mutationally different species variants of orthologous and paralogous peptide sequences, represent the natural molecular diversity of the neuropeptides. Such libraries can provide a wide range of structural information establishing comparative functional analyses. Since DNA sequencing data are rapidly increasing, more development in the natural peptide library approach is expected.
Mechanisms involved in chronic neuropathic pain after spinal root avulsion injury

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Background. Vehicle accidents are the most common cause of avulsion of nerve roots from the spinal cord. This results in chronic intractable pain, refractory to pharmacotherapy. Poor therapeutic outcome is largely due to lack of information on underlying mechanisms, and an established animal model to test potential treatment options. Dorsal root rhizotomy (DRR) is commonly used to model avulsion injury but does not damage the spinal cord, as often happens clinically. The neuroanatomical effects of DRR and avulsion (DRA) in the spinal cord have been compared. Secondly, design of a more clinically representative behavioural model of spinal root avulsion injury (SRA) has been assessed. Finally, therapeutic efficacy of drugs prescribed to treat general neuropathic pain, and those used to treat conditions like motoneurone disease and spinal cord injury has been assessed.

Methods. Rats underwent L3-L6 DRR or DRA and assessed histologically at time points post injury. Antibodies specific to glia, blood vessels, neurones, leukocytes, and apoptosis were applied to spinal cord sections and quantitatively analysed. Additional animals underwent L5 SRA, and behaviourally assessed for the development of neuropathic pain, using tactile and thermal plantar probes. SRA animals were treated with an antidepressant (Amitriptyline), anticonvulsant (Carbamazepine), anti-inflammatory (Minocycline), and anti-excitotoxic (Riluzole) agents, for therapeutic assessment.

Results. DRA produced a greater and prolonged gliosis, inflammatory response, and vascular and neuronal loss than DRR. SRA induced neuropathic pain behaviour. Neuroinflammatory responses were observed in the adjacent L4 spinal segments, but not in the L4 DRG neurones, suggesting the pain is mediated centrally within the intact spinal cord. Amitriptyline or carbamazepine transiently ameliorated pain, confirming their limited efficacy seen clinically. Minocycline prevented pain development correlated with microglial/macrophage inhibition. Encouragingly, it reversed established pain, persisting beyond drug wash-out period. Riluzole had a limited histological and behavioural therapeutic efficacy.

Conclusion. This work outlines the mechanisms of avulsion-induced pain. The establishment of a behaviourally reproducible animal model finally provides a platform to test pharmacological candidates for treatment.

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- Work carried out in accordance with the Animal Scientific Procedures Act (1986)
A role for the scaffolding molecule PSD-95, which assembles signalling complexes with glutamate receptors, in chronic pain

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Background and Aims. Chronic pain is a pervasive clinical condition that lacks adequate therapeutic treatment, making the identification of novel targets for drug development a priority. Underlying the development of this pathological pain state is a process of neuronal plasticity that results in hyperexcitability of sensory neurons in the spinal cord. Chronic pain hypersensitivity can be dependent on NMDA receptors however use of NMDA receptor antagonists in the clinic is limited because of adverse side effects. The aim of this study was to investigate the role of post synaptic density 95 (PSD-95), a scaffolding molecule in the NMDA receptor complex, in chronic pain.

Methods. The role of the Src homology 3 (SH3) domain of PSD-95 was investigated using mice carrying a single point mutation in the polyproline binding site of the SH3 domain. Adult mutant and wild-type mice behavioural responses were assessed in two models of inflammatory pain; a) formalin and b) Complete Freund’s Adjuvant (CFA)-induced inflammation and in the chronic constriction injury (CCI) model of neuropathic pain. Behavioural reflex sensitisation following the intrathecal administration of inhibitors was assessed in wild-type mice. Co-immunoprecipitation of PSD-95 complexes was carried out to identify protein-protein interactions underlying the behavioural responses.

Results. Development of neuropathic behavioural reflex sensitisation was intact in mutant mice, with sensitisation to inflammation specifically attenuated, thus indicating a particular role for the SH3 domain in response to inflammation. The molecular mechanism of this response was found to involve the recruitment of phosphoinositide 3-kinase-C2alpha (PI3K-C2α) to PSD-95, which was increased following inflammation in wild-type mice and absent in mutant mice. Administration of wortmanin (a PI3K-C2α inhibitor) or a PI3K-C2α decoy peptide in wild-type mice blocked inflammatory induced sensitisation.

Conclusions. These results show peripheral tissue damage produces a neuronal response in the spinal cord involving the recruitment of PI3K-C2α to the SH3 domain of PSD-95. Blocking this recruitment with either a mutation or a peptide competitor attenuates the expected sensitised response, as does the administration of a PI3K-C2α inhibitor. This study suggests that PI3K-C2α is a potential therapeutic avenue for chronic pain of inflammatory origin.

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Botulinum toxin A in a rat model of migraine and/or trigeminal neuropathy

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Background and Aims. Botulinum toxin A is used in treating painful states associated with muscular overactivity. Its use was also reported to be useful in relieving symptoms of chronic pain states like tension-type headaches, peripheral neuropathies and migraine. In this experiments we wanted to assess the effects of a single unilateral injection of botulinum toxin type A (BoNT/A, 3.5 U/kg) on mechanical allodynia and dural extravasation induced by infraorbital nerve constriction injury (IoNC) in rats.

Methods. Male Wistar rats were operated and the infraorbital nerve constricted. After two weeks BoNT/A was administered into the whisker pad either ipsilaterally or contralaterally to the side of nerve injury. In one experiment the effect of BoNT/A on bilateral pain was assessed for a period of one month by measuring mechanical allodynia using the von Frey filaments. In a second experiment, animals which developed mechanical alldynia were administered with Evans blue intravenously and dural extravasation of Evans blue - plasma protein complexes was measured. The experiments were approved by the Ethical Committee of the University of Zagreb, School of Medicine (permit No. 07-76/2005-43).

Results. Mechanical alldynia was reduced by a single BoNT/A injection on the side of the toxin injection, and on the opposite side, too. The antinociceptive effect started on day 3 following the toxin application and lasted for at least 17 more days. IoNC-induced neuropathy elicited significant bilateral increase in dural extravasation of plasma proteins. Additionally, BoNT/A completely abolished increased dural extravasation on both sides. BoNT/A was effective on both allodynia and dural extravasation regardless of whether the site of injection was ipsilateral or contralateral to the site of nerve injury.

Conclusion. Lasting reduction of bilateral mechanical alldynia by a single peripheral unilateral BoNT/A injection, together with the abolished dural protein extravasation, imply central site of the BoNT/A antinociceptive actions.

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Sigma-1 receptors are involved in the visceral pain induced by intracolonic administration of capsaicin in mice

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Background and Aims. Visceral pain is the most common form of pain produced by disease, and one of the most frequent reasons why patients seek medical attention. We have reported that Sigma-1 (σ1) receptors play a key role in different modalities of somatic pain, but it is almost unexplored whether these receptors have any role in visceral pain. To test this possibility we performed studies on an intracolonic-capsaicin administered model.

Methods. Wild-type (WT) and σ1-receptor KO female CD-1 (24-30 g) mice were used. Mice were always handled in accordance with ethical principles for the evaluation of pain in conscious animals (Zimmerman, 1983) and with the European Communities Council Directive of 24 November 1986 (86/609/ECC). For behavioural testing, mice were placed on a raised grid and 0.05 ml of vehicle or capsaicin (0.01-1%) was administered by inserting a fine cannula into the colon via the anus. We recorded both behavioural responses to chemical stimulation of the colon and responses to mechanical stimulation of the abdomen using Von Frey hairs. Visceral pain-related behaviours were immediately counted for 20 min after intracolonic (i.cl.) administration of capsaicin. Referred hyperalgesia was quantified 20 min after the i.cl. instillation of capsaicin by measuring the response to the stimulus with the Von Frey hair and thresholds were measured using the up-down paradigm.

Results. The i.cl. administration of capsaicin induced dose-dependent visceral pain behaviours and referred hyperalgesia in WT mice. The peak behavioural responses and maximal referred hyperalgesia were evoked by 1% and 0.3% capsaicin, respectively in WT mice. σ1-receptor KO mice showed a significant reduction in the number of pain-related behaviours, but they presented a very similar response to mechanical stimulation with von Frey hairs. The selective σ1-receptor antagonist BD-1063 administered subcutaneously 30 min before i.cl. capsaicin, dose-dependently inhibited the behavioural responses and the referred hyperalgesia in WT. In contrast, BD-1063 did not affect both pain behaviours and referred hyperalgesia in σ1-receptor KO mice in comparison with WT treated animals.

Conclusions. These results suggest that σ1-receptors could play a role in the mechanisms underlying capsaicin-induced visceral pain.

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Background and Aims. Sex hormones may have a pivotal role in mediating the sex differences in pain. A number of chronic pain conditions such as migraine, temporomandibular joint disorder, fibromyalgia, arthritis and interstitial cystitis are more prevalent in women than on men. Furthermore, research has suggested that the sex hormones may have a pivotal role in mediating the sex differences in pain. Experimental studies have demonstrated activational role of estrogen on pain behavior in female rats using the model of inflammatory pain. (Aloisi, Ceccarelli, 2000). However, critical role for progesterone but not for estradiol in mediating enhanced female tactile and thermal hypersensitivity in neurophaty pain following L5 nerve root ligation was established recently (Lacroix-Fralish et al., 2006). The involvement of sex steroids in modulation of pain sensitivity may depend on the context, dose, and length of exposure as well as highly dependent on genetic background and differences in pain models. The aim was to determine if gonadal hormones mediate pain sensitivity in rats with neuropathic pain.

Methods. In the first experiment mechanical allodynia following sciatic nerve ligation (SNL) was estimated in male and female rats on day 7 and 14 after surgery. Sex differences were observed in the magnitude of allodynia in rat: females demonstrated decreased thresholds to tactile and cold stimuli as compared to males. The aim second experiment was to determine the role of gonadal hormones in enhanced mechanical and cold sensitivity in female rats following SNL. Adult female rats were ovariectomized or sham-operated 6 weeks before SNL. First group of ovariectomized females was chronically treated with estradiol. Second group of ovariectomized rats and group of cycling females were treated with vehicle. Treatment started two days before SNL. The development of mechanical and cold allodynia was monitoring for eight weeks following SNL.

Results. Hormonally intact females demonstrated significantly lower withdrawal mechanical thresholds compared to ovariectomized females during all observation period. Furthermore, allodynic response to cold stimuli was significantly higher in both hormonally intact and estradiol-treated ovariectomized females compared to vehicle-treated ovariectomized females.

Conclusions. Our results suggest that the adult activational effects of female gonadal hormones mediate the enhanced female mechanical and cold sensitivity following SNL. Furthermore, increased female sensitivity to cold stimuli is modulated, at least in part, by estradiol. We are planned to increase number of animals in group for carrying out statistic analysis on a greater file data. Subsequently it is planned to carry out pilot research of a difference sensitivity painful syndromes in human.
Phenomics and QTL mapping of sensitivity to noxious heat and mechanical stimuli in naive A/J, C57BL/6J and their 23 AXB-BXA descendant recombinant inbred (RI) mouse lines

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Background and Aims. The 23 RI lines of mice of the AXB-BXA set were produced by crossing offspring of the inbred mice lines A/J & C57BL/6J. The aims of this study were to use the AXB-BXA RI set to identify: (i) QTLs for sensitivity to noxious heat & mechanical stimuli, (ii) gender & genetic differences across lines, (iii) if a rostrocaudal sensitivity gradient to these stimuli exists across body sites including the inner auricles, hindpaws, & dorsal/ventral tail.

Methods. 394 mice were tested for withdrawal response rates to a Von Frey hair (0.2g bending force). Each body site was tested 7 times & responses categorized as 0=no response, 1=threshold withdrawal, 2=a vigorous suprathreshold response. Brief noxious heat pulses (250msec) were delivered from a diode laser (980nm) to 294 mice, 3 trials/body site/side. Responses to stimulation of the inner auricles and tail were categorized using the same scale as above. For the hindpaw, the response time (sec) was measured from stimulus onset until the animal replaced the paw on the floor.

Results. No gender differences were found in mechanical and noxious heat sensitivities when the genetic background was not taken into consideration. However 3/25 and 8/25 lines showed significant body region differences in heat and mechanical sensitivity between genders, respectively. For both stimulus modalities, the inner auricle was the most sensitive, then the ventral tail, and lastly the hindpaw. The lines were ranked (per body site) based on sensitivity to these stimuli, revealing highly significant correlations between noxious heat sensitivity in the ear and tail; no significant correlation was found between the ear and hindpaw. Only the paw and tail were highly significantly correlated for mechanical sensitivity. No significant correlation was found between the two modalities in all tested body sites, indicating that sensitivity to noxious heat and mechanical stimuli are independent traits, justifying the use of each trait for QTL mapping. Mapping these traits using WebQTL software (www.genenetwork.org) revealed a number of suggestive QTLs. A QTL for noxious heat sensitivity in the ear was identified on chr. 3 (spanning from 125-135MBps), harbouring several candidate genes including Tacr3. A QTL for mechanical pain in the ear was identified on chr. 1 (spanning from 132-142MBps), harbouring the gene Nfasc.

Conclusion. Our data indicate that the AXB-BXA RI set offers robust tools for pain phenomics and mapping QTLs for mechanical and heat pain.

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Novel behavioral models for the assessment of the emotional component of pain and for spontaneous pain in rats

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Background and Aims: Current preclinical pain models concentrate on the sensory, rather than the emotional, component of pain and measure evoked, as opposed to spontaneous, pain. It was the aim of this study to develop rat models that reliably dissociate (a) the sensory from the emotional component of pain and (b) evoked from spontaneous pain.

Methods: Model 1: In order to dissociate the sensory from the emotional component of inflammatory pain (0.5% carrageenan intraplantar), we used a paw pressure test combined with conditioned place aversion and preference (CPP) paradigms, and tested the efficacy of morphine (0.01–10mg/kg i.p.) in each model. Model 2: In order to dissociate evoked from spontaneous pain we subjected neuropathic (chronic constriction injury, CCI) and sham-operated animals to a cold plate paradigm combined with a CPP paradigm and tested the efficacy of morphine (1–10mg/kg i.p.) in each model.

Results: Model 1: The minimal effective dose (MED) of morphine for producing anti-hyperalgesic effects was 1mg/kg, whereas the MED for anti-aversive effects was 0.03mg/kg, and the MED for producing CPP in carrageenan-treated and sham-treated rats was 10 and 1mg/kg, respectively. Model 2: The anti-allodynic MED of morphine was 3.16mg/kg, and the MED for producing CPP in CCI and sham-operated animals was 0.3 and 2.15mg/kg, respectively.

Conclusions: The present study suggests (a) that morphine more potently reduces the emotional than the sensory component of pain and that this differential sensitivity is not confounded by the intrinsic rewarding effects of the drug, and (b) that morphine more potently reduces spontaneous pain than evoked pain.
5-HT$_7$ receptor-mediated modulation of mechanical nociception in naïve versus neuropathic rats – Implication of Cl$^-$ transport dynamics

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**Background and aims.** Among 5-HT receptors, the excitatory 5-HT$_7$R subtype is expressed by both primary afferent fibers (PAF) and inhibitory interneurons in the dorsal horn of the spinal cord. Both locations are potentially critical for 5-HT control of nociceptive signalling. Our studies aimed at investigating which effects 5-HT$_7$R activation exerts on mechanical nociception in neuropathic- versus naïve healthy-rats.

**Methods.** Neuropathy was caused by unilateral chronic constriction injury to the sciatic nerve (CCI-SN), and nociception was assessed by determination of hindpaw pressure thresholds (PT) to trigger paw withdrawal (PW) and vocalization (V). Two weeks after CCI-SN, hyperalgesia was evidenced by significant decreases in PW-PT (-30%) and V-PT (-35%).

**Results.** In naïve rats, acute treatment with the 5-HT$_7$R inverse agonist SB269970 (3 mg/kg i.p.) significantly decreased PW-PT (-45%) and V-PT (-40%), whereas the 5-HT$_7$R agonist E-55656 exerted no effect on mechanical nociception. In contrast, in CCI-SN rats, SB269970 produced limited and transient increases in PT and E-55656 exerted marked anti-hyperalgesic effects that could be prevented by SB269970. Because Cl$^-$ transport dynamics through the sodium-potassium-chloride co-transporter NKCC1 differ markedly in neuropathic- versus naïve-rats, bumetamide, a specific blocker of NKCC1, was tested to assess whether the differential effects of 5-HT$_7$R ligands could be mediated through Cl$^-$ transport changes in CCI-SN- versus naïve-rats. On its own, intrathecal injection of bumetamide (1.75 µg/rat) did not modify PT values in both naïve and CCI-SN rats. However, pretreatment with bumetamide prevented totally both the anti-nociceptive effect of E-55656 in CCI-SN rats and the pronociceptive effect of SB269970 in naïve rats.

**Conclusions.** In PAF, NKCC1 activity is known to produce high [Cl$^-$]int and, in turn, shunt-mediated tonic presynaptic inhibition. In neuropathic rats NKCC1 overactivation has been reported to further elevate [Cl$^-$]int, which causes Ca$^{2+}$-dependent PAF excitation. Our results suggest that 5-HT$_7$R stimulation by E-55656 promotes PAF inhibition through a GABA-mediated stabilization of Cl$^-$ conductance, thereby exerting an antinociceptive action in CCI-SN rats. In contrast, the pronociceptive and antinociceptive effects of the 5-HT$_7$R inverse agonist SB269970 in naïve and CCI-SN rats, respectively, might be mediated through a reduction in PAF Cl$^-$ conductance.
Nucleotides excite sensory neurons via two P2Y receptors and a dual signaling cascade

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Background and Aims. Sensory neurons innervating the skin provide information about physical contact between organisms and the environment including stimuli that lead to pain sensation. Metabotropic P2Y receptors have been suggested to be important in the signaling of sensory neurons, but their effects and signaling mechanisms remained controversial.

Methods. Patch-clamp recordings were performed in primary cultures of dorsal root ganglion (DRG) neurons from neonatal rats. P2Y receptor ligands and signaling interceptors were applied.

Results. ADP (EC$_{50}$: 7.5 uM), ATP (EC$_{50}$: 0.5 uM), UTP (EC$_{50}$: 0.8 uM) and thio-UTP (EC$_{50}$: 0.4 uM) increased the number of action potentials fired in response to current injection; UDP failed to affect action potential firing. The effect of ADP was attenuated by a P2Y$_1$ antagonist (MRS 2179). This enhancement of excitability was abolished by flupirtine (30 uM), a Kv7 channel opener, and slightly, but insignificantly attenuated by iodoresiniferatoxin (0.3 uM). Under voltage clamp, the same nucleotides inhibited currents through Kv7 channels in a concentration dependant manner with similar EC$_{50}$ values. The P2Y$_1$ specific agonist MRS2365 also caused an inhibition of Kv7 channels (EC$_{50}$ value of 8.68 nM), and the P2Y$_1$ antagonist MRS2179 attenuated the inhibition by ADP. Treatment of sensory neurons with the phospholipase C inhibitor U73122, with the Ca$^{2+}$-ATPase inhibitor thapsigargin, or the Ca$^{2+}$ chelator BAPTA-AM abolished the inhibition of Kv7 channels by ADP. Moreover, ADP and tUTP increased the amplitudes of currents TRPV1 receptors evoked by capsaicin.

Conclusion. Activation of P2Y$_1$ and P2Y$_2$ receptors increases the excitability of sensory neurons via a dual mechanism: an inhibition of Kv7 channels via phospholipase C and increase in intracellular Ca$^{2+}$, and a sensitization of TRPV1 receptors, with the former mechanism being the decisive one.

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