European Pain School 2009

at the University of Siena, Italy

a PENS Summer School

Molecular Mechanisms of Pain Response

Venue: University of Siena, Tuscany, Italy
Certosa di Pontignano (historical site in Chianti)

Date: June 13 to 20, 2009

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

Programme Committee

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University of Siena
53100 Siena, Italy
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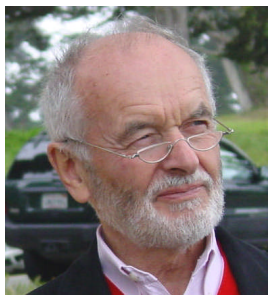
Prof. Marshall Devor
Dept. Cell & Animal Biology
Hebrew University
91904 Jerusalem, Israel
e-mail: marshlu@vms.huji.ac.il

Prof. Manfred Zimmermann
Neuroscience and Pain
Research Institute
69123 Heidelberg, Germany
e-mail: epsiena@aol.com



School Director

Prof. Anna Maria Aloisi
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Local Organization Team at the Dept. of Physiology, University of Siena, 53100 Siena, Italy

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The European Pain School appreciates partial funding by PENS,
Joint venture of IBRO, the International Brain Research Organization, and
FENS, the Federation of European Neuroscience Societies, www.fens.org/pens

Chairman of FENS School Committee

Prof. Roberto Caminiti, University “La Sapienza” Rome,
Dept. of Physiology and Pharmacology, Rome, Italy

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- Regione Toscana www.regione.toscana.it

- The University of Siena www.unisi.it

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.

The first School in 2003 was elected a FENS-IBRO Summer School by FENS, the Federation of European Neuroscience Societies. In 2009 the European Pain School again was awarded FENS School status and is receiving financial support. Additional funding was provided by an educational grant of Grünenthal.

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 thrilled by 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 extendings to the large round tables in the Cloisters 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

Aloisi Professor	Anna Maria	Dept. of Physiology University of Siena	Siena, Italy aloisi@unisi.it
Carli Professor	Giancarlo	Dept. of Physiology University of Siena	Siena, Italy carlig@unisi.it
Devor Professor	Marshall	Dept. of Cell & Animal Biology Hebrew University of Jerusalem	Jerusalem, Israel marshlu@vms.huji.ac.il
Kress Professor	Michaela	Dept. of Physiology, University of Innsbruck, Austria	Innsbruck, Austria michaela.kress@uibk.ac.at
Krishtal Professor	Oleg	Dept. of Cellular Membranology Bogomoletz Institute of Physiology	Kiev, Ukraine krishtal@biph.kiev.ua
Lötsch Professor	Jörn	Dept. of Clinical Pharmacology, University of Frankfurt	Frankfurt, Germany j.loetsch@em.uni-frankfurt.de
Malcangio Senior Lecturer	Marzia	Wolfson Ctr. for Age Related Diseases King's College, University of London	London, UK marzia.malcangio@kcl.ac.uk
Przewlocka Professor	Barbara	Institute of Pharmacology, Polish Academy of Science	Krakow, Poland przebar@if-pan.krakow.pl
Sapunar Professor	Damir	Dept of Anatomy, Pain Laboratory, University of Split Medical School	Split, Croatia ds@mefst.hr
Serra Professor	Jordi	Dept. of Neurology MC Mutual, Barcelona	Barcelona, Spain jserrac@meditex.es
Tölle Professor	Thomas	Dept. Neurology, Medical Faculty, TU Munich	Munich, Germany toelle@lrz.tu-muenchen.de
Wood Professor	John N.	Dept. of Biology University College London	London, UK j.wood@ucl.ac.uk
Zimmermann Professor Em.	Manfred	Dept. of Physiology, Medical Faculty, University of Heidelberg	Heidelberg, Germany epsiena@aol.com

European Pain School, Siena 2009
Programme

June 13, Saturday

11:00 - 17:00 Arrival of Scholars and Faculty

at the Certosa di Pontignano

15:00 - 17:00 Registration in the Certosa

18:00 - 19:00 Session I Opening Ceremony

Welcome Addresses

- Siena University, Prof. Alberto Auteri, President of Medical School
- FENS School Committee, Prof. Roberto Caminiti, Rome
- The European Pain School, Prof. Anna Maria Aloisi, School Director
- Pain in Europe, Prof. Marshall Devor, Jerusalem
- The Certosa di Pontignano, Prof. Giancarlo Carli, Siena
- Monasteries, Forerunners of Universities and Hospitals,
  Prof. Manfred Zimmermann, Heidelberg

Scholars' Self-introduction - to be continued informally

at the subsequent wine reception

Synoptic Table of the 30 Scholars see on page 13 f

Abstracts of Scholars' oral presentations see on page 17 f,

arranged in alphabetical order of Scholars' names

June 14, Sunday

Sunday, 9:00 – 12:45, Session II

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

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

Scholar 1: Glyceroltrinitrate facilitates stimulated CGRP release but not gene expression in rat trigeminal ganglia. Miriam Eberhardt, Erlangen, Germany

Scholar 2: Molecular mechanisms of sensitization of pain transducing receptors on mouse trigeminal neurons. Elsa Fabbretti, Nova Gorica, Slovenia, and Trieste, Italy

Scholar 3: Cortical spreading depression in transgenic migraine mouse models.
Reinald Shyti, Leiden, TheNetherlands

Discussions conducted by Faculty and Scholar

Sunday, 15:00 – 19:00, Session III

Lecture 3: Pain Studies on Humans: Insights from functional imaging with MRI and PET
Thomas Tölle, Munich, Germany

Lecture 4: Neuropathic Pain: The Role of Cytokines and the Immune system
Michaela Kress, Innsbruck, Austria

Scholar 4: Expression of Fractalkine and ILβ mRNA in rat brain following persistent and acute inflammatory pain. Helen Jerina, Bath, United Kingdom

Scholar 5: Role of the chemokine Bv8/PK2 in the neutrophil-dependent inflammatory pain as studied in rats and mice. Annalisa Nicotra, Rome, Italy

Scholar 6: Differential associations between brain 5-HT_{1A} receptor binding and response to pain versus touch in human subjects**.** Ilkka Martikainen, Turku, Finland

Discussions conducted by Faculty and Scholar

June 15, Monday

Monday, 9:00 – 12:45, Session IV

Lecture 5: Endogenous Modulators in Nociception: Facts, Myths and Mysteries
Oleg Krishtal, Kiev, Ukraine

Lecture 6: Receptor Proteins, Kinases and Cannabinoids in pain
Jörn Lötsch, Frankfurt, Germany

Scholar 7: Substance MCS-18 isolated from Helleborus purpurascens is a potent antagonist of the capsaicin receptor, TRPV1. Cristian Neacsu, Bucharest, Romania

Scholar 8: Regulation of KCNQ2/3 potassium ion channels in dorsal root ganglion neurons by the transcriptional repressor REST in nociception, studied in a rat model of neuropathic pain. Lezanne Ooi, Leeds, United Kingdom

Scholar 9: Distribution and neurochemistry of high affinity binding sites for Botulinum toxin type A in Rat, Guinea-pig and Human urinary bladder. Ana Coelho, Porto, Portugal

Discussions conducted by Faculty and Scholar

Monday, 15:00 – 19:00, Session V

Lecture 7: Targeting the Neurochemistry of Pain: Lessons learned from in vivo and ex vivo Imaging of the Human Brain. Thomas Tölle, Munich, Germany

Lecture 8: Chronic Widespread Pain - The Fibromyalgia Syndrome
Giancarlo Carli, Siena, Italy

Scholar 10: Peripheral NMDA receptors and Temporomandibular Disorder (TMD) pain mechanisms in human volunteers. Eduardo Enrique Castrillon, Stockholm, Sweden, and Trujillo, Peru

Scholar 11: The effect of peripheral inflammation on brain activity and pain sensitization, studied on animals and humans. Bianka Karshikoff, Stockholm, Sweden

Scholar 12: Effect of a sensorymotor training on pain and cortical reorganization in patients with complex regional pain syndrome (CRPS). Anne-Christine Schmid, Tübingen, Germany

Discussions conducted by Faculty and Scholar

June 16, Tuesday

Tuesday, 9:00 – 12:45, Session VI

Lecture 9: Neuropathic Pain: The point of view of a Neurologist. Jordi Serra, Barcelona, Spain

Lecture 10: Inflammatory Mediators, Viruses and Ca^{2+} in Nociceptor Excitation and Sensitization. Michaela Kress, Innsbruck, Austria

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Scholar 13: Functional and structural changes in central pain circuitry in patients with chronic pain and recurring herpes simplex virus infections. Nuutti Vartiainen, Helsinki, Finland

Scholar 14: Increased expression of neuronal injury markers in the DRG of osteoarthritic rats. Sara Adães, Porto, Portugal

Scholar 15: Peripheral nerve injury alters dopamine receptor numbers in the nucleus accumbens in a subpopulation of rats, which display sensory and affective disabilities. Paul J. Austin, Sydney, Australia, and London, United Kingdom

Group Discussions conducted by Faculty and Scholar

Tuesday, 15:00 – 19:00, Session VII

Lecture 11: Evaluating the pathological role of ectopic activity in neuropathic pain Jordi Serra, Barcelona, Spain

Lecture 12: What can rats tell us about neuropathic pain? Critical evaluation of behavioral tests used in rodent pain models. Damir Sapunar, Split, Croatia

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Scholar 16: Transplantation of cell/biomatrix complexes in experimental spinal cord injury. Ronald Deumens, Maastricht, The Netherlands

Scholar 17: Activation of signaling pathways in the dorsal root ganglia of mice following intense nociceptive stimulation. Vijayan Gangadharan, Heidelberg, Germany

Scholar 18: Serotonergic mechanisms are involved in pain relief with spinal cord stimulation: Study in an animal model of mononeuropathy. Zhiyang Song, Stockholm, Sweden

Discussions conducted by Faculty and Scholar

June 17, Wednesday

Wednesday, 9:00 – 12:45, Session VIII

Lecture 13: Endogenous opioids and opioid drugs - role for acute and chronic pain
Barbara Przewlocka, Krakow, Poland

Lecture 14: Drugs against pain – Pharmacokinetics, pharmacogenetics and drug development
Jörn Lötsch, Frankfurt, Germany

Scholar 19: Drug-drug interaction studies in healthy subjects evaluating the effects of commonly co-administered medications on the pharmacokinetics of Tapentadol, a novel centrally acting analgesic. Elke Kleideiter, Aachen, Germany

Scholar 20: The non-opioid analgesic Flupirtine is a modulator of ion channels in cultured rat primary sensory neurons. Felicia Popovici, Vienna, Austria

Scholar 21: Abnormalities of calcium signaling in sensory DRG neurons of rats with different forms of diabetic neuropathy: reversal effect of Nimodipine. Eugen V. Khomula, Kiev, Ukraine

Discussions conducted by Faculty and Scholar

Wednesday, 14:30 – 24:00 Excursion to Siena Old Town

14:30 Bus departs from the Certosa

15:00 – 17:00 Guided tour to historical Siena, City Hall and Cathedral

17:00 – 24:00 Free time in Siena

24:00 Bus return to the Certosa di Pontignano

June 18, Thursday

Thursday, 9:00 - 12:45, Session IX

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 22: Agonists of the novel estrogen receptor GPR30 induce PKCε-dependent mechanical hyperalgesia in rats by altering the microtubule-TRPV1 interaction.
Julia Kuhn, Berlin, Germany

Scholar 23: Stress-induced analgesia in mice: evidence for interaction between endocannabinoids and cholecystokinin. Kaido Kurrikoff, Tartu, Estonia

Scholar 24: Inhibition of both central and peripheral COMT activity sensitize mice to pain.
Oleg Kambur, Helsinki, Finland

Discussions conducted by Faculty and Scholar

Thursday, 15:00 - 19:00, Session X

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

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

Scholar 25: Sphingosine-1-phosphate causes heat hyperalgesia in C57Bl6J mice.
Camilla Benetti, Innsbruck, Austria

Scholar 26: TNF-α inhibition by thalidomide attenuates neuropathic pain and modifies voltage -gated sodium channels mRNA expression in sensory neurons of rats.
Laura Casals-Díaz, Barcelona, Spain

Scholar 27: Regulation of Voltage Gated Sodium Channel Nav 1.7 by ubiquitin ligases and beta auxiliary subunits in Human Embryonic Kidney cells (HEK).
Cédric Laedermann, Lausanne, Switzerland

Discussions conducted by Faculty and Scholar

June 19, Friday

Friday, 9:00 - 12:45, Session XI

Lecture 19: Genetic Approaches to Pain Mechanisms and Treatment.
John Wood, London, UK

Lecture 20: The Ethics of Pain Research and Therapy

Manfred Zimmermann, Heidelberg, Germany, and invited discussants

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Scholar 28: Nociceptive stimulation induces Arc/Arg3.1 expression in the rat spinal cord with a preference for neurons that contain enkephalin.
Aram S. M. Hossaini, Rotterdam, The Netherlands

Scholar 29: Glial cell regulation and involvement of the $P2X_7$ receptor in a mouse model of bone cancer pain. Rikke Rie Hansen, Copenhagen, Denmark

Scholar 30: *Csf2rb,* a gene in the *Pain1* autotomy locus in mice, is associated with neuropathic pain in humans and in mice. Merav Yarkoni-Abitbul, Toronto, Canada

Discussions conducted by Faculty and Scholar

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

Friday, 15:00 – 24:00, Farewell Session

15:00 **Bus Transfer to Rapolano Terme, a hot mineral water Spa in Toscana**

16:00 - 19:00 **Stay and relax at "Terme Antica Querciolaia", Rapolano Terme**

Bathing in the waters of a hot mineral spring known and used by the Romans

19:30 - 24:00 **Farewell Dinner and Party in Restaurant "Le Scuderie del Granduca" in Asciano**

24:00 **Bus return to the Certosa di Pontignano**

June 20, Saturday, Departures

Name, Age, M/F, Email	Contry of Work/ Citizenship Workplace(s)	Academic levels, Study subjects	Mentor(s)	No. of papers abstracts	Fields of experience, Methods	Abroad where?	Abstract, Title of Thesis, other main subjects
Adaes, Sara, 28 yrs, F, sara.adaes@gmail.com	Portugal U of Porto	PhD Student	JM Castro-Lopes	3 7	Biochem, Cell Biol, Animals		A: Increased expression of neuronal injury markers in the DRG of osteoarthritic rats
Austin, Paul, 28 yrs, M, paus-tin@anatomy.usyd.edu.au	Australia/UK U of Sydney U of Bath	PostDoc Fellow PhD Neuroscience MSc Pharmacol	Susan Duty Melanie Welham Kevin A Keay	1 7	Mol Biol Parkinson, Phar-macology, Animals	USA Palo Alto Industry 4 yrs	A: Peripheral nerve injury alters dopamine receptor numbers in the nucleus accumbens in a subpopulation of rats, which display sensory and affective disabilities
Benetti, Camilla, 28 yrs, F, camilla.benetti@i-med.ac.at	Austria/Italy U of Innsbruck U of Padova	PhD Student Neu-rosci MSc Chem & Pharmacy	Michaela Kress	1 3	Neuroscience, Rat in vivo	Austria, Univ Innsbruck	MSc: Effects of CB1-antagonist, SR1417161A, in the dentate gyrus before and after the induction of Long Term Potentiation (LTP): *in vivo* electrophysiology studies A. PhD: Sphingosine-1-phosphate causes heat hyperalgesia in C57Bl6J mice
Casals-Diaz, Laura, 27 yrs, F laura.casals82@gmail.com	Spain Univ Autònoma de Barcelona	PhD Student MSc Neuroscience	Xavier Navarro	2 3	Mol Biol, Neuropath Pain		A: TNF alpha inhibition by thalidomide attenuates neuro-pathic pain and modifies voltage -gated sodium channels mRNA expression in sensory neurons
Castrillon, Eduardo, 39 yrs, M ecastrillon@odont.au.dk	Sweden/Peru Karolinska Inst Stockholm, Odontology U of Peru	PostDoc Fellow PhD Orofac Pain MSc Public Health MD Dentistry	Malin Ernberg Lars Arendt-Nielsen Peter Svensson	10 9	Human pain studies, EMG, Hyperalgesia, Analgesic mechanisms, Coping, NMDA mechanisms, Prevention	Aalborg, Denmark Aarhus Denmark	A PhD: Peripheral NMDA receptors and TMD pain mecha-nisms in human volunteers Postdoc work on craniomandibular disorders
Coelho, Ana Cristina, 23 yrs, F anacoelho@med.up.pt	Portugal U of Porto	PhD Student, Neuroscience MSc Neuroscience	António Avelino Francisco Cruz	1 4	Mol Biol, Genetics, Animal research and care, Polyclonal antibody production		PhD Project: Mechanisms of action of B otulinum toxin in the treatment of overactive bladder A MSc: Distribution and neurochemistry of high affinity binding sites for Botulinum toxin type A in Rat, Guinea-pig and Human urinary bladder
Deumens, Ron, 30 yrs, M, r.deumens@np.unimaas.nl	Netherlands U of Maastricht Radboud U Nijme-gen	PostDoc Fellow PhD Neuroscience MSc Neuroscience BSc Biology	H.W.M. Steinbusch E.A.J. Joosten	20 29	Cell Biol, Animal Res, Histology, Neuropath Pain, Neuroregeneration, Spinal cord Injury	San Diego USA Aachen Germany London UK	A PhD thesis : Transplantation of cell/biomatrix complexes in experimental spinal cord injury in rats
Eberhardt, Mirjam, 26 yrs, F eber-hardt@physiologie1.uni-erlangen.de	Germany U of Erlangen	PostDoc Fellow Research Assistant MD Pain Science	Peter Reeh Karl Messlinger Michael Fischer	2 3	Animal Model of Headache, Neurophysiology	Orkney Islands, UK	A MD thesis: Glyceroltrinitrate facilitates stimulated CGRP release but not gene expression in rat trigeminal ganglia
Fabbretti, Elsa, 40 yrs, F fabbre@sissa.it	Slowenia U Nova Gorice Italy U of Trieste	Postdoc, Lecturer PhD Biotechnol Biology	C Schneider O Burrone	18 10	Molecular Genetics	Paris, France Nova Gorica U Slovenia	A PhD: Mechanisms Mediating the Enhanced Gene Tran-scription of P2X3 Receptor by Calcitonin Gene-related Peptide in Trigeminal Sensory Neurons

Name, Age, M/F, Email	Contry of Work/ Citizenship Workplace(s)	Academic levels, Study subjects	Mentor(s)	No. of papers abstracts	Fields of experience, Methods	Abroad where?	A: Abstract, Title of Thesis, other main subjects
Gangadharan, Vijayan, 28 yrs, M vijayan.gangadharan@pharma.uni-heidelberg.de	Germany/India U of Heidelberg U of New Delhi	PhD Student molecular neuropharmacology MSc Biochemistry	Rohini Kuner		Molecular Neuropharmacology		A: Activation of signaling pathways in the dorsal root ganglia of mice following intense nociceptive stimulation
Hansen, Rikke Rie, 30 yrs, F rrh@farma.ku.dk	Denmark U of Pharmacol Copenhagen	PhD Student, Cand Pharm	Anne-Marie Heegaard	5	Biochemistry, Gene technology	U Leiden, Netherlands	A: Involvement of the P2X$_7$ receptor in bone cancer pain
Hossaini, Aram, 27 yrs, M s.hossaini@erasmusmc.nl	Netherlands Erasmus U Rotterdam	PhD Student Neuroscience MSc Neuroscience	JC Holstege	4	Brain histochemistry, Animal behavior, Gene KO mice		A: Nociceptive stimulation induces Arc/Arg3.1 expression in the spinal cord with a preference for neurons that contain enkephalin
Jerina, Helen, 30 yrs, F h.s.l.jerina@bath.ac.uk	UK U of Bath, School for Health	PhD Student BSc Biol Sciences	CR Stevens DR Blake Jenny Lewis	0 3	Biochemistry, Brain inflammation, Cytokines, Rheumatic patients		A: Expression of Fractalkine and ILβ mRNA in rat brain following persistent and acute inflammatory pain
Kambur, Oleg, 27 y, M oleg.kambur@helsinki.fi	Finland U Helsinki	PhD Student, Neuroscience MSc pharmacy	Eija Kalso PT Männistö	1 1	Neuroscience, Pharmacology, Animal behaviour		A: Inhibition of both central and peripheral COMT activity sensitize mice to pain
Karshikoff, Bianka, 31 yrs, F bianka.karshikoff@ki.se	Sweden Karolinska Inst Stockholm	PhD Student Clin. Neuroscience MSc Molecular Biology	John Axelsson Martin Ingvar Mats Lekander Caroline Olgart Höglund	1 2	Immune system activation. Humans and animals, Gender biology		A: The effect of peripheral inflammation on brain activity and pain sensitization, studied on animals and humans
Khomula, Eugen V., 28 yrs, M eugen_kh@biph.kiev.ua	Ukraine Int. Ctr. Mol. Physiol. NASU Kiev Ukraine	PhD Student Biophysics MSc Appl Physics	Nana V Voitenko Platon G Kostyuk	2 10	Neurophysiology, Ion channels, Biophysics, Patch clamp, Ca^{++} Imaging, Animal behaviour		A: Abnormalities of calcium signaling in sensory DRG neurons of rats with different forms of diabetic neuropathy: reversal effect of nimodipine
Kleideiter, Elke, 35 yrs, F Elke.Kleideiter@grunenthal.com	Germany U of Tuebingen U of Muenster Gruenenthal Laboratories, Aachen	PostDoc researcher PhD Pharmacology MSc Pharmacy	U Klotz HPC Ammon	8 22	Molecular Biology, Pharmacy, Clinical drug studies Telomerase		A: Drug–drug Interaction Studies in Healthy Subjects Evaluating the Effects of Commonly Co-administered Medications on the Pharmacokinetics of Tapentadol, a Novel Centrally Acting Analgesic PhD: Telomeres & Telomerases as prognostic tumor markers and their potential for pharmacological interventions
Kuhn, Julia Annabelle, 29 yrs, F kuhn@molgen.mpg.de	Germany FU and MPI Berlin Studienstiftung Fellow	PhD Student Diploma in Biochemistry	Tim Hucho Jon D Levine Petra Knaus	3 3	Hormonal regulation of pain, Sensitization, Animals	UCSF Pain Center, USA	A: Agonists of the novel estrogen receptor GPR30 induce PKCε-dependent mechanical hyperalgesia in rats by altering the microtubule-TRPV1 interaction

Name, Age, M/F, Email	**Contry of Work/ Citizenship Workplace(s)**	**Academic levels, Study subjects**	**Mentor(s)**	**No. of papers abstracts**	**Fields of experience, Methods**	**Abroad where?**	**A: Abstract, Title of Thesis, other main subjects**
Kurrikoff, Kaido, 32 yrs, M Kaido.Kurrikoff@ut.ee	Estonia U of Tartu	PhD Student MSc Biomedicine BSc Psychology	Ero Vasar Sulev Koks	10	Animal pain models, Gene KO mice, Cell biology, Biochemistry, Pharmacology, 8 years of Pain research	London King's College, UK Helsinki Univ	PhD project: Stress-induced analgesia in mice: evidence for interaction between endocannabinoids and cholecystokinin A: Interactions of the neuropeptide cholecystokinin with endogenous opioids and cannabinoids in the regulation of pain and endogenous analgesic mechanisms
Laedermann, Cedric, 28 yrs, M cedric.laedermann@unil.ch	Switzerland U of Lausanne	PhD Student MSc Neuroscience BSc Biology	Hugues Abriel Isabelle Decosterd	0 5	Ionic channels, Electrophysiology, Molecular biology, Immunocytochemistry		A: Regulation of Voltage Gated Sodium Channel Nav 1.7 by ubiquitin ligases and beta auxiliary subunits in Human Embryonic Kidney cells (HEK)
Martikainen, Ilkka Kristian, 29 yrs, M ilkrima@utu.fi	Finland U of Turku	PhD Student MD, licensed physician	Antti Pertovaara	8	Psychophysics, human brain imaging, transcranial magnetic stimulation	Univ Mainz & Univ Heidelberg, Germany	A: Differential associations between brain 5-HT_{1A} receptor binding and response to pain versus touch in human subjects
Neacsu, Cristian, 33 yrs, M neacsu@biologie.kappa.ro	Romania U of Bucharest	MSc Student Neurobiol BSc Biochemistry	Alexandru Babes Marie-Luisa Flonta	1 4	Ion channels, Pain related receptors, In vitro technique		MSc thesis: Effect of NGF, GDNF and BDNF on the expression of ion channels TRPV1, TRPM8 and TRPA1 in rat DRG neurons A: Substance MCS-18 isolated from Helleborus purpurascens is a potent antagonist of the capsaicin receptor, TRPV1
Nicotra, Annalisa, 30 yrs, F annalisa.nicotra@uniroma1.it	Italy U of Rome U of Palermo	PostDoc fellow PhD Pharmacol MSc Biol. Science		3 1	Animal behavioral pharmacology		A: Role of chemokine Bv8/PK2 in the neutrophil-dependent inflammatory pain in rats and mice PhD: Taxolo resistance of breast cancer cells
Ooi, Lezanne, 29 yrs, F l.ooi@leeds.ac.uk	UK U of Leeds	PostDoc fellow PhD Biochemistry BSc Biochemistry	Nikita Gamper	7	Biochemistry, Genetics, Transcriptional regulation, Electrophysiology, Nociceptor sensitization		PhD thesis: Analysis of repressor element 1-silencing transcription factor interactions with its target genes A: Regulation of KCNQ2/3 K^+ ion channels in dorsal root ganglion neurons by the transcriptional repressor REST in nociception, studied in rat model of neuropathic pain
Popovici, Felicia, 28 yrs, F felicia.popovici@meduniwien.ac.at	Austria/Romania U of Vienna	PhD Student MD	Stefan Boehm	0 2	Medicine, Genetics, Cell Biology, Electrophysiology, Patch clamp		A: The non-opioid analgesic flupirtine is a modulator of ion channels in cultured rat primary sensory neurons MD Thesis: Chromosome 1p microsatellite markers for molecular genetic testing of oligodendroglial neoplasms
Schmid, Anne-Christine, 28 yrs, F anne-christine.schmid@uni-tuebingen.de	Germany U of Tuebingen	PhD Student MSc Biology	Niels Birbaumer Raimund Apfelbach	0	Human Brain Imaging fMRI, MEG, Transcranial Stimulation		A: Effect of a sensorymotor training on pain and cortical reorganization in patients with complex regional pain syndrome (CRPS)

Name, Age, M/F, Email	**Contry of Work/ Citizenship Workplace(s)**	**Academic levels, Study subjects**	**Mentor(s)**	**No. of papers abstracts**	**Fields of experience, Methods**	**Abroad where?**	**A: Abstract, Title of Thesis, other main subjects**
Shyti, Reinals, 27 yrs, M r.shyti@lumc.nl; reinshyti@yahoo.gr	Netherlands/Albania U of Leiden	PhD Student MSc Neuroscience BSc Psychology	AM van den Maag-denberg C Barrett Michael Ferrari	0	Neurophysiology, Transgene animals, Migraine models		A: Cortical spreading depression in transgenic migraine mouse models MSc Thesis: Enhanced secretory activity of midbrain Ucn1 neurons in two different mouse models with reduced alcohol consumption
Song, Zhiyang, 33 yrs, M zhiyang.song@ki.se	Sweden/China Karolinska Inst Stockholm	PostDoc Fellow PhD Neurophysiol PhD (China) MD ophthalmol	Bjorn A. Meyerson Bengt Linderoth	3	Neurophysiology, Patch clamp, Molecular cell biol-ogy, Stereotaxic brain sur-gery		A: Serotonergic mechanisms are involved in pain relief with spinal cord stimulation: Study in an animal model of mononeuropathy
Vartiainen, Nuutti, 30 yrs, M nuutti@neuro.hut.fi	Finland U of Helsinki	PhD Student MD Anesthesiology	Riita Hari Nina Forss	6 4	fMRI, MEG on humans, Neuropathic pain		A: Functional and structural changes in central pain cir-cuitry in patients with chronic pain and recurring herpes simplex virus infections
Yarkoni, Merav, 38 yrs, F merav.yarkoni@utoronto.ca	Canada/Israel U of Toronto Hebrew U Jerusalem	PostDoc Researcher PhD Pain genetics MSc Mol Genetics	Zeev Seltzer Barry Sessle	2 2	Human and animal genetics, Biostatistics, Neurophysiol-ogy, Biochemistry, Cell biology	USA, U of Massa-chusetts	PhD Project: Identification of genes for chronic pain in mice and humans: whole genome and candidate gene approaches. *A: Csf2rb*, a gene in the *Pain1* autotomy locus in mice, is associated with neuropathic pain in humans and in mice

Sara Adães

Increased expression of neuronal injury markers in the DRG of osteoarthritic rats.

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Background & Aims: Pain is the most prevalent symptom of osteoarthritis (OA), but its mechanisms remain to be elucidated. We have recently observed that the number of neurons innervating the OA joint, identified by retrograde labelling with Fluorogold (FG), decreases 40% in animals with 31 days of disease progression. This could result from neuronal cell death, or from a decreased FG uptake due to fibre retraction or another type of neuronal damage. Since the total number of L3, L4 and L5 dorsal root ganglia (DRG) cells was not altered in OA rats, but a slight increase in the total number of medium-large size cells was observed, we hypothesized that cellular hypertrophy might be occurring in the DRGs of OA animals, which has been described as a possible consequence of neuronal damage. To study this possibility, we evaluated the time-course expression of ATF-3, a neuronal injury marker, and of NPY, normally not present in the DRG but shown to be increased as a result of neuronal damage. Since the total number of DRG cells remained unaltered, and since ATF-3 has also been associated with the regeneration of injured cells, we also evaluated its co-localization with the regeneration marker GAP-43.

Methods: This work was approved by the Ethical Committee for Health of the Hospital S. João, Porto, Portugal. OA was induced by intra-articular injection of 2mg of mono-iodoacetate (MIA) in the left knee joint of adult male Wistar rats. Control animals were injected similarly with saline. Animals were sacrificed at 3, 7 and 14 days post-injection. Seven days prior to their sacrifice, FG was injected in the same joint. L3, L4 and L5 ipsilateral DRG were used for immunohistochemical analysis of NPY, ATF-3 and ATF-3 + GAP-43 double labelling. The areas of all the cell profiles analysed were measured with image analysis software.

Results: A significant increase in the number of ATF-3 positive cells was observed as early as 3 days after the induction of OA. Such increase diminished over time, but an increase in the percentage of ATF-3 cells positive for GAP-43 was observed at days 7 and 14. NPY expression showed a similar pattern as ATF-3 expression, with a significant increase immediately at 3 days of OA progression.

Conclusion: The increase in the expression of ATF-3 and NPY, known to be induced as a consequence of neuronal injury, supports the hypothesis that damage in DRG neurons innervating the OA joint may be occurring. This may explain the decrease in the number of FG backlabelled cells in OA animals. Interestingly, the increased co-localization of ATF-3 and GAP-43 over time indicates that neuronal regeneration may be taking place as a response to neuronal damage in the MIA model of OA.

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Paul Austin

Peripheral nerve injury alters dopamine receptor numbers in the nucleus accumbens in a subpopulation of rats, which display sensory and affective disabilities

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Background and Aims: Clinically chronic neuropathic pain is characterised by sensory changes, disability and altered affect. In *all* rats, sciatic nerve constriction injury (CCI) evokes sensory changes (hyperalgesia & allodynia), in addition we have shown that a *subpopulation* (30%) also show disabilities (altered social behaviour, sleep and endocrine function) and affective changes [1]. Studies in both rats and humans have suggested: (i) that altered affect in chronic pain states, may be a consequence of changes in the mesolimbic dopaminergic 'reward-aversion' circuitry targeting the nucleus accumbens (NAcc); and (ii) that sensory "pain" thresholds correlate specifically to dopamine D2 receptor (D2R) availability in the striatum [2]. The aim of these studies was to quantify topographically the density of D2R expressing neurons in both the NAcc and striatum in rats *with* (n=5) and *without* (n=5) disability following CCI.

Methods: Rats underwent von Frey and thermal sensory testing, as well as resident-intruder social interactions testing for 5 days prior to, and 6 days after CCI. Following testing the brains were processed post-mortem for D2R immunoreactivity (-IR) and analysed stereologically throughout the entire rostrocaudal extent of the striatum and nucleus accumbens. The research plan was approved by the University of Sydney, Animal Ethics Committee.

Results: In the striatum there were no topographic differences in the numbers of D2R-IR neurons in rats with, or without, disabilities following CCI when compared with uninjured control animals. However, in the NAcc there was a topographically specific decrease in the number of D2R-IR neurons at ~1.0 mm anterior to bregma in rats with CCI-evoked disabilities (-32.7% vs. non-disabled [$p>0.05$]; -37.2% vs. control [$p>0.01$]).

Conclusions: Thus, in the NAcc we have demonstrated a decrease in density of D2R-IR neurons at a distinct rostrocaudal level, in the subpopulation of rats that show disability and affective change following CCI. Plasticity in NAcc D2R expression likely contributes to the affective changes characteristic of chronic neuropathic pain, and the observed decreases may be triggered by altered activity in direct inputs arising from both the spinal cord and mesolimbic dopaminergic nuclei.

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Camilla Benetti

Sphingosine-1-phosphate causes heat hyperalgesia in C57Bl6J mice

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Background and Aims: Sphingosine 1-phosphate (S1P) is an important lipid mediator which has been implicated in many biological processes including inflammation. S1P is recently emerging as an important component in the regulating nociceptor excitability [1]. It is a physiologically active sphingolipid that contributes to cellular signaling either by acting as intracellular second messenger, or as external messenger binding to G protein coupled receptors. Biologically active lysophosphatidic acid has been found essential for development of neuropathic pain [2]. However, the role of S1P in the regulation of peripheral nociception has not been addressed. We therefore studied if S1P can induce pain and we address possible mechanisms that could be involved.

Methods: In this study, behavioural and electrophysiological methods were used. For *in vivo* behavioural testing, withdrawal latencies were determined for heat stimulation (Hargraves test) and correlative data were obtained from single fibre recordings in an *in vitro* skin nerve preparation. Whole cell voltage clamp recordings were performed in isolated dorsal root ganglion neurons chemically stimulated with the Dynaflow® system.

Results: *In vivo*, intraplantar injection of S1P (100 µM) induced heat hyperalgesia within 15 min which partially recovered after three hours. Accordingly, polymodal nociceptors showed a higher discharge rate in nociceptive nerve endings after incubation with S1P in response to a heat stimulus *in vitro*. Heat and capsaicin, a selective agonist of the nociceptor specific ion channel TRPV1, induced ionic currents which were sensitized by conditioning stimulation with S1P. Application of S1P in isolated dorsal root neurons induced itself a small ionic current mainly in capsaicin sensitive cells. In line with these data, deletion of of the nociceptor specific heat transducer gene TRPV1 significantly reduced the heat hyperalgesia induced by S1P *in vivo*. The presence of $S1P_1$, $S1P_2$, $S1P_3$ receptors in sensory neurons was demonstrated.

Conclusion: Together, our data suggest that peripheral S1P induces heat hyperalgesia in mice by a fast modulation of TRPV1. S1P itself can induce an ionic current and it may be speculated that S1P can induce pain by direct activation of the heat transducer channel TRPV1.

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Laura Casals-Diaz

TNF-α inhibition by thalidomide attenuates neuropathic pain and modifies voltage -gated sodium channels mRNA expression in sensory neurons of rats

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Background and Aims. There is evidence that TNF-α plays a key role in the development of inflammatory and neuropathic pain. Moreover, transcriptional changes have been described for alpha subunits of voltage-gated sodium (Nav) channels after peripheral nerve injuries, potentially underlying hyperexcitability in painful conditions. We propose that TNF-α might activate signalling pathways which modify Nav channels expression.

Methods. Two groups of female Sprague-Dawley rats were subjected to the spared nerve injury (SNI) model with preservation of the sural branch. We administered either vehicle (VEH, n=8) or thalidomide (THA, n=10) as a TNF-α inhibitor (50 mg/kg i.p., 10% DMSO), 2 hours before surgery and onwards daily. Algesimetry tests were performed to evaluate mechanical allodynia and thermal hyperalgesia up to 1 month. At 7 and 28 days postinjury (dpi) rats were sacrificed and L4 and L5 DRGs were dissected and processed for mRNA purification. Gene-specific mRNA analysis by real-time PCR for Nav1.3, Nav1.7, Nav1.8 and Nav1.9 was performed. At the same time points, intact sural nerves from perfused rats were harvested and embedded in Epon resin. Transverse semithin sections were examined by light microscopy. Experimental procedures were approved by the Ethics Committee of our institution, and followed the European Communities Council Directive 86/609/EEC.

Results. VEH rats showed lowered mechanical and thermal thresholds in the injured paw. In contrast, THA rats showed an attenuation of mechanical allodynia during the whole follow-up and the reversal of thermal hyperalgesia up to 14 dpo. Real-time PCR analysis showed that the SNI induced the upregulation of Nav1.3 and downregulation of Nav1.7, Nav1.8 and Nav1.9, which were still observed by 28 dpo. This changes were significantly enhanced in THA rats at 7 dpo, while by 28 dpo Nav1.3 increased expression was mantained but Nav1.7, Nav1.8 and Nav1.9 expression returned near to basal levels. Semithin sections of the sural nerve from both groups showed no alterations in myelin appearance, size of the axons or vascularization.

Conclusions. Inhibition of TNF by THA leads to the attenuation of neuropathic pain in the SNI model. The effect on thermal hyperalgesia is evident during the first two weeks of treatment. THA interferes in the cascade inducing transcriptional changes of Nav channels after nerve injury. Altered expression of Nav channels on THA rats can not be attributed to a neuropathy induced by THA.

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Eduardo Enrique Castrillon

Peripheral NMDA receptors and Temporomandibular Disorder (TMD) pain mechanisms in human volunteers

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Background and Aim: Ketamine (KET) can attenuate glutamate-evoked pain which suggests that glutamate (GLU) acts on NMDA receptors to evoke muscle pain. This work is aimed at finding out if increased concentrations of GLU in the masticatory muscles is involved in the development and maintenance of chronic myofascial TMD pain (M-TMD).

Methods: This study on M-TMD patients and healthy volunteers was approved by the Ethics Committee at Aarhus University. Experimental pain was induced by injections of 0.2 ml of sterile solutions of monosodium GLU into the deep masseter muscle. The order of injections was randomized and performed in a double blind manner. Microdialysis was performed to measure interstitial GLU levels in the masseter muscle. Pain was assessed by an electronic VAS, drawings of pain area and the McGill Pain Questionnaire. A numerical rating scale was used to assess pain relief and pain unpleasantness. Mechanical sensitivity was assessed by pressure pain thresholds and tolerance. Function and psychosocial variables were assessed.

Results: Even though healthy controls experiencing glutamate-evoked pain compared with M-TMD patients showed to share some characteristics [1], a co-injection of KET could not attenuate glutamate-evoked pain or mechanical sensitization in the masseter muscle in healthy young females with no observed differences considering their use of oral contraceptives [2]. Local intramuscular injection of KET did not affect pain or jaw function in M-TMD patients [3] and those patients showed elevated interstitial concentrations of GLU in the masseter muscle compare with healthy controls.

Conclusion: Nevertheless, it was demonstrated that elevated concentrations of interstitial GLU are found in the masseter muscle of M-TMD patients, the lack of differences on healthy young women taking or not oral contraceptives in the effect of GLU or the lack of ability of the combined injection of GLU and KET to decrease the effect of GLU [2], plus the lack of major effects of local injections of KET on masseter muscle pain in M-TMD patients [3] suggest that different mechanisms may underlie the sensitization in women than in men.

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Ana Coelho

Distribution and neurochemistry of high affinity binding sites for Botulinum toxin type A in Rat, Guinea-pig and Human urinary bladder

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Background & Aims. Pain and bladder hyperactivity are the main symptoms of BPS/IC (bladder painful syndrome/interstitial cystitis). Botulinum toxin type A (BoNT/A) has been used as a new treatment for these pathologies. The specificity of BoNT/A is due to the presence of membrane receptors (Synaptic Vesicle protein, SV2) that are exposed during neurotransmitter exocytosis. Once internalized, BoNT/A undergoes a pH-dependent conformational change that causes the dissociation of the heavy and light chains. The latter has endopeptidase activity that cleaves specific sites of the SNAP-25 protein, preventing the assembly of the synaptic fusion complex SNARE and blocking neurotransmitter release. In the present study, we studied the distribution of SV2 and SNAP-25 in three different species, including human. The neurochemistry of BoNT/A sensitive structures was investigated using markers for parasympathetic, sympathetic and sensory fibers. This could be one of the first steps in the understanding of BoNT/A mechanism of action in the treatment of BPS/IC.

Methods. Human bladders were obtained from cadaveric organ donors (19-65 years) after permission from the Ethics Committee of Hospital S. João. Rodent bladders were injected with 10U of BoNT/A and were collected at 1, 3, 8 15 days and 1 month (rat) and 1, 3 and 8 days (guinea pig). Cryostat sections were processed for single or dual immunofluorescent staining using antibodies against SV2, SNAP-25 (cleaved and uncleaved), vesicular acetylcholine transporter (VAChT), tyrosine hydroxilase (TH) and calcitonin-gene related peptide (CGRP).
Results. Immunoreactive (IR) fibers for SV2 and SNAP-25 were found in the mucosa and muscular layers of all the species. No staining was found in epithelial or muscular cells. Double labeling showed extensive co-localization of both proteins. SV2 exhibited a high degree of co-localization with all types of nerve fibers. Immunostaining for cleaved SNAP-25 in BoNT/A treated animals showed sparse fibers at all time points studied in the rat and abundant fibers in the guinea pig muscular layer. Cleaved SNAP-25 immunoreactivity was most abundant in VAChT-IR fibers.

Conclusion. Our data show that: I) SV2 and uncleaved SNAP-25 co-expression is high. II) BoNT/A targets and by-products of BoNT/A action are more abundant in cholinergic, parasympathetic fibers. III) The amount of SNAP-25 cleavage in the bladder after a similar BoNT/A dose is orders of magnitude more intense in the guinea-pig than in the rat.

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Ronald Deumens

Transplantation of cell/biomatrix complexes in experimental spinal cord injury in rats

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Background and Aims. Previous studies have shown that transplantation of olfactory ensheathing cells into relatively small spinal lesion sites can stimulate plasticity of various axon pathways and thereby stimulate improved motor outcome after experimental spinal cord injury. However, additional plasticity of nociceptive fibers innervating the spinal cord may result in enhanced pain, especially if this plasticity involves aberrant fiber sprouting. Here we aimed to reduce aberrant fiber sprouting and increase directional axon regrowth by implanting highly orientated biodegradable matrices seeded with olfactory ensheathing cells into spinal lesion gaps. It was assessed whether this approach leads to reduced central pain and enhanced motor outcome [1].

Methods. Adult Lewis rats received low-thoracic unilateral hemisection injuries. Highly orientated biodegradable matrices seeded with olfactory ensheathing cells were acutely implanted into the lesion gap. Animals were weekly tested for locomotor performance using CatWalk gait analysis and for pain behavior using von Frey hair filaments. Control groups consisted of animals receiving either no treatment or receiving a non-cell-seeded matrix. In a subset of animals, the corticospinal tract was anterogradely traced. After a post-injury survival period of about 10 weeks, animals were anesthetized and perfused. Plastic changes in both motor-related and pain-related fibers were analyzed. The research plan was approved by the Ethical Committee of the Maastricht University Medical Center.

Results. Spinal hemisection injury resulted in a small set of behavioral deficits detectable with the CatWalk gait analysis. Acute implantation of cell-seeded biomatrices resulted in minor improvements of motor function and in subtle attenuation of mechanical allodynia in the forelimbs. In addition, numerous axon fibers were detected within the implant, none of which were of corticospinal origin. However, corticospinal axons were found to sprout rostrally to the lesion site. Furthermore, nociceptive fibers are currently analyzed and these data will be discussed at the meeting.

Conclusions. Implanting a highly orientated biomatrix seeded with axon-growth promoting cells such as olfactory ensheathing cells induces plasticity of injured motor axons, which may underlie a moderate improvement in motor outcome. Importantly, this strategic intervention does not exacerbate, but rather attenuates mechanical allodynia after experimental spinal cord injury.

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Mirjam Eberhardt

Glyceroltrinitrate facilitates stimulated CGRP release but not gene expression in rat trigeminal ganglia

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Background and Aims. Nitric oxide (NO) donors induce delayed headaches in migraineurs and in a rat model cause delayed ongoing activity in central trigeminal neurons which process intracranial afferent input. Cellular models imply that NO may increase the release or production of calcitonin gene-related peptide (CGRP), a key mediator in primary headaches. We investigated whether NO donors can modulate CGRP release and gene transcription in rat trigeminal ganglia (TG).

Methods. Reversible, inflammatory mediator (IM: pH 6.1; PGE_2, bradykinin, histamine, 5-HT, all 10 µM) -stimulated CGRP release was measured by EIA in a recently established model using intact isolated TG of rats. Effects of an NO donor on stimulated CGRP release were examined with one TG exposed to NONOate (10µM) and the contralateral TG untreated as control (n = 6). In further *in vivo* studies glyceroltrinitrate (GTN, 250µg/kg/h i.v.) or saline was infused over 2 hours under isoflurane (1.5%) anaesthesia. After additional 0.5h animals were sacrificed. CGRP content and IM-stimulated release were measured from TG (n = 11). In a similar set of experiments mRNA was extracted from TG 0.5 and 6 h after GTN or saline infusion. After reverse transcription mRNA levels of CGRP and the CGRP receptor components RAMP1, CLR and RCP were determined by qRT-PCR, ß-actin served as control (n = 38). The experimental protocol was reviewed by an ethics committee and approved by the local government.

Results. IM caused a 130% increase in CGRP release, whereas application of NONOate did not affect basal or stimulated CGRP release. After infusion of GTN, IM-induced CGRP release was 80% greater compared to controls, while total CGRP content in TG was not different. The mRNA levels of CGRP or its receptor components remained unchanged compared to saline treated rats.

Conclusions. The present data suggest that prolonged increase in NO levels facilitates stimulated CGRP release from trigeminal ganglion cells. The underlying mechanism seems not to be based on increased CGRP production. Delayed headaches induced by NO may not be due to changes in expression of CGRP or CGRP receptors.

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Elsa Fabbretti

Molecular mechanisms of sensitization of pain transducing receptors on mouse trigeminal neurons

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Background & Aims. Migraine headache originates from stimulation of nerve terminals of trigeminal ganglion neurons that innervate meninges. Characteristic features of migraine pain are its delayed onset and persistent duration. Current theories propose that endogenous substances released during a migraine attack like the neuropeptide CGRP (Calcitonin gene related peptide) and the neurotrophin NGF (Nerve growth factor) sensitize trigeminal neurons to transmit nociceptive signals to the brainstem, though the mechanisms remain poorly understood. CGRP receptor antagonists are currently under clinical trials on migraine patients. Recent studies indicate that acute, long-lasting sensitization of trigeminal nociceptive neurons occurs via distinct processes involving enhanced expression and function of ATP-gated P2X3 receptors known to play a role in chronic pain [1].

Methods. Trigeminal ganglia from C57-Black mice were cultured for 24 hours and used then for immunofluorescence, western blot, immunoprecipitation or functional patch-clamp or calcium imaging experiments. In selected experiments NGF, neutralizing anti-NGF antibodies or CGRP were added to the cultures. Behavioral nociceptive tests induced by the P2X3 receptor agonist were performed after approval of National Italian of Health ethical committee.

Results. We demonstrated that CGRP induces an up-regulation of the ionic currents mediated by P2X3 receptors by enhancing receptor trafficking to the neuronal membrane and activating their gene transcription. Such up-regulated receptors acquire the ability to respond repeatedly to extracellular ATP, thus enabling long-lasting signalling of painful stimuli. In contrast, NGF induces rapid, reversible up-regulation of P2X3 receptor function via protein kinase C activation, an effect counteracted by in vivo NGF neutralisation. Furthermore, we deeply studied the role of differential P2X3 receptor phosphorylation states in the modulation of its function.

Conclusion. The diverse intracellular elements used by CGRP and NGF show that sensitization of P2X3 receptor function depends from the complex integrated action of multiple cellular pathways. These findings imply that combinatorial strategies to inhibit a chronic pain attack might be most efficient approaches, and their efficacy might highly depend on the time of administration.

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Vijayan Gangadharan

Activation of signaling pathways in the dorsal root ganglia of mice following intense nociceptive stimulation.

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Background and Aims: Previous studies have shown that inhibitors of PKG-I (cGMP dependent protein kinase) block inflammatory pain. Furthermore, mice lacking PKG-I globally show reduced nociceptive behaviour [1]. However, the underlying signaling mechanisms are poorly understood. Being a kinase, PKG-I phosphorylates several downstream targets in various cell types which mediates important biological processes. PKG-I and some of its known targets are expressed in small diameter DRG neurons. Here, we investigated that PKG-I and some of its substrates in small diameter DRG neurons are indeed activated upon nocicptive stimulation and whether these changes modulate nociception and inflammatory pain.

Methods: All animal experiments were approved by the local ethical committee (Regierungspräsidium Karlsruhe). Mice lacking PKG-I specifically in peripheral nociceptors were generated using Cre-loxP mediated recombination system without affecting its expression in spinal cord and brain, being referred to as SNS-PKG-I $^{-/-}$. To induce pain, both SNS-PKG-I $^{-/-}$ and corresponding control wild-type mice received an intraplantar injection with 1% formalin, while PBS was injected as placebo. L4-L5 DRGs were collected after 15 min, and phosphorylation status of PKG-I substrates was identified using western blot analysis. Intrathecal catheters were chronically implanted in mice to deliver pharmacological inhibitors ML-7 and 2-APB to the L4-L5 spinal segments.

Results: We found no difference in the expression levels of PKG-I substrates such as VASP (Vasodilator-stimulated phosphoprotein), IP_3R1(inositol 1,4,5-triphosphosphate receptor 1), and MLC (myosin light chain) in SNS-PKG-I $^{-/-}$ mice compare to wild type control mice naïve state and following formalin injection. Within 15 minutes after formalin injection the phosphorylation status of VASP, IP_3R1 and MLC increases in control mice but not in SNS-PKG-I $^{-/-}$ mice. Finally, pharmacological inhibition of MLC and IP3R1 by ML-7 and 2-APB, respectively, led to diminished phase II response in the formalin pain test.

Conclusions: We have thus established a system for quantitatively assessing the activation of PKG-I target proteins in nociceptive neurons following nociceptive stimulation in vivo. Our results show that the PKG-I targets IP_3R1 and MLC are causally linked to the modulation of spinal nociceptive processing.

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Rikke Rie Hansen

Glial cell regulation and involvement of the $P2X_7$ receptor in a mouse model of bone cancer pain

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Background and aims. Bone cancer pain is a common and debilitating complication to primary or metastatic bone cancer, and is difficult to control with the current drug regiments. The mechanisms of bone cancer pain are believed to consist of a mix of neuropathic and inflammatory pain, but recent results have demonstrated that bone cancer pain might have a separate mechanism. Spinal cord astrocytes and microglia cells are activated in models of the neuropathic pain, and it is believed that they play a role in the development and maintenance of the pain. Furthermore it has been shown that the purinergic receptors $P2X_3$, $P2X_4$ and $P2X_7$, which are expressed on glial cells, play a role in neuropathic pain. The aim of this project is to investigate the regulation of glial cells and the role of the $P2X_7$ receptor in bone cancer pain.

Methods. Syngeneic 4T1 breast cancer cells were inoculated into the right femora of BALB/cJ mice. Three pain related behaviours were assessed: limb use of injured hind limb, weight distribution on hind limbs and mechanical allodynia, which was tested with von Frey monofilaments. Activation of spinal cord astrocytes and microglia cells were determined by fluorescent immunohistochemistry with their respective markers glial fibrillary acidic protein (GFAP) and Iba1. The effect of the $P2X_7$ receptor antagonist, A-438079, on pain related behaviour was investigated. Wild type BALB/cJ mice were inoculated with 4T1 breast cancer cells, and A-438079 (300 µmol/kg s.c.) were tested in mice with clear signs of pain related behaviour. All animal experiments were approved by the the Danish Animal Ethics Council.

Results. Mice with bone cancer had a significant lower limb use score and a significant change in their weight distribution on hind limbs. A strong activation of astrocytes was seen, whereas no microglia activation was seen. Treatment with the $P2X_7$ receptor antagonist did not affect the pain related behaviours in the mice.

Conclusion. Astrocyte activation but not microglia activation was seen in the model of bone cancer pain [1]. Also our results indicate that the $P2X_7$ receptor does not play a role in the maintenance of bone cancer pain. This is in contrast to what is seen in neuropathic pain, and supports the idea that bone cancer pain has its own mechanisms.

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Aram S.M. Hossaini

Nociceptive stimulation induces Arc/Arg3.1 expression in the rat spinal cord with a preference for neurons that contain enkephalin

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Background and aims: The immediate early gene Arc/Arg3.1 plays an essential role in long term synaptic plasticity in several brain regions. Spinal pain processing is also characterized by plastic changes. However, it is unknown whether Arc/Arg3.1 is involved in spinal plasticity. The aim of this study was to investigate the expression pattern of Arc/Arg3.1 in the rat spinal cord using various pain models and to assess the phenotypic changes in nociception of Arc/Arg3.1 knockout (KO) mice.

Methods: All animal experiments were performed according to the guidelines of Rotterdam Animal Ethical Committee. For acute pain we applied capsaicin, formalin, CFA, or mustard oil. For chronic pain, the CFA and spared nerve injury model were used for induction of inflammatory and neuropathic pain, respectively. After overdoses with pentobarbital, the rats were perfused with paraformaldehyde and the spinal cord was dissected. Arc/Arg3.1 mRNA and protein were visualized in free floating sections by means of (fluorescent) in situ hybridization and immunohistochemistry, respectively. For colocalization experiments, these techniques were combined. In the Arc/Arg3.1 knockout (KO) and wild type (WT) mice, the mechanical and thermal thresholds were assessed in CFA and formalin induced pain models.

Results: Only after a nociceptive stimulus, Arc/Arg3.1 is observed in the superficial dorsal horn, especially lamina II. The expression of Arc/Arg3.1 is intensity dependent, and is highly colocalized with the much more abundantly expressed activation marker c-Fos. We found low expression of Arc/Arg3.1 (4%) in neurons positive for GAD67 mRNA (GABAergic neurons), neurons expressing PKC-γ (8%), calbindin (10%) and NK-1 (19%). However, Arc/Arg3.1 is highly colocalized (68%) with pre-pro-enkephalin mRNA (enkephalinergic neurons). The Arc/Arg3.1 KO mice displayed only a mild hyposensitivity in the mechanical threshold after acute and chronic pain stimuli.

Conclusion: Our data showed that nociceptive stimuli induced Arc/Arg3.1 in the superficial laminae of dorsal horn, preferentially in enkephalinergic neurons. In addition, knockdown of Arc/Arg3.1 did not result in a major change in pain behavior. Our findings suggest that Arc/Arg3.1 is involved in spinal pain processing, mainly through enkephalinergic neurons located in lamina II.

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Helen Jerina

Expression of Fractalkine and ILβ mRNA in rat brain following persistent and acute inflammatory pain

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Background and Aims: Studies have shown that chronic pain leads to central sensitisation that is partially regulated by release of proinflammatory molecules within the CNS. Most work has concentrated on the role of the spinal cord and little is known about changes in supraspinal regions. Here I have compared two animal models of inflammatory pain and investigated the expression of cytokine (TNFα, IL-1β) and fractalkine (FRA) mRNA in the brain. Carrageenan (CAR) induces an acute inflammatory response while Complete Freund's Adjuvant (CFA) induces prolonged inflammation and persistent pain.

Methods: Adult male Wistar rats received an intraplantar injection of either 100µl of 0.1% CFA (n=4), 100µl 1% CAR (n=3) or 100µ1 physiological saline (SAL) (n=3) into the right hind paw. Swelling of both hind paws was measured by plethysmometry at 3 hrs, 6 hrs and daily thereafter. Rats were sacrificed at day 7. Brains were cut into six sections, the left and right "front", "mid" and "hind" brain and total RNA extracted. Expression of TNFα, IL-1β and FRA mRNA was measured using semi-quantitative RT-PCR. This work was UK Home Office licensed.

Results: CAR animals showed maximal swelling of right hind paws on day 3, returning to baseline by day 7. By contrast, inflammation persisted in CFA animals, increasing up to day 7. Left hind paws of all animals showed no significant difference to SAL controls. IL-1β mRNA increased significantly in the right frontal cortex, both right and left midbrain and left hindbrain of CFA rats compared with SAL controls ($P<0.05$, unpaired t-test). In CAR animals a significant increase was seen only in the right hindbrain ($P<0.05$). There was no significant difference in TNFα expression. Of particular interest, FRA mRNA expression was increased in the midbrain region of both CFA and CAR animals.

Conclusions: In CAR animals, although paw inflammation had subsided, IL-1β mRNA remained elevated, primarily in the hind brain. In the CFA model, where pain persists, IL-1β mRNA was highly increased throughout the whole brain. Although numbers are small, the CFA animals showed much higher expression of IL-1β mRNA in the fore and midbrain in comparison to CAR animals, providing further evidence of a role for this cytokine in the development of chronic pain and related symptoms. Although FRA has been proposed as a signalling chemokine in the spinal cord, this is the first evidence of pain related upregulation in the brain. Inflammation in the mid and fore brain of CFA rats suggests a role for IL-1 β and FRA in modulating the cognitive and emotional effects of chronic pain. I am currently performing immunohistochemical analysis and investigating further time points to build on these results.

This work is funded by the University of Bath.

Oleg Kambur

Inhibition of both central and peripheral COMT activity sensitize mice to pain

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Background and Aims. Catechol-*O*-methyltransferase (COMT) modulates pain and opioid analgesia in humans and rodents [1]. Some of those effects have been attributed to downstream changes in central opioid system. Involvement of peripheral and/or spinal mechanisms has also been suggested but has not been studied before. Here we describe for the first time the effects of central (dinitrocatechol) and peripheral (nitecapone) COMT-inhibitors on nociception in mice.

Methods. Male mice of the C57BL/6J background, aged 3-4 months and weighting 26-34 g were used in the experiments. The animals were habituated to handling and to the testing environment for four consequent days before the experiments. After that animals were randomly divided into three parallel groups that were used in all behavioural assays. Groups received dinitrocatechol (30mg/kg, i.p., n=16), nitecapone (30mg/kg, i.p., n=16) or vehicle (i.p., n=21) for 6 days. Thermal nociception was assessed using paw flick and hot plate tests and mechanic nociceptive thresholds were measured with digital force gauge. Nociception was assessed on days 1 and 5. On the 6th day of experiment, immediately after administration of drugs inflammation was induced by intraplantar injection of carrageenan and nociceptive responses were measured again. COMT activity was measured in a separate group of animals from striatal samples, as described earlier [2]. All experiments were approved by the institutional animal investigation committee and the provincial government of Southern Finland.

Results. As expected, dinitrocatechol inhibited COMT activity in the brain and increased mechanic, thermal and carrageenan-induced nociception in several tests. These effects remained on the fifth day of treatment. Nitecapone did not inhibit COMT-activity in the brain but showed increase in nociception similarly to dinitrocatechol.

Conclusions. These results show for the first time that COMT is involved in modulation of nociception outside the brain and pronociceptive effects of COMT inhibitors are at least partly mediated via spinal and/or peripheral mechanisms.

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Bianka Karshikoff

The effect of peripheral inflammation on brain activity and pain sensitization, studied on animals and humans

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Background & Aims: My thesis work aims to explore the neuroimmune communication involved in pain and sickness behaviour, i.e. how signalling molecules of the immune system (cytokines) influence the CNS and elicit the behavioural changes that follow illness. Pain sensitivity is an important part of the sickness response [1] and is influence by cytokines. Recently, increased overall bodily pain has been shown in children with allergy, a chronic inflammatory disease [2].

Methods: a) An animal study is currently in progress studying the activation of sensory neurons in the lung during asthmatic inflammation in mice. 20 ovalbumine (OVA) sensitized Balb/c mice undergo intranasal OVA challenge. Markers of neuronal activity and glia involvement, such as phosphorylation of mitogen activated protein kinases (MAPK), c-FOS, GFAP and IBA1, are measured in the spinal cord using western blotting. b) An experimental study of low dose endotoxin stimulation in 30 healthy volunteers in a cross-over design is planned. This acute inflammatory model induces flu-like symptoms for ca. 6 hours, during which we will test for mood changes using validated questionnaires and draw blood samples to measure cytokine levels. The subjects will also undergo two cognitive tests during fMRI scanning; a pain sensitivity test using heat pain and a test of interoceptive awareness. c) A second human study will investigate comorbidity of pain diagnoses in a chronically allergic population, compared to non-allergics. An existing database of 1.200.000 Swedes will be used that encompasses information about all public and private health care during 2000-2007. Ethical permits were received from the regional ethics committee Karolinska Institutet, Stockholm.

Results & Hypotheses: a) An alteration in spinal MAPK activity was detected in OVA-sensitized mice indicating that allergic inflammation drives molecular changes at the level of the spinal cord. b) We hypothesize that pain sensitivity and interoceptive awareness will increase during immune activation, as well as anxiety and fatigue. We believe that these changes will be correlated to brain activity in the cingulate and insular cortices and peripheral cytokine levels. c) We hypothesize that there will be a greater prevalence of pain diagnoses in the allergic population as compared to a control non-allergic population.

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Eugen Khomula

Abnormalities of calcium signaling in sensory DRG neurons of rats with different forms of diabetic neuropathy: reversal effect of nimodipine

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Background & Aims. Vanilloid receptors 1 (TRPV1) and T-type voltage operated calcium channels (T-type VOCCs) are important participants of molecular mechanisms underlying nociception in primary sensory neurons. They are also actively involved in neuronal calcium signaling. Previous contributions of our laboratory demonstrated a significant positive effect of prolonged treatment of diabetic animals with nimodipine. Here we have focused on diabetes-induced changes in small isolectin B4 (IB4)-positive capsaicin sensitive rat DRG neurons. In a model of streptozotocin (STZ)-induced diabetes we investigated changes of TRPV1 and T-type VOCCs functioning under diabetic neuropathy and analyzed if a prolonged treatment with nimodipine could rescue abnormalities developed in these Ca^{2+}-regulating systems.

Methods. Using a whole cell patch clamp and calcium imaging T-type VOCCs and capsaicin-induced currents as well as respective cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) transients were measured in small IB4-positive DRG neurons among 5 groups of animals of the same age (9 weeks): control, diabetic (6 weeks of diabetes) with thermal hypo-, hyperalgesia, and nimodipine treated diabetic with hypo-, hyperalgesia. A nimodipine treatment was performed orally (20mg/kg) daily with meal for 3 weeks in 3 weeks of diabetes induction. Diabetes was induced by injection of STZ (60 mg/kg) in P21 rats. The research plan was approved by the Ethical Committee of the Bogomoletz Inst. of Physiology.

Results. A peak T-type VOCCs current density as well as amplitudes of corresponding $[Ca^{2+}]_i$ transients were significantly increased in both hypo- and hyperalgesic diabetic rats. Density of capsaicin-induced currents and amplitudes of respective $[Ca^{2+}]_i$ transients were decreased in neurons of hypoalgesic rats and increased in neurons of hyperalgesic ones. The responses were partially but significantly changed toward control values under chronic nimodipine treatment.

Conclusions. Diabetic neuropathy was associated with significant changes in functioning of T-type VOCCs and TRPV1 in small IB4-positive DRG neurons, which are involved in thermal nociceptive signaling. These specific alterations in calcium signaling may contribute to the development of diabetes-induced thermal hypo- and hyperalgesia. Nimodipine treatment can partially reverse many diabetic-induced abnormalities of different Ca^{2+}-regulating systems showing its potential as an approach to cure diabetic neuropathy.

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Elke Kleideiter

Drug–drug Interaction Studies in Healthy Subjects Evaluating the Effects of Commonly Co-administered Medications on the Pharmacokinetics of Tapentadol, a Novel Centrally Acting Analgesic

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Background and Aims. Tapentadol is a novel, centrally acting analgesic with 2 mechanisms of action, μ-opioid receptor agonism and noradrenaline reuptake inhibition, in a single molecule. Tapentadol is metabolized predominantly by direct O-glucuronidation followed by renal clearance of the metabolites [1]. The aim of these studies was to investigate whether commonly co-administered medications had clinically relevant effects on tapentadol pharmacokinetics (PK).

Methods. Two randomized, open-label studies were conducted in healthy subjects to evaluate the effects of acetaminophen (2-way crossover; N = 24; Treatments: tapentadol; tapentadol + acetaminophen) and naproxen or acetylsalicylic acid (ASA; 3-way crossover; N = 38; Treatments: tapentadol; tapentadol + naproxen; tapentadol + ASA) on the PK of a single oral 80-mg dose of tapentadol IR. The studies were approved by an ethics committee (Antwerp, Belgium) or an institutional review board (Plantation, FL, USA). Tapentadol serum concentrations and plasma concentrations of acetaminophen, naproxen, and ASA were determined in the 2 studies. Statistical analyses were performed on log-transformed tapentadol PK data using mixed-effects models.

Results. Mean serum concentrations were similar after tapentadol IR administration and after coadministration with acetaminophen or ASA; the 90% confidence intervals (CIs) for the ratios of geometric means for maximum serum concentration (Cmax)and area under the curve (AUC) of the combined treatments to tapentadol IR were well within the accepted range for bioequivalence (80% to 125%). Co-administration of naproxen did not significantly alter the Cmax of tapentadol, but increased the mean AUC parameters by 17% relative to tapentadol; the 90% CIs for AUC to last measurement and AUC to infinity were slightly outside the 125% upper bound for bioequivalence (126.47% and 126.14%, respectively). Plasma concentrations of acetaminophen, naproxen, and ASA were within the expected ranges.

Conclusions. These results indicate that there were no clinically relevant changes in the serum concentrations of tapentadol, if tapentadol was given concomitantly with acetaminophen, naproxen, or acetylsalicylic acid.

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Julia Kuhn

Agonists of the novel estrogen receptor GPR30 induce PKCε-dependent mechanical hyperalgesia in rats by altering the microtubule-TRPV1 interaction

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Background/Aims: Therapy-induced pain is a major side effect occurring in ~ 20 % of fulvestrant treated breast cancer patients. Recently we found the agonists estrogen (E2), G-1 and fulvestrant of the novel estrogen receptor GPR30 to induce PKCε-dependent mechanical hyperalgesia. Further, microtubules were identified to regulate PKCε-dependent nociceptor sensitization. Now we investigated, if GPR30 agonists alter PKCε-dependently the interaction of microtubule with ion channels such as TRPV1 and if this alteration is a critical step in GPR30-agonist induced mechanical hyperalgesia.

Methods: Dissociated DRG neurons from male Sprague-Dawley rats were cultured for 3 days for live cell microscopic studies of morphological responses to E2 (1 nM), G-1 (10 nM), or fulvestrant (10 nM). Response to E2/G-1 (1 min) were studied on DRG neuron derived F-11 cells transfected with TRPV1-GFP constructs and in presence or absence of PKC inhibitors. *In situ*-cytoskeleton was prepared and microtubules analyzed immunofluorescently by laser scanning microscopy. *In vitro,* purified TRPV1 was phosphorylated by PKCε, followed by pull down to analyze tubulin binding to TRPV1/pTRPV1 and evaluated by Western Blotting. Mechanical pain thresholds were quantified by Randall-Selitto paw pressure tests 30 min after G-1 (1µg) application. Drugs were applied 30 min prior to G-1 intradermally. To knockdown TRPV1 40 µg antisense or mismatch ODNs were administered intrathecally over 3 days. All animal experiments were approved by the Institutional Animal Care and Use Committee at UCSF and the Committee for Research and Ethical Issues of IASP.

Results: E2, G-1 and fulvestrant induced rapid microtubule disassembly in a subset of DRG neurons. In F-11 cells destabilization of microtubules after E2- or G-1-treatment was observed only in TRPV1-expressing but not in TRPV1-negative cells. Block of PKCs as well as mutation of the PKCε phosphorylation site TRPV1-S800A blocked E2/G-1 actions on microtubules. *In vitro,* PKCε mediated phosphorylation of TRPV1-C terminus reduced tubulin binding to TRPV1. In behavior experiments, application of the microtubule disruptor nocodazol prior to G-1 blocked the mechanical hyperalgesia normally induced by G-1, while stabilization of microtubules with taxol had no effect. Knockdown of TRPV1 reversed the effects of microtubule altering drugs.

Conclusions: Our results indicate that GPR30 agonists alter in nociceptive neurons PKCε-dependently the microtubule-TRPV1 interaction resulting in mechanical hyperalgesia. Thus, we provide a cellular mechanism which might be responsible for fulvestrant induced pain during chemotherapy.

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Kaido Kurrikoff

Stress-induced analgesia in mice: evidence for interaction between endocannabinoids and cholecystokinin

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Background & Aim. Stress-induced analgesia (SIA) is a mechanism in response to stressful stimuli. Opioid peptides and endocannabinoids are known mediators of SIA. We were interested whether the endocannabinoid tone and the related SIA could be modulated by activity of the neuropeptide cholecystokinin (CCK). The rationale for this hypothesis is the evidence for a cellular colocalisation of CCK and cannabinoid CB_1 receptors in CNS structures. Our aim was to test whether abolishing the endogenous CCK tone might influence cannabinoid-mediated SIA.

Methods. CCK_2 receptor deficient male mice were used. Electric foot-shocks (0.2, 0.4, 0.6, 0.9 mA, 3 min) were used to induce stress. SIA was estimated with tail-flick latency. Each animal received only one stress-application. Gene expression levels were measured via real-time PCR. We measured the expression levels of CCK and opioid ligands and receptors, as well as cannabinoid receptor CB_1 and ligand synthesizing and metabolizing enzymes. The tissues (lumbar spinal cord, brainstem, midbrain, striatum and mesolimbic area) were collected 20 min after termination of stress. The permission (No. 39, 7.10.2005) for the present study was given by the Estonian National Board of Animal Experiments in accordance with the EC Directive of 24.11.1986 (86/609/EEC).

Results. As expected, CB_1 antagonist rimonabant (1 to 3 mg/kg) prevented the analgesia in response to footshock-induced stress in wild-type mice. In contrast, CCK_2 receptor deficient mice developed SIA regardless of rimonabant treatment. Naloxone (0.01 - 10 mg/kg), an opioid antagonist, antagonised SIA in both wild-type and CCK_2 receptor deficient mice. This finding suggests that CCK, through CCK_2 receptors, modulates the action of endocannabinoids. Gene expression analysis revealed an upregulation of CCK_2 receptor gene in the lumbar spinal cord and mesolimbic area in wild-type mice in response to stress. In addition, wild-type mice displayed upregulation of genes implicated in endocannabinoid-mediated neurotransmission – elevation of CB_1 receptor, 2-AG and anandamide synthesizing enzymes DAGLa and NAPE-PLD – in response to stress in the lumbar spinal cord and mesolimbic area. We did not find any of these changes in CCK_2 receptor deficient mice.

Conclusions. Altogether, behavioural and gene expression studies reflect an involvement of CCK_2 receptors in the development of endocannabinoid mediated SIA.

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Cédric Laedermann

Regulation of Voltage Gated Sodium Channel Nav 1.7 by ubiquitin ligases and beta auxiliary subunits in Human Embryonic Kidney cells (HEK).

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Background / Aims: Neuropathic pain (NP) is a disorder that follows a lesion or a dysfunction of the nervous system. NP causes a positive shift toward hyperexcitability of the peripheral nervous system. This peripheral activity is mainly carried by voltage-gated sodium channels (VGSC), among them Nav1.7 isoform is important for pain signalling. VGSC contain an α-subunit, the pore of the channel, and β-subunits responsible for the regulation of channel density at the cell membrane. Ubiquitin ligases of the Nedd4 family are also known to regulate the channel turnover**.** The aim of this study was to investigate the molecular and cellular mechanisms involved in the regulation of the membrane density of Nav1.7 that may be altered in NP.

Methods: *In vitro* whole-cell patch clamp on HEK 293 cells transfected with Nav1.7 allowed the recording of sodium currents INa, reflecting the density of channel at the membrane. Nedd4-2 and β-subunits were co-transfected to test their effect on Nav1.7 current. The interaction between Nedd4-2 and Nav isoforms was previously reported to depend on a consensus sequence, the PY-motif in the C-term. Mutations of the PY-motifs allow us to demonstrate that this sequence is important for interacting with Nedd4-2. Biochemistry experiments were performed to study this interaction. GST-fusion proteins (GST fused with the 66 last amino-acids of Nav1.7) were used to pull-down Nedd4-2 from HEK293 lysates.

Results: Co-transfection experiments with Nedd4-2 decreased the current amplitude by ~80% ($n = 36$, $p<0.001$), without modifying the biophysical properties of INa. This effect was dependent on the PY-motif since mutations in this sequence abolished the down-regulatory effect of Nedd4-2. The importance of this motif was confirmed by pull down experiments since the PY mutants completely abolished the interaction with Nedd4-2. Co-transfection of β-subunits showed an overall opposite effect since β1, β2, and β3 increased the INa: for β1 by ~100% ($n=22$, $p<0.001$); for β2 by ~ 70% ($n=22$, $p<0.01$), and β3 of ~200% ($n=17$, $p<0.001$) and no effect of β4. β1 and β2 subunits also caused a positive shift in the voltage-dependence of both activation and inactivation.

Conclusion and Perspectives: In HEK293 cells, Nedd4-2 and β-subunits have opposite effects in terms of regulating the density of Nav1.7 at the cell surface. We need to confirm *in vivo* these results combining experimental pain animal models together with knock-out mice for these two genes in order to correlate the cell surface modulation of Nav1.7 with differences in pain sensitivity.

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Ilkka Martikainen

Differential associations between brain 5-HT$_{1A}$ receptor binding and response to pain versus touch in human subjects

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Background and Aims. Recently, we found that a low serotonin 5-HT$_{1A}$ receptor binding potential (BP) in dorsal raphe, neocortex and limbic cortex predicts high intensity rating to cold pressor pain [1]. Here we tested the hypothesis that the serotonergic influence on pain is rather due to modulation of non-sensory factors, such as response bias, than to a specific effect on the sensory signal. Furthermore, we tested whether the influence of 5-HT$_{1A}$ receptors varies with the modality of cutaneous stimulation, and whether 5-HT$_{1A}$ receptors also influence short-term memory for pain.

Methods. Psychophysical performance was assessed in 16 healthy subjects who had participated in a positron emission tomography study using [carbonyl-^{11}C]WAY-100635 ligand for the assessment of 5-HT$_{1A}$ BP. Sensitivity to tactile stimuli was tested with von Frey hairs, and sensitivity to heat pain and heat pain short-term memory were tested with cutaneous heat pain stimuli delivered with Medoc TSA-2 NeuroSensory Analyzer. Signal detection theory was applied to allow separate analysis of the subject's sensory-discriminative capacity and attitude toward reporting a sensation (response criterion; non-sensory factor). The research plan was accepted by Joint Ethical Committee of Turku University Central Hospital and University of Turku, Turku, Finland.

Results. The results indicate that the subject's response criterion, but not discriminative capacity, for heat pain was inversely correlated with 5-HT$_{1A}$ BP in the dorsal raphe, middle temporal gyrus, orbitofrontal cortex and posterior cingulum whereas the subject's discriminative capacity, but not criterion, for touch was inversely correlated with 5-HT$_{1A}$ BP in the cingulum, inferior temporal gyrus and medial prefrontal cortex. Certainty ratings of the responses, but not hit rates, in the heat pain short-term memory task were correlated with 5-HT$_{1A}$ BP in the dorsal raphe.

Conclusions. The results indicate that with respect to touch, 5-HT$_{1A}$ receptors predominantly influence discriminative capacity and with respect to pain, subject's response criterion. Moreover, 5-HT$_{1A}$ receptors influence subjective performance in short-term memory task for pain.

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Cristian Neacsu

Substance MCS-18 isolated from Helleborus purpurascens is a potent antagonist of the capsaicin receptor, TRPV1

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Background and aims. In Balkan area extracts of *Helleborus Purpurascens* are traditionally used for pain treatment. Our aim was to test if MCS-18 (a pure compound derived from *Helleborus* roots) may inhibit the vanilloid receptor TRPV1 expressed in the native tissue (rodent DRG neurons in primary culture) and in expression systems (HEK-293 cells).

Methods. Dorsal root ganglion (DRG) neurons were obtained from all spinal levels of adult Wistar rats (as described in Reid et al., 2002), with the approval of the Ethics Committee of the University of Bucharest. HEK-293 cells were transiently transfected with rat TRPV1. Recordings were made with calcium microfluorimetry and the patch-clamp technique.

Results. MCS-18 reversibly inhibits capsaicin- and proton-, but not heat-evoked increases in intracellular calcium concentration ($[Ca^{2+}]_i$) in TRPV1-expressing DRG neurons in the rat (by ~86%). Capsaicin-induced currents were inhibited in both whole-cell and outside-out configurations, and the effect was concentration-dependent. The substance had no effect on the responses mediated by acid-sensing ion channels (ASICs) or the irritant receptor TRPA1. MCS-18 also inhibits capsaicin-evoked increases in $[Ca^{2+}]_i$ (~70%) and capsaicin-induced currents in rTRPV1-transfected HEK-293 cells (by ~60%).

Conclusions. MCS-18 is a strong, selective, concentration-dependent and reversible inhibitor of the polymodal receptor TRPV1; it inhibits the activation of TRPV1 by capsaicin and protons (partially), but not by heat. These results provide support for a possible use of *Helleborus* extracts in the treatment of chronic pain.

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Annalisa Nicotra

Role of the chemokine Bv8/PK2 in the neutrophil-dependent inflammatory pain as studied in rats and mice

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Background and Aims: In the past years, several groups have generated convincing evidence that neutrophil-associated hyperalgesia results from the neutrophil release of proinflammatory pronociceptive factors including cytokines. Bv8/Prokineticin 2 (PK2) is a new chemokine characterized by a structural motif comprising five disulfide bonds. This family comprehends the mammalian Prokineticin 1 (PK1 or EG-VEGF) and Prokineticin 2 (PK2) or mammalian Bv8 and the amphibian homolog Bv8 (1). Bv8/PKs activate two G protein-linked receptors (PKR1 and PKR2) localized on primary sensory neurons, neutrophils, macrophages and endothelial cells. Activation of PKRs sensitizes nociceptors to thermal, mechanical and chemical stimuli, promotes chemotaxis and cytokine release and stimulate angiogenesis. PKs are expressed in inflamed tissues and are involved in many aspects of the immune response. The goal of the present experiments is to demonstrate that the granulocyte-derived Bv8/PK2 is responsible for inflammatory pain and highly contributes to initiate inflammation.

Methods: We used two animal models of inflammatory pain: a) oyster-glycogen induced peritonitis; b) CFA-induced paw inflammation. a) Male rats were intraperitoneally (i.p.) injected with 1% oyster glycogen. Six hours later peritoneal extravasated granulocytes were collected lysed and fractioned by ion exchange, gel filtration and HPLC. Collected fractions were assayed "in vivo" and "in vitro" to identify Bv8-like activity. b) Male rats or mice were i.pl. injected with Complete Freund's Adjuvant (CFA) and the mRNA expression levels of PKs and PKRs, at different time after inflammation were evaluated in injected and in contralateral paw. Thereafter we sorted from the inflamed paw of mice, granulocytes and macrophages, and we measured PK2 expression in each cell population.

Results: 1) Peritoneal granulocytes express high levels of PK2. By chromatographic purification we succeeded in isolating a granulocyte-derived Bv8-like protein that induced hyperalgesia, which was abolished by an anti-Bv8 antibody, and bound PKRs. 2) In rat and mouse CFA-injected paws, the PK2 expression dramatically increases in the skin of inflamed paw (1000- and 200-fold above non inflamed skin in rats and in mice), reaching a maximum in 12 hours and returning to basal levels in 2-3 days, exactly matching the time course of inflammation induced hyperalgesia. The increase in PK2 mRNA, besides on the increased number of infiltrating cells, depends on a marked upregulation of Bv8, mainly in neutrophils. Neutrophil extracted Bv8 protein induced hyperalgesia.

Conclusions: In animal models of inflammatory pain we demonstrated that pain temporally correlates with the expression levels of Bv8/PK2; we, also, identified the neutrophils as the main inflammatory cells that synthesize Bv8/PK2.

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Lezanne Ooi

Regulation of KCNQ2/3 potassium ion channels in dorsal root ganglion neurons by the transcriptional repressor REST in nociception, studied in a rat model of neuropathic pain

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Background and Aims. Understanding how excitability of sensory neurons is regulated is an important goal since this excitability underlies pain transmission and unfortunately almost everyone will suffer from inflammatory pain at some point in their life. Recent studies have identified expression of M-type K^+ channels (encoded by KCNQ genes) in damage-sensing (nociceptive) sensory neurons, where they are thought to control excitability. Accordingly, receptor-induced inhibition of M-current in these neurons has been shown to contribute to peripheral sensitisation and inflammatory pain. The aim of this study is to identify novel mechanisms for the direct regulation of KCNQ channel expression in nociceptors and to determine whether this regulation contributes to neuropathic pain.

Methods. Bioinformatic analyses were coupled with biochemical assays, including electrophoretic mobility shift assays, reporter assays, RT-PCR and western blotting, to identify putative regulators of KCNQ expression. These data were confirmed with electrophysiological experiments and immunohistochemistry. To test these findings we used a rat neuropathic pain model (partial sciatic nerve lesion, PSNL), in which the sciatic nerve is exposed and cut on the ipsilateral side and approximately half of the axons in the sciatic nerve are ligated. Dorsal root ganglia (DRG) were sampled at 30 days after nerve injury and changes in channel expression studied. The research plan was approved by the Ethical Committee of the University of Leeds.

Results. We identified binding sites for the transcriptional repressor REST within both the KCNQ2 and KCNQ3 genes. Neonatal rat (P7) DRG neurons cultured in the presence of a REST-expressing adenovirus showed 7.39±0.11 fold ($p \leq 0.05$) increased REST protein, which led to a concomitant 2.20±0.09 fold ($p \leq 0.05$) decrease in KCNQ2 protein and a corresponding 7.65±0.49 fold ($p \leq 0.01$) reduction in M-current in DRG neurons, compared to vehicle control. We further showed that REST protein expression was increased 3.65±0.80 fold in cultured DRG neurons in response to inflammatory stimulation (1 µM bradykinin, 1 µM histamine, 1 µM ATP, 10 µM PAR2-AP and 1 µM substance P for 48 hrs). Increases in REST correlated with a 1.76±0.38 ($p \leq 0.05$) fold decrease in KCNQ2 immunoreactivity. Similarly we observed a significant increase in REST mRNA (2.11±0.01 fold) and protein levels and a reciprocal downregulation of KCNQ2 (1.75±0.07 fold) and KCNQ3 (1.43±0.01 fold) transcripts in DRGs from animals in the neuropathic pain model (PSNL).

Conclusions. Transcriptional regulation of KCNQ channels by REST has profound effects on neuronal excitability and contributes to the mechanisms of peripheral sensitisation in chronic pain.

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Felicia Popovici

The non-opioid analgesic flupirtine is a modulator of ion channels in cultured rat primary sensory neurons

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Background and Aim. Flupirtine (Katadolon®) is a centrally acting, non-opioid analgesic drug with muscle relaxant and neuroprotective properties. Although it is routinely used in the clinic, the mechanism of action is not yet fully understood. It has been suggested to antagonize NMDA receptors, to activate G protein coupled inward rectifier K+ (GIRK) channels and to activate KCNQ K+ channels. Since loss of GABAergic transmission in the superficial dorsal horn of the spinal cord underlies several forms of chronic pain, it has been shown recently that pronounced analgesia can be achieved by specifically targeting spinal $GABA_A$ receptors containing the α2 and/or α3 subunits (Knabl J et al., 2008). This study investigates for the first time the interaction of flupirtine with $GABA_A$ receptors involved in pain sensation.

Methods. Using the perforated patch clamp technique and different primary neuronal cell cultures (hippocampal, dorsal root and superior cervical ganglion), we measured GABA-induced as well as voltage-induced currents in the presence and absence of flupirtine.

Results. Flupirtine caused a potentiation of currents through $GABA_A$ receptors, which was dependent on both cell type and GABA concentration. In DRG neurons, EC_{50} values for GABA-induced currents were decreased from 33.3 µM in the absence, to 7.8 µM in the presence of 30 µM flupirtine. When GABA was applied at concentrations corresponding to the EC_5 value, 30 µM flupirtine enhanced the current amplitudes by a factor of 4.15 $\pm$ 0.29. In hippocampal neurons, EC_{50} values for GABA were reduced by flupirtine from 5.4 to 2.1 µM, and the factor of potentiation was 3.2 $\pm$ 0.26 ($p < 0.05$ vs. DRG). At saturating GABA concentrations, flupirtine decreased current amplitudes by 10.3 and 31.5 % in hippocampal and DRG neurons, respectively. Further experiments showed that flupirtine enhanced currents through KCNQ/K_v7-channels in a concentration-dependent manner (EC_{50} 4.6 µM). In voltage-current ramps measured in DRG neurons, application of flupirtine modulated both, $GABA_A$ receptors at voltages more negative than -50mV predominantly and KCNQ/K_v7-channels at voltages more positive than -50mV exclusively.

Conclusions. These results reveal flupirtine as preferential modulator of $GABA_A$ receptors involved in pain sensation. Its analgesic action may thus result from a combined action on $GABA_A$ receptors and KCNQ channels in sensory neurons.

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Anne-Christine Schmid

Effect of a sensorymotor training on pain and cortical reorganization in patients with complex regional pain syndrome (CRPS)

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Background and Aims. Previous imaging studies have shown that chronic pain syndromes (e.g., phantom limb pain, chronic regional pain syndrome [CRPS]) lead to cortical reorganization in primary sensory (S1) and primary motor (M1) cortex. Furthermore, the magnitude of reorganization has been demonstrated to be associated with the intensity of the pain. Based on this, it can be hypothesized that these reorganization processes are maladaptive and thus inverting maladaptive cortical reorganization to a physiological pattern might improve the chronic pain syndromes.

Methods. CRPS patients performed a specific sensorymotor training developed by our group, for two weeks. Cortical organization was studied with magnetoencephalography (MEG). During MEG recording a tactile stimulation of the thumb (D1) and the pinkie (D5) was performed to determine the hand representation. The unaffected hand was used as the control. MEG data was collected from the patients before and after the two-week training. The patients had to fill out a pain-diary and a training-diary, where they were asked questions about their training habits. Subjective pain levels were rated using a visual analog scale (VAS) three times a day (morning, midday, evening) as well as before and after training. We used the pain disability index (PDI), a questionnaire which measures the subjective disturbance. The study was approved by the ethics committee of the University Clinics Tübingen.

Results. The Pain ratings on the VAS before and after treatment decreased in all subjects after training. MEG data showed cortical reorganization in form of a diminished affected hand in comparison to the unaffected hand in form of a smaller distance between D1 and D5 at the affected compared to the unaffected hand. After the two weeks of training we could see an enlargement of the D1/D5 distance of the affected hand from a pathological to a physiological pattern. The cumulative value of the PDI decreased in all patients.

Conclusion. The sensorymotor training leads to an enlargement of the cortical region of the affected side. This was accompanied by a reduction of pain. This proof of principle was achieved in a small sample and has to be reproduced in larger studies to bring the treatment from the bench to the daily clinical bedside.

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Cortical spreading depression in transgenic migraine mouse models

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Background & Aims. Migraine attacks are characterized by a throbbing, often unilateral headache, preceded in part of patients by a visual aura which is caused by cortical spreading depression (CSD). CSD is a transient wave of neuronal and glial cell depolarization that propagates slowly through the cerebral cortex and may trigger the headache mechanism. Familial hemiplegic migraine (FHM) is a rare monogenic subtype of migraine with aura, in which the aura is characterized by transient hemiparesis. To study the *in vivo* consequences of FHM mutations, we generated transgenic knock-in mice carrying the pathogenic FHM R192Q missense mutation in voltage-gated $Ca_V2.1$ calcium channels [1]. Here, we set out to assess, quantify and compare susceptibility to CSD and its behavioral and neurological manifestations in migraine and wild-type mice. Next, we will identify brain regions that are activated by CSD using expression markers of neuronal activation.

Methods. Mutant and wild type mice were anesthetized with isoflurane and two burr holes were drilled through the skull for electrophysiological recordings. A glass capillary microelectrode was inserted into the brain at a depth of 300 μm to record extracellular steady (DC) potentials and electrocorticogram. For induction of CSD a cotton ball soaked with KCl was placed on the dura. Mice were allowed to recover from surgery and their neurological status was assessed with the wire-grip test at different time points after CSD. Subsequently, mice were transcardially perfused with 4% PFA and their brains were processed for *c-fos* immunostaining. Experiments were approved by the Animal Experiments Ethical Committee of the Leiden University Medical Center.

Results. Our results revealed an increased susceptibility of mutant mice to CSD. We have preliminary data that a differential brain regional CSD susceptibility exists only in mutant mice. Interestingly, mutant mice exhibited severe neurological deficits on the contralateral side of the body after CSD.

Conclusion. Our results confirm and extend previous reports [1, 2] of increased susceptibility to CSD and neurological deficits in mutant mice. Studies are going on to identify brain regions that are activated by CSD.

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Zhiyang Song

Serotonergic mechanisms are involved in pain relief with spinal cord stimulation: Study in an animal model of mononeuropathy

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Background and Aims. Spinal cord stimulation (SCS) may effectively relieve neuropathic pain, but the underlying mechanisms for its beneficial effects are still only partially known. In a previous study it was demonstrated that SCS may induce an increased release of serotonin in the cat spinal dorsal horn[1], and the inhibitory effect on pain of serotonin has been well documented. In the present study, we further examine the possible role of serotonin in the effect of SCS on signs of neuropathy in an animal model.

Methods. The experiments were performed in accordance with the recommendations of the Committee for Research and Ethical Issues of the IASP (1983). Under 4% Isoflurorane anesthesia, a monopolar electrode system for SCS was implanted at the level of T11 in mononeuropathic rats produced by partial injury of the sciatic nerve. The microcontacts of the SCS electrodes were connected to a Grass S44 stimulator and stimulation was applied for 30 min with a frequency of 50 Hz and a pulse width of 0.2 ms. The rat responded to SCS by displaying a tendency towards normalization of the tactile withdrawal thresholds in the injured side paw (WT≥15g). Animals were deeply anesthetized with sodium pentobarbital and sacrificed. Dorsal horn tissue from SCS-responding (with/without SCS), non-responding (with/without SCS) as well as from normal rats were subjected to ELISA and immunohistochemical analysis. Moreover, serotonin, muscarinic M_4 and $GABA_B$ receptor antagonists were administered i.t. to SCS non-responders in order to further explore the mode of action of serotonin in SCS.

Results. The 5-HT content in the dorsal quadrant of the spinal cord ipsilateral to nerve injury was increased following SCS in responding rats, but not in other groups. Immunohistochemical examination showed a relatively high density of 5-HT stained terminals in the dorsal horn superficial laminae (I-II) in responders following stimulation and in intact control rats. Moreover, it was found that i.t. administration of a sub-effective dose of serotonin in SCS non-responding rats markedly enhanced the effect of SCS on tactile and cold hypersensitivity, but not on heat hyperalgesia. This enhancing effect on tactile hypersensitivity could be partially blocked by a $GABA_B$ receptor antagonist but not by a muscarinic M_4 receptor antagonist.

Conclusions. SCS increases the release of 5-HT in spinal dorsal horn suggesting that serotonin may be involved via a supraspinal loop in the pain relieving effect of SCS.

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Nuutti Vartiainen

Functional and structural changes in central pain circuitry in patients with chronic pain and recurring herpes simplex virus infections

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Background and Aims. A patient group suffering from spontaneous chronic pain in widespread areas on one side of the body was described recently [1]. The patients had a history of recurrent herpes simplex virus (HSV) infections due to immunological abnormalities. The clinical picture suggested a connection between the HSV infections and the pain, and the wide distribution of pain suggested central nervous system involvement, but supporting evidence was lacking. With functional and structural brain imaging, we searched for changes in the central nervous system that would support the hypothesis for central origin of pain.

Methods. We used functional magnetic resonance imaging to measure pain- and touch-related brain activations in eight patients and in eleven healthy control subjects, while they received painful heat stimuli and innocuous tactile stimuli to the hands. Voxel-based morphometry was used to assess possible changes in the gray matter density of the brain. The research plan was approved by the Ethics Committee of the Helsinki and Uusimaa Hospital District.

Results. The brain activations to painful heat were statistically significantly weaker in the patients than in the control subjects in the bilateral insular cortices, in the anterior cingulate cortex (ACC), and in the thalamus, while the brain activations to innocuous tactile stimuli were the same in both groups. The gray matter density in the frontal and prefrontal cortices and in the ACC was smaller in the patients than in the control subjects.

Conclusion. We observed both functional and structural changes in the central pain circuitry in our chronic pain patients [2]. On the basis of these changes and the clinical features of the patients, we speculate that the recurrent infections by the neuroinvasive HSV have caused dysfunction in the central nervous system, resulting in central pain. Further studies are needed to clarify the role of HSV in central pain.

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Merav Yarkoni

Csf2rb, a gene in the *Pain1* autotomy locus in mice, is associated with neuropathic pain in humans and in mice

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Background and Aims. *Pain1,* a genetic locus on mouse chromosome 15, harbours unidentified gene(s) controling the contrast in autotomy in A/J (A) and C57BL/6J (B) inbred mice lines and across the 23 AXB-BXA recombinant lines that were derived by crossing A and B mice [1]. In the present study we used a genome-wide expression approach to identify genes in dorsal root ganglia (DRGs) and spinal cord (SC) of A and B mice. While this study was designed as a whole genome screen, special attention was given to finding genes in *Pain1*, and to test whether these genes also correlate with human neuropathic pain (NP).

Methods. The hindpaw of 10 A and 5 B adult male mice was denervated by sciatic and saphenous neurectomy. Five A and 5 B mice underwent sham operation and in 5 A and 5 B mice the hindpaw was left intact, in total we report on 35 mice. On the first week postoperatively, the mice were perfused transcardially with RNA later to preserve RNA integrity. Using 70 Agilent microarrays (4X44K) we profiled the expression levels of all genes in L3-L6 DRGs and spinal cord (1 array/tissue/mouse). Data analysis was performed using the Partek Genomic Suite software package, version 6.0. Expression levels of denervated mice were normalized by average levels of the respective sham groups to ascertain that significant differences are autotomy-specific.

Results. *Csf2rb,* encoding granulocyte-macrophage-colony-stimulating-factor (GM-CSF) receptor 2-beta, was the only gene in *Pain1* satisfying the criteria of being a gene controlling autotomy via expression levels. In the DRG of naïve mice, *Csf2rb* was expressed 1.7 times more in A *vs* B (p=0.03). In the spinal cord of denervated mice, A with high autotomy expressed *Csf2rb* 1.9 times more than low autotomy A mice (p=8.7E-05) and 1.7 times more than low autotomy B mice (p=3.0E-05). The human *Csf2rb* was then genotyped in 85 traumatic leg amputees and 336 women post-mastectomy (with or without NP) using 13 tagging SNPs. Two SNPs (rs2075943 and rs960739) were significantly associated with phantom limb pain (p=0.007 and p=0.045), and post-mastectomy pain (p=0.047 and p=0.04, respectively).

Conclusions. *Csf2rb* is a good candidate for the gene in *Pain1* that controls autotomy variability in mice and NP in humans. Increased levels of CSF2RB, the receptor of the cytokines GM-CSF, IL3 and IL5, may mediate the glial response in the CNS, thereby affecting NP levels.

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