Program Book

INRC

International Narcotics Research Conference

July 9-14, 2017 | Chicago IL USA
Hyatt Centric Magnificent Mile

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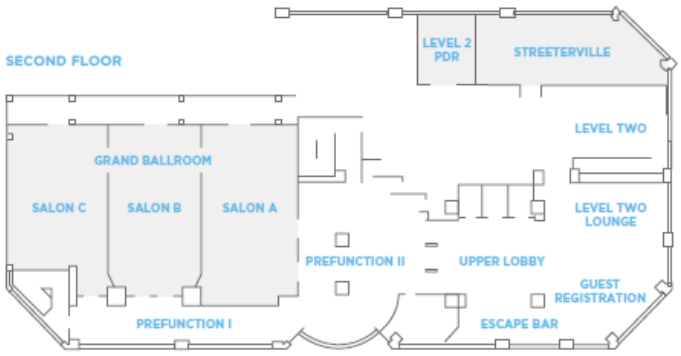

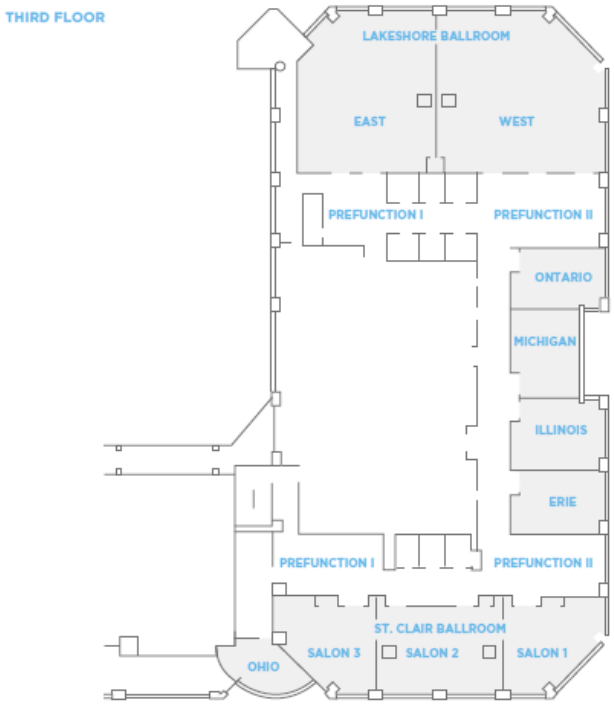

INRC 2017 AWARDEES

Founders' Award – Professor John Traynor

Dr. John Traynor is Professor of Pharmacology and Associate Chair for Research at the University of Michigan Medical School. He holds the Edward F Domino Research Professorship in Pharmacology and is Principal Investigator of a NIDA-funded training grant on the biology of drug abuse and holder of a NIDA Merit Award. Also, Dr. Traynor chairs the Neuropharmacology Division of the American Society for Pharmacology and Experimental Therapeutics and is a standing member of Neurobiology of Motivated Behavior Study Section of CSR (Center for Scientific Review) of the National Institutes of Health. He has served as Director of the University of Michigan Substance Abuse Research Centre for a four years term and was a member of the Medical School's Biomedical Research Council from 2010-2016. Dr. Traynor was a permanent member of The National Institute on Drug Abuse (NIDA) Training and Career Development Committee and has served on the editorial boards of Journal of Neurochemistry and the Journal of Medicinal Chemistry. He is currently on the editorial board of the British Journal of Pharmacology.

Dr. Traynor earned degrees in Pharmacy (B.S.) and Medicinal Chemistry (Ph.D.), both from the University of Aston in the United Kingdom. His post-doctoral training at the University of Gottingen in Germany concentrated on Biochemical Pharmacology. For more than nearly three decades, Dr. Traynor's research has focused on opioids and includes areas such as structure-activity relationships, signaling and behavior. He has trained a large number of PhD students and postdoctoral fellows as well as many Masters and Undergraduate students.

Dr. Traynor's laboratory employs a wide range of techniques from molecular biology to behavioral pharmacology. Strong analgesic drugs such as morphine are extremely important both for the treatment of pain and for the medical and social consequences of opiate (heroin) addiction. The laboratory uses in vitro and in vivo models to characterize the mechanism of action of these drugs. In recent years, Dr. Traynor's research has focused on alternative ways to better harness the analgesic effects of endogenous opioid peptides by targeting a specific family of intracellular proteins (RGS proteins) that act as negative regulators of a variety of neurotransmitters, and/or developing allosteric compounds that modulate opioid receptors in unique ways. The long-term aim of these studies is to identify novel targets and medications to treat pain, drug dependence and depression.

Dr. Traynor is also widely appraised for his role in organizing symposia in national and international conferences. He has been a frequent attendant at the INRC meetings throughout his career and together with his students and collaborators provided outstanding contributions to our organization. He was President of the International Narcotics Research Conference (INRC, 2010-2014) and continues to serve on the Executive Committee of INRC as the immediate past president. Over the years he has really been an active attendant at the INRC meetings and participated in discussion of actual topics in a way that reveal an outstanding competence and experience in questions related to the field of opioids. Unambiguously, Dr. Traynor belongs to the core of successful and prominent researchers that has been essential for development and maintenance of INRC as an organization promoting high-quality science in the area of opioid research.

Young Investigators Award – Dr. Aashish Manglik

Dr. Aashish Manglik is currently a Stanford Distinguished Fellow and Instructor of Molecular and Cellular Physiology at Stanford University School of Medicine. He has an independent position in which he has his own laboratory and funding, including an Early Independence Award from the NIH Director.

Dr. Manglik has been recognized for his work on G protein-coupled receptor signaling and in in silico drug discovery. His work has elucidated the structural basis of opioid receptor function, thereby yielding insight into the mechanism of action of fundamental and widely used analgesics like morphine and codeine. Using these structures, he has pioneered the development of novel opioid analgesics, identified by in silico methods, which potentially possess reduced dose-limiting side effects. Dr. Manglik's work has been published in more than twenty peer-reviewed articles, and he was a key contributor to the invention of the core technologies at a company he co-founded called Ab Initio Biotherapeutics.

Dr. Manglik received his B.A. in Biology and Chemistry at Washington University in St. Louis, where he was awarded the Spector Prize for his outstanding academic and research achievements. At Stanford, he was awarded an American Heart Association Pre-doctoral Fellowship in 2012. In his research there, Dr. Manglik used biophysical techniques such as X-ray crystallography and NMR spectroscopy. Obtaining purified opioid receptors and subsequent crystal structures is a tremendous achievement and requires the very highest abilities and perseverance. These results also rely on the development of new tools to stabilize these challenging proteins. Several of his papers and patents describe the development and design of single domain antibodies (nanobodies) for different conformational states of the mu-opioid receptor. Dr. Manglik trained as an MD as well as a PhD, and his medical training allows him to cover a broad picture generated by the uncovering of these structures. For example, his most recent achievement, and work that he presented at INRC 2016, has been published in Nature. It describes the structure-based discovery of PZM21, an opioid with reduced side-effects in rodent models. This was a mammoth effort across the laboratories of prominent and well-reputed scientists and demonstrates not only Dr. Manglik's ability to interact and collaborate with top researchers but also his breadth of knowledge.

Dr. Manglik has presented many of his findings at meetings and universities in the US but also abroad, e.g. at the above mentioned INRC meeting in Bath (UK) in 2016. At the meeting in Bath, he gave a highly impressive talk on his work on the mu-opioid receptor structure and the discovery of PZM21. His engagement at this meeting attracted many INRC members and his creative attitude will certainly bring an excitement to INRC and encourage other young scientists working in related areas to become regular attendees.

Schedule for INRC 2017

Sunday July 9, 2017

3:00-5:30 PM - Registration

5:30-6:00 PM – Pre-welcome reception for trainees

6:00-8:00 PM - Welcome Reception

Monday July 10, 2017

7:00-8:30 AM - Breakfast

8:15-8:30 AM - Welcome Address

Plenary Lecture

8:30-9:30 AM - Mark von Zastrow – University of California San Francisco, CA, USA - Cell biology of opioid drug action

Opioid Receptor Trafficking & Signaling

Chair: Aki Ozawa - Torrey Pines Institute, FL, USA

Discussion Leader: Louis Gendron – Université de Sherbrooke, QC, Canada

9:30-9:55 AM – Manoj Puthenveedu – Carnegie Mellon University, PA, USA- Endocytic control of functional selectivity of opioid receptors

9:55-10:20 AM – Lee-Yuan Liu-Chen – Temple University, PA, USA– Characterization of a mutant mouse line expressing a fusion protein of kappa opioid receptor and tdTomato

10:20-10:35 AM – Hot Topic 1 – Dominique Massotte - Institut des Neurosciences Cellulaires et Integratives, Strasbourg, France - Trafficking & signaling of endogenous mu-delta heteromers.

10:35-10:55 AM - Coffee Break

10:55-11:20 AM - Nathaniel Jeske – University of Texas Health Science Center at San Antonio, TX, USA – Peripheral Opioid Receptor Regulation

11:20-11:45 AM - Meritxell Canals – Monash University, Melbourne, Australia - GPCR signaling platforms for pain and analgesia

11:45-12:00 PM – Hot Topic 2 - Marta Filizola - Icahn School of Medicine at Mount Sinai, NY, USA - Structural and Dynamic Elements of μ-Opioid Receptor Functional Selectivity

12:00-12:15 PM – Hot Topic 3 – William Birdsong – Oregon Health and Sciences University, OR, USA - Opioid modulation of synaptic transmission in affective pain circuitry

12:15-12:30 PM – Discussion

12:30-1:30 PM – Lunch

Kappa opioid and nociceptin peptides in reward and aversion circuits.

Chair: Ream Al-Hasani - St. Louis College of Pharmacy and Washington University, MO, USA

Discussion Leader: Larry Toll – Torrey Pines Institute, FL, USA

1:30-1:55 PM - Ream Al-Hasani - St. Louis College of Pharmacy and Washington University, MO, USA - Circuit dynamics of *in vivo* dynorphin release in the nucleus accumbens shell

1:55-2:20 PM - Hugo Tejeda - Bonci Lab, NIDA-IRP, MD, USA - Kappa-opioid receptor control of nucleus accumbens synaptic integration differentially gates D1 and D2 MSN activity

2:20-2:45 PM - Olivier George – Scripps Research Institute, CA, USA - Upregulation of dynorphin in the central nucleus of the amygdala mediates the negative emotional states of nicotine withdrawal but not escalation of nicotine intake.

2:45-3:05 PM – Coffee Break

3:05-3:30 PM - Andrew Hardaway - Kash Lab, University of North Carolina, NC, USA, - Central amygdala Prepronociceptin-expressing neurons mediate palatable food consumption & reward

3:30-3:55 PM - Kyle Parker - Bruchas Lab Washington University, MO, USA - Midbrain nociceptin neurons modulate reward behaviors

3:55-4:10 PM – Hot Topic 1 - Anushree N. Karkhanis - Wake Forest School of Medicine, NC, USA - Adolescent social isolation increases kappa opioid receptor function in the nucleus accumbens and basolateral amygdala of rats.

4:10-4:25 PM – Hot Topic 2 - Kabirullah Lutfy – Western University, CA, USA - Male but not female mice lacking nociceptin exhibit higher anxiety and enhanced reward following exposure to the elevated plus maze test.

4:25-4:40 PM – Discussion

4:40-5:10 PM – **Data blitz Session 1**

5:10-7:00 PM – **Poster Session A**

Tuesday July 11, 2017

7:00-8:30 AM - Breakfast

Plenary Lecture

8:30-9:30 AM - Michael Salter – Hospital for Sick Children & University of Toronto, Toronto, Canada - Sex, Pain and Microglia

Targeting opioid receptors to treat alcohol use disorders

Chair: Meredith Robins – Purdue University, IN, USA

Discussion Leader: Kabirullah Lutfy – Western University, CA, USA

9:30-9:55 AM – Richard van Rijn – Purdue University, IN, USA - The potential of delta opioid receptors as novel target for alcohol use disorders

9:55-10:20 AM – Subhash Pandey - University of Illinois at Chicago & Jesse Brown VA, IL, USA - A role of epigenetically regulated dynorphin/kappa-opioid receptor and neuropeptide Y system in alcohol tolerance

10:20-10:35 AM – Hot Topic 1- Braulio Munoz – Indiana University School of Medicine - Ethanol disrupts synaptic specific mu opioid receptor-mediated long term depression in dorsal striatum

10:35-10:55 AM - Coffee Break

10:55-11:20 AM - Annika Thorsell – Linkoping University, Linkoping, Sweden – The OPRM1 A118G SNP in drug reinforcement and treatment response to naltrexone: Evidence from a humanized mouse model.

11:20-11:45 AM - Linda Rorick-Kehn – Lilly Laboratories, Eli Lilly and Company, IN,USA - The novel nociceptin receptor antagonist LY2940094 reduces ethanol-seeking and self-administration in animal models and significantly reduces heavy drinking in alcohol-dependent subjects.

11:45-12:00 PM – Hot Topic 2 – Lucia Hipolito - University of Valencia, Valencia, Spain – Inflammatory pain alters the vulnerability to alcohol relapse without impacting the intensity of the relapse in rats.

12:00-12:15 PM – Hot Topic 3 – Moriya Yuki - Tohoku University, Sendai, Japan - The effects of chronic stress on alcohol consumption in μ-opioid receptor knockout mice

12:15-12:30 PM – Discussion

12:30-1:30 PM – Lunch

Opioids and Pain

Chair: Nicolas Massaly – Washington University, MO, USA

Discussion Leader: Cathy Cahill – University of California Irvine, CA, USA

1:30-1:55 PM – Jim Wang - University of Illinois at Chicago, IL, USA - Protein kinase mechanisms in opioid-induced hyperalgesia

1:55-2:20 PM – Greg Scherrer – Stanford University, CA, USA - Circuits and synaptic mechanisms for pain control by endogenous and exogenous opioids

2:20-2:35 PM – Hot Topic 1 - Shiwei Liu – University of California Irvine, CA, USA - Kappa opioid receptor up-regulation contributes to mood and reward dysregulation in neuropathic pain.

2:45-3:05 PM – Coffee Break

3:05-3:30 PM – Hiroshi Ueda – Nagasaki University, Nagasaki, Japan - Therapeutic recovery from diminished morphine analgesia in chronic pain state

3:30-3:55 PM – Vanna Zachariou, Icahn School of Medicine at Mount Sinai, NY, USA - Targeting RGSz1 to optimize the actions of opioid analgesics.

3:55-4:10 PM – Hot Topic 2 – Moe Watanabe - Hoshi University, Tokyo, Japan - Characterization of the endogenous small-sized peptides released in the nucleus accumbens related to pain modulation

4:10-4:25 PM – Hot Topic 3 – Gregory Corder – Stanford University, CA, USA - A neural network for abstracting nociceptive information into an aversive pain perception

4:25-4:40 PM – Discussion

4:40-5:10 PM – **Data blitz Session 2**

5:10-7:00 PM – **Poster Session B**

Wednesday July 12, 2017

7:00-8:30 AM - Breakfast

Founders' Lecture

8:30-9:30 AM – John Traynor – University of Michigan, MI, USA - Opioid Research: past achievements – future challenges

Emerging Techniques & Hot Topics

Chair: Amanda Fakira - Icahn School of Medicine at Mount Sinai, NY, USA

Discussion Leader: Michael Bruchas – Washington University, MO, USA

9:30-9:45 AM – Brigitte Kieffer – McGill University/Douglas Institute, Montreal, Canada – Probing whole-brain connectivity in live mice: receptors and drugs

9:45-10:00 AM – Elena Romanova – University of Illinois Urbana-Champaign, IL, USA – Unbiased characterization of intercellular signaling peptides using mass spectrometry

10:00-10:15 AM – Emma Childs – University of Illinois at Chicago, IL, USA – Human laboratory paradigms to study conditioned cues

10:15-10:30 AM – Michael Morgan – Washington State University, WA, USA - Preclinical Assessment of opioid analgesia, side effects, and withdrawal

10:30-10:50 AM - Coffee Break

10:50-11:05 AM - Damien Jullie - University of California San Francisco, CA, USA - Probing axonal trafficking with live cell imaging of pHluorin tagged Mu Opioid Receptor

11:05-11:20 AM - Hot Topic 1 – Louis Gendron – Université de Sherbrooke, QC, Canada - Investigating delta opioid receptors in a new conditional knockin mouse

11:20-11:35 AM – Hot Topic 2 – Aliza Ehrlich – McGill University, Douglas Research Center, QC, Canada - Distinct MOR agonist profiles revealed by visualizing Mu opioid receptor trafficking with MOR-Venus mice

11:35-11:50 AM – Hot Topic 3 – Emily Jutkiewicz - University of Michigan, MI, USA - Allosteric activation of delta opioid receptors in vivo: promising behavioral profile

11:50-12:05 PM – Hot Topic 4 – Sidney Williams – Washington University, MO, USA - Development of a Virtual Reality Paradigm for in vivo Hippocampal Imaging During Morphine Conditioned Place Preference

12:05-12:30 PM – Discussion

12:30-1:30 PM - Lunch

1:30 PM – Free afternoon and breakout session on Antibodies & Mutant Mice (Christopher Evans - University of California Los Angeles USA, and Brigitte Kieffer - McGill University - Douglas Institute, Canada)

Thursday July 13, 2017

7:00-8:30 AM - Breakfast

Plenary Lecture

8:30-9:30 AM - Plenary Lecture – Catherine Bushnell, National Center for Complementary and Integrative Health, NIH, Bethesda, MD, USA - Alterations of the opioid system in chronic pain

Opioid Epidemic

Chair: Kyle Windisch - Rockefeller University, NY, USA

Discussion Leader: Jose Moron-Concepcion – Washington University, MO, USA

9:30-9:55 AM – Theodore J Cicero – Washington University, MO, USA - History of Prescription Opioid abuse and the Recent Transition to Heroin

9:55-10:20 AM – Sandra Comer – Columbia University and the New York State Psychiatric Institute, NY, USA – Pharmacological Therapies for Opioid Use Disorder: Implications of Fentanyl Availability on Treatment Effectiveness

10:20-10:35 AM – Hot Topic 1 – Barbara Belli - Orexigen Therapeutics, CA, USA - OREX-1019; a small molecule with potential for addiction maintenance treatment and relapse prevention

10:35-10:55 - Coffee Break

10:55-11:20 AM - Andrew Charles – University of California Los Angeles, CA, USA – Prescription opioids and migraine: the basic and clinical science of a major public health issue

11:20-11:45 AM Tuan Trang – Hotchkiss Brain Institute, University of Calgary, Calgary, Canada - Blocking microglial pannexin-1 channels alleviates opioid withdrawal,

11:45-12:00 PM – Hot Topic 2 - Laura Moye – University of Illinois at Chicago - Identifying the role of peripheral delta opioid receptors in chronic migraine

12:00-12:15 PM – Hot Topic 3 – Seven Tomek – Arizona State University, AZ, USA - Effects of Heroin on Prosocial Behavior in Rats.

12:15-12:30 PM – Discussion

12:30-1:30 PM – Lunch

Young Investigator Symposium – Ligand Directed Signaling at Opioid Receptors

1:30-2:15 PM – Aashish Manglik - Stanford University, CA, USA - Structural dynamics in G protein-coupled receptor signaling

2:15-2:40 PM - Susruta Majumdar – Memorial Sloan Kettering Cancer Center, NY, USA - Kratom derived natural products as leads to design analgesics with mu opioid G-biased agonism and delta opioid antagonism

2:40-2:55 PM – Hot Topic 1 - Mariana Spetea - University of Innsbruck, Innsbruck, Austria - Ligand-receptor and structure-function relationship studies on differently substituted diphenethylamines interacting with the κ-opioid receptor

2:55-3:15 PM – Coffee Break

3:15-3:35 PM – Jonathan Violin – Trevena Inc, PA, USA - Discovery and development of oliceridine, a G protein-biased ligand targeting the mu-opioid receptor

3:35-3:50 PM – Hot Topic 2 - Kylie B. McPherson - Oregon Health & Science University, OR, USA – Agonist Functional Selectivity determined by RGS proteins.

3:50-4:05 PM – Hot Topic 3 – Indrajeet Sharma – University of Oklahoma, OK, USA – Collybolide, a novel biased agonist to study kappa-opioid receptor pharmacology.

4:05-4:20 PM – Discussion

4:20-5:00 PM – **INRC Business Meeting**

5:00 PM – Conference Close

6:00-7:00 PM – **Closing Reception - Odyssey Cruise Boarding**

7:00-9:30 PM – **Cruise Banquet and Dancing**

Friday July 14, 2017

Departure

Poster Presentations

1 - Political Priority for Tobacco Control among Adolescents in Local Governments Areas, Osun State, Nigeria
Ademola Adelekan

2 - Effectiveness of Provider Initiative Approach for Smoking Cessation among Pregnant Women in Osun State, Nigeria
Ademola Adelekan

3 - Ring Substitution in the Macrocyclic Opioid Peptide CJ-15,208 Alters the Opioid Activity Profile in vivo
J. V. Aldrich

4 - Identification of a novel interaction site of the mu-delta opioid receptor heterodimers
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5 - Low Abuse Liability of the Endomorphin Analog ZH853 is Supported by Reduced Psychomotor Activation and Withdrawal.
Ariel Amgott-Kwan

6 - Morphine influences cell proliferation, migration and apoptosis of tumor cell lines endogenously expressing mu opioid receptors
Andrea Bedini

7 - OREX-1019; a small molecule with potential for addiction maintenance treatment and relapse prevention
Barbara Belli PhD

8 - OREX-1038; a potent analgesic molecule with potential for reduced abuse liability
Barbara Belli PhD

9 - Rewarding effects of opioidergic projections from the ventral pallidum to substantia nigra
Julio Bernardi

10 - Opioid receptors modulate skin ageing and melanocytic disorders
Bigliardi PL1

11 - μ-Opioid receptor trafficking in human keratinocytes
Bigliardi-Qi M*

12 - Opioid modulation of synaptic transmission in affective pain circuitry
William Birdsong

13 - Antidepressant activity of the buprenorphine analog BU10119
Caroline A. Browne

14 - Peripherally restricted opioid combination therapy synergizes in multiple pain states
Daniel J. Bruce

15 - Characterization of opioid receptor subtypes and biased signaling as factors for modulation of alcohol use in mice.
Robert J. Cassell

16 - Nucleus accumbens mu-opioid receptors are recruited and necessary for the enhancement of motivated behaviors
Daniel C Castro

17 - Characterization of Sigma 1 Receptor (S1R) antagonist CM-304 and a related analog, AZ-66: Evaluating novel therapeutics for allodynia and induced pain
Thomas J. Cirino

18 - Oxycodone-induced conditioned place preference and locomotor activity in male and female Oprm1 A112G mice
Devon Collins

19 - A neural network for abstracting nociceptive information into an aversive pain perception
Gregory Corder

20 - On Target effects of Mu opioid receptor activation on brain activity and connectivity identified by mouse fMRI
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21 - Differential regulation of delta opioid receptor-mediated behaviors by arrestin2 in mice
Isaac J. Dripps

22 - Distinct MOR agonist profiles revealed by visualizing Mu opioid receptor trafficking with MOR-Venus mice
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23 - Percutaneous Electrical Nerve Stimulation (Electro-Acupuncture) elevates Agmatine in Spinal Cords of Nerve-Injured Rats.
Samuel J. Erb

24 - Evaluation of opioid-nociceptin bifunctional ligands by biochemical, pharmacological and structural modeling tools
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25 - How did the Opioid System Evolve?
Chris Evans

26 - The role of striatal mu opioid receptor populations in reward-related behaviors.
Christopher Evans

27 - The role of the deorphanized receptor, GPR83, in stress and reward.
Amanda K. Fakira

28 - Assessment of ZH853, a novel endomorphin analog, versus morphine in acute and long term dosing for chronic pain.
Amy Feehan

29 - Structural Insights into Opioid Peptidomimetics with Bifunctional Mu-Opioid Agonism and Delta-Opioid Antagonism
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30 - Structural and Dynamic Elements of μ-Opioid Receptor Functional Selectivity
Marta Filizola

31 - Characterizing the biochemical and synaptic properties of CNIH3, a novel component in individual risk for opioid dependence
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32 - Mechanistic insights into opioid-induced chemoresistance in breast cancer cells: DOR stimulation increases P-glycoprotein abundance by exploiting EphA2/c-src signaling
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33 - Investigating delta opioid receptors in a new conditional knockin mouse
Louis Gendron

34 - Oxycodone conditioned place preference alters hippocampal mossy fiber leu-enkephalin levels in a sex-dependent manner
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35 - Delta-opioid receptor (DOR) antagonism/inverse agonism reduces the development of mu-opioid receptor (MOR) tolerance
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36 - Selective Inhibition of M5 Muscarinic Acetylcholine Receptors Attenuates Remifentanil Self-Administration without Blocking Morphine-induced Analgesia in Rats
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37 - Functional consequences of OPRM1 A118G (MOR N40D) on human neurotransmission
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38 - Crucial role of the κ-opioid receptor system in tumor angiogenesis
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43 - Dermatolgical manifestations in substance abuse and how the community responses in developing country
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44 - Opioid receptors reveal a parallel cycle of regulated presynaptic membrane trafficking
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45 - Probe Dependence of High and Low Affinity Agonist Binding to the Delta Opioid Receptor is Allosterically Regulated by BMS-986187 and Na+ Ions
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46 - Allosteric Agonism at the Delta Opioid Receptor: Potential Mechanism for Functional Selectivity
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47 - Allosteric activation of delta opioid receptors in vivo: promising behavioral profile
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48 - Analysis of medication overuse headache after repeated morphine or THC administration using home cage wheel running
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51 - Norbuprenorphine pharmacokinetics and pharmacodynamics: First in man evaluation
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52 - Serum and urine concentrations of morphine and morphine metabolites in patients with advanced cancer receiving continuous intravenous morphine: an observational study
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53 - Evaluation of the dual kappa and delta opioid receptor agonist MP1104 in rat models of cocaine self-administration, analgesia and behavioural side-effects
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54 - Regulation of morphine sensitivity and adenylate cyclase sensitization by carboxyl-terminus of V1b vasopressin receptors
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55 - Generation and characterization analysis of human iPS cell derived-sensory neurons as a useful research tool for pain and opioid systems
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56 - Factors associated with ketamine use in pancreatic cancer patient in a single hospice center
Kyung min Kwon

57 - Sex differences in opioid signaling
Emily Leff

58 - Genetic deletion of mu opioid receptors from Kölliker-Fuse neurons reduces morphine-induced respiratory depression
Erica Levitt

59 - Cannabinoid CB2 receptor plasticity in rostral ventromedial medulla in inflammatory pain
Ming-Hua Li*

60 - The CB2 cannabinoid receptor agonist LY2828360 suppresses chemotherapy-induced neuropathic pain with sustained efficacy and attenuates morphine tolerance and dependence
Xiaoyan Lin

61 - Kappa opioid receptor up-regulation contributes to mood and reward dysregulation in neuropathic pain
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63 - AT-328, a selective NOP agonist, reduces the rewarding action of ethanol and cocaine
Kabirullah Lutfy

64 - The role of mu opioid receptors in hyperlocomotion and sensitization induced by amphetamine
Kabirullah Lutfy

65 - Male but not female mice lacking nociceptin exhibit higher anxiety and enhanced reward following exposure to the elevated plus maze test
Kabirullah Lutfy

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67 - Dissecting dopamine pathways altered by pain-induced dysfunction in opioid signaling
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Nicolas Massaly

69 - Design and Synthesis of Collybolide Probes for Kappa-Opioid Receptor
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70 - Trafficking and Signaling of Endogenous Mu-Delta Heteromers
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71 - Measurement of Heroin Antibody Binding Affinity Using Microscale Thermophoresis
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72 - Neuroinflammatory effects of HIV-1 Tat protein correlate with elevated brain dopamine levels and increased morphine consumption and conditioned place preference (CPP), and are prevented by indomethacin treatment
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76 - Identifying the role of peripheral delta opioid receptors in chronic migraine
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80 - The effects of commonly used opioids in cell cultures
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81 - Metformin acting through the mammalian target of rapamycin complex 1 (mTORC1) attenuates morphine efficacy in a mouse model of neuropathic pain.
Ilona Obara

82 - Development of a Selective Antagonist for the Mu-Delta Heterodimer
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85 - A novel non-canonical role of RGS4 in δ-opioid receptor mediated neuronal outgrowth and differentiation
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101 - Effect of Magnetic Acupuncture Attachment on Neigun(acupoint P6) on Autonomic Nervous System
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110 - Overlapping mechanisms between opioid induced hyperalgesia and chronic migraine
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111 - Influence of tramadol in global cerebral ischemia-induced mechanical allodynia
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112 - In Vitro And In Vivo Profile Of PPL-101 And PPL-103: Mixed Opioid Partial Agonist Analgesics With Low Abuse Potential
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113 - Effects of Heroin on Prosocial Behavior in Rats
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115 - Automated detection of Mu Opiod Receptor recycling reveals distinct subpopulations of exocytic events
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116 - Development of a Virtual Reality Paradigm for in vivo Hippocampal Imaging During Morphine Conditioned Place Preference
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117 - Activation, Desensitization and Trafficking of total phosphodeficient MORs in the MOR-knockout rat
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118 - Inflammatory Pain Changes The Expression and Function Of CB1 Receptors in The Periaqueductal Gray
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122 - Alternatively spliced mu opioid receptor C termini impact the diverse actions of morphine
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Speaker Abstracts

Plenary 1 - An expanding view of functional selectivity in GPCR-directed drug action

Mark von Zastrow; UCSF

The concept of functional selectivity in GPCR-directed drug action has its early roots in the cell biological study of opioid receptors and, although highly controversial when first proposed, is now generally accepted to the point of motivating modern drug discovery campaigns. The biochemical and cellular basis of functional selectivity, however, remains enigmatic. An early hypothesis that is now widely recognized, and presently called biased agonism, posits that functional selectivity among ligands is determined through differential coupling of ligand-occupied receptors to heterotrimeric G proteins relative to β-arrestins. I will briefly discuss the biochemical genesis of such agonist bias at opioid receptors, focusing on ligand discrimination mediated by receptor multi-phosphorylation. I will then discuss emerging evidence suggesting that there exists a discrete and additional source of functional selectivity among opioid receptor-directed ligands, determined by differences in the subcellular location at which receptors are activated. Such 'spatial bias' is particularly striking in neurons and has interesting implications because it reveals cellular effects produced by alkaloid drugs such as morphine that are not produced by peptide agonists. I will then generalize the concept of spatial bias to other GPCRs, focusing on mechanistic insight into spatial drug discrimination gained through study of adrenergic receptors. Finally, I will discuss ongoing efforts to more incisively elucidate functional selectivity using proximity labeling and quantitative proteomic methodologies.

Session 1: Endocytic control of functional selectivity of opioid receptors

Zara Y. Weinberg, Tiffany Phan, Manojkumar Puthenveedu PhD; Carnegie Mellon University, Pittsburgh, PA 15213

Functional selectivity at the μ opioid receptor (μR) has been a major focus for drug discovery in the recent past. Efforts to modify functional selectivity has focused on developing new orthosteric or allosteric modulators that target μR itself. We test the hypothesis that functional selectivity of any given agonist can be independently modified by trafficking events that regulate spatial localization of μR. We identify specific sequence elements on μR that determine the dynamics of μR residence in specialized endocytic domains, along with arrestin. Different ligands can leverage sequence interactions on μR to regulate receptor lifetimes in spatially separated membrane domains, and, therefore, regulate the magnitude of arrestin signaling. Independently manipulating endocytic dynamics was sufficient to modulate arrestin signaling without changing G protein mediated signals. Our results show that functional selectivity in the opioid system can be independently tuned by changing the spatial localization of receptors.

Session 1: mTOR pathway is involved in KOR agonist-induced aversion

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The KOR agonist nalfurafine is used in Japan for treatment of uremic pruritus in hemodialysis patients; significantly, at the therapeutic doses, dysphoria was not observed as an adverse reaction. We took a "bedside to bench" approach to investigate possible mechanisms underlying KOR-mediated aversion. Nalfurafine produced conditioned place aversion (CPA) in mice at higher dose than the doses causing analgesia and anti-scratch, whereas the opposite was true for MOM-SalB and U50,488H, two structurally distinct KOR agonists. At ~A_{50} in the anti-scratch test, U50,488H, but not nalfurafine, significantly increased the baseline threshold in intracranial self-stimulation. Thus, our findings on nalfurafine mimicked clinical observations. In mouse brains, at the lowest doses producing maximal anti-scratch effects, U50,488H and MOM-SalB promoted robust KOR phosphorylation at T363 and S369, but nalfurafine did not. Shotgun phosphoproteomic analyses provide an unbiased view of changes in downstream phosphorylation events. In mouse striatum, more than 390 phosphosites were different between U50,488H and nalfurafine treatment. In addition, the two agonists activated distinct downstream pathways in different brain regions. For example, proteins in the mTOR signaling pathway were phosphorylated by U50,488H, but not by nalfurafine, in striatum and cortex; however, no differential phosphorylation of mTOR pathway was found in hippocampus. Inhibition of the mTOR pathway by rapamycin abolished U50,488H-induced CPA, without affecting analgesic and anti-scratch effects. These results indicate that the mTOR pathway is involved in U50,488H-induced CPA and lack of CPA by nalfurafine may be related to its inability to cause KOR phosphorylation and to activate the mTOR pathway.

Session 1: Peripheral Opioid Receptor Regulation

Nathaniel A. Jeske PhD; University of Texas Health Science Center of San Antonio

Opioid receptors expressed in peripheral sensory neurons have the ability to serve as a significant analgesic target in multiple pain phenotypes. Furthermore, targeting peripheral opioid receptors significantly reduce addiction potential and avoid other serious, negative side effects associated with central nervous system opioid receptor activation. However, opioid receptors expressed in nociceptors exist in an analgesically desensitized state. New research from our group has uncovered the mechanism behind this reduced responsiveness, and has identified a target for pharmaceutical consideration that would increase analgesic efficacy of opioids in the periphery. This avenue for treatment would reduce systemic dosing requirements to alleviate pain, thereby addressing treatment concerns associated with the current and ongoing opioid epidemic.

Session 1 - GPCR signaling platforms for pain and analgesia

Meritxell Canals PhD; Monash University, Australia

The realization that G protein-coupled receptors (GPCRs) can continue to signal once internalized has triggered major revision of the classical view of GPCRs as proteins predominantly signaling from the plasma membrane. In addition, it has become apparent that the localization of a GPCR *within* the plasma membrane can also dictate its signaling outcomes. It has therefore become important to understand how both receptor and signal compartmentalization dictate cellular and physiological responses. I will present work that illustrates these concepts in the context of the mu-opioid receptor (MOPr) and the neurokinin 1 receptor (NK_1R), two receptors that are key in the (patho)physiology of pain and analgesia. First, using a range of biophysical approaches (including FRET and BRET biosensors) we have shown how the localization of the MOPr within the plasma membrane once activated by different ligands dictates distinct spatiotemporal signaling profiles. Second, combining FRET and BRET with *ex vivo* and *in vivo* experiments, we have recently shown that the NK_1R generates signals from endosomes that underlie pain and reveal a critical role for endosomal signaling of GPCRs in complex pathophysiological processes. Taken together, our results illustrate the importance of understanding molecular mechanisms that govern the formation and location of GPCR signaling platforms in order to provide new venues for the generation of safer and more effective analgesics.

Session 2: Circuit dynamics of in vivo dynorphin release in the nucleus accumbens shell

Ream Al-Hasani[1,2,3], Jenny-Marie T. Wong[6], Jordan G. McCall[1,2,3], Omar S. Mabrouk[6], Gavin Schmitz[1,2], Dionnet L. Bhatti[1], Kirsten Porter-Stransky[4], Brandon Aragona[4], Robert T. Kennedy[4], Michael Bruchas[1,3]; [1]Washington University, St. Louis, MO; [2]St. Louis College of Pharmacy; [3]Washington University School of Medicine; [4]University of Michigan, Ann Arbor, Michigan

We recently used an optogenetic approach to demonstrate that stimulation of dynorphinergic cells in the ventral nucleus accumbens shell (vNAcSh) elicits robust aversive behavior and photostimulation of dorsal NAcSh dynorphin (dNAcSh) cells induces a place preference and is positively reinforcing. Both of which appear to be dependent on kappa opioid receptor (KOR) activation. To follow these recently published findings, we are investigating how KOR is able to mediate these opposing behaviors in two distinct regions of the NAcSh. We are using an opto-microdialysis approach which combines optogenetics with microdialysis for use in awake, freely moving mice. This system allows quantification of neuropeptide release while directly modulating cell-type specific neuronal firing in the NAcSh. We have identified that the amount of dynorphin and met-enkephalin released during optogenetic stimulation is equal in the dNAc and vNAc. Interestingly, release of leu-enkephalin and dopamine is only detectable following photostimulation in the dNAc release. To further understand the circuitry driving the opposing unique behaviors and distinct neuropeptide release profiles, we are mapping the projections to and from discrete regions with the dyn-reporter mouse (dyn-$Cre^{tdTomato}$) and using tracing approaches (Rabies, canine adenovirus and cholera-toxin B). Thus far we have identified projections from the lateral septum, dorsal and ventral tegmental area (VTA). Together these experiments will help us understand how these distinct populations of dynorphin neurons in the NAcSh are engaged, altered, and recruited in stress and reward-related behaviors. Supported by NIDA K99/R00 DA038725 (RA), NIDA R01 DA037152 (MRB) and R01 DA037152 EUREKA NIDA (MRB).

Session 2: Kappa-opioid receptor control of nucleus accumbens synaptic integration differentially gates D1 and D2 MSN activity

Hugo Tejeda; National Institute on Drug Abuse

Endogenous dynorphin signaling via the kappa-opioid receptor (KOR) in the nucleus accumbens (NAcc) powerfully mediates negative affective states and stress reactivity. Excitatory inputs from the hippocampus and amygdala play a fundamental role in shaping the activity of both NAcc D1 and D2 MSNs, which encode positive and negative motivational valences, respectively. However, a circuit-based mechanism by which KOR modulation of excitation-inhibition balance modifies D1 and D2 MSN activity is lacking. Here, we provide a comprehensive synaptic framework wherein presynaptic KOR inhibition decreases excitatory drive of D1 MSN activity by the amygdala, but not hippocampus. Conversely, presynaptic inhibition by KORs of inhibitory synapses on D2 MSNs enhances integration of excitatory drive by the amygdala and hippocampus. In conclusion, we describe a circuit-based mechanism showing differential gating of afferent control of D1 and D2 MSN activity by KORs in a pathway specific manner.

Session 2: Upregulation of dynorphin in the central nucleus of the amygdala mediates the negative emotional states of nicotine withdrawal but not escalation of nicotine intake.

Olivier George PhD; The Scripps Research Institute

Abstinence from nicotine often results in emergence of a negative emotional state that predict relapse and escalation of nicotine intake in humans and rats. Prodynorphin and activation of kappa receptors have been shown to produce withdrawal-like symptoms, suggesting that activation of the dynorphin-kappa system may be responsible for the emergence of a negative emotional state leading to escalation of nicotine intake. However, the causal role of activation of the dynorphin-kappa system on measures of negative emotional states and nicotine intake in dependent animals remains to be demonstrated. To test this hypothesis we tested the effect of viral vector downregulation of prodynorphin in the central nucleus of the amygdala on withdrawal induced-pain, conditioned place aversion, escalation of nicotine intake and stress-induced reinstatement. Immunohistochemical analysis showed that prodynorphin's content was increased in the CeA in nicotine dependent rats during abstinence, but not in non-dependent rats. Downregulation of CeA prodynorphin using AAV-shPdyn did not affect nicotine escalation, but significantly decreased withdrawal-induced hyperalgesia, aversion to withdrawal and stress-induced reinstatement using the pharmacological stressor Yohimbine (1.25mg/kg). These results demonstrate that while activation of kappa receptors mediates both the negative emotional state of withdrawal and the increased motivation for nicotine after abstinence, increased prodynorphin levels in the CeA only mediates the negative emotional state of nicotine withdrawal. This report provides preclinical evidence for the efficacy of kappa antagonists in reducing the motivational effects of nicotine withdrawal, and identify that upregulation of prodynorphin in the CeA is responsible for the emergence of the negative emotional state of nicotine withdrawal.

Session 2: Central amygdala Prepronociceptin-expressing neurons mediate palatable food consumption and reward

J. Andrew Hardaway[1], Christopher M. Mazzone[1], Dipanwita Pati[1], Michelle Kim[1], Jennifer Jensen[1], Jeffrey F. Diberto[1], Ami Shiddapur[1], Jonathan A. Sugam[1], Michael P. Saddoris[1], Greg Tipton[1], Zoe McElligott[1], Michael R. Bruchas[2], Garret D. Stuber[1], Thomas C. Jhou[1], Cynthia M. Bulik[1], Thomas L. Kash[1]

[1]UNC Chapel Hill, [2]Washington University

Food palatability is one of many sensory factors that drive food consumption, and the drive to feed for its rewarding properties is a key contributor to obesity and binge eating. We have identified a population of cells expressing prepronociceptin, the gene encoding the opioid-like neuropeptide nociceptin, in the central amygdala (*Pnoc*^{CeA}) that are activated by palatable food consumption. Ablation or chemogenetic inhibition of these cells reduces palatable food consumption. Further, ablation of *Pnoc*^{CeA} cells reduces the increase bodyweight and adiposity accompanied by continuous high fat diet access. *Pnoc*^{CeA} neurons project to the vBNST, PBN, and NTS, and activation of axons in the PBN and NTS produces reward behavior. These data suggest that the *Pnoc*^{CeA} hindbrain network is critical for promoting the reinforcing and rewarding properties of palatable food. In my current work, I am using slice electrophysiology and *in vivo* Ca^{2+} imaging to measure the recruitment of *Pnoc*^{CeA} cells during palatable food consumption and the impact of nociceptin receptor signaling on synaptic physiology.

Session 2: Midbrain nociceptin neurons modulate reward behaviors

Kyle Parker PhD; Washington University School of Medicine in St. Louis

Nociceptin/Orphanin FQ Opioid Receptor (NOPR) and its endogenous ligand, nociceptin (NOFQ) are widely distributed throughout the brain and have been demonstrated to have a role in mediating pain, stress, anxiety, feeding, and reward behaviors. In particular, NOPR stimulation has been shown to inhibit mesolimbic dopamine transmission and reduce reward-related behaviors. However, the endogenous sources of nociceptin within these reward circuits are not completely understood. Using a novel transgenic prepronociceptin (PNOC)-Cre mouse and retrograde tracing techniques, we examined a population of nociceptin neurons located within the paranigral and paraintrafascicular nuclei of the ventral tegmental area (VTA). We found that these nociceptin neurons have monosynaptic projections onto dopamine cells and ablation of these cells enhances sucrose seeking under fixed and progressive ratio operant tasks. In addition, we found that chemogenetic stimulation of these nociceptin neurons reduces operant responding under the same progressive ratio task and can drive a conditioned place aversion (CPA). It was also found that optogenetic stimulation of these VTA-projecting cells during a real-time place Preference task drive aversion-like behaviors as well as reduce operant behavior performance. These findings show a previously unknown population of nociceptin-containing neurons that are positioned to tonically suppress reward motivation via dopamine cell inhibition. Understanding how this discrete population may be critical to the regulation of normal reward seeking behaviors could provide insight into behaviors dysregulated during motivational states such as addiction.

Plenary 2: Sex, Pain and Microglia

Michael W. Salter MD PhD; Hospital for Sick Children & University of Toronto, Toronto, Canada

Neuron-microglial interactions are increasingly recognized as being key for physiological and pathological processes in the CNS. Microglia are found to play a causal role in neuropathic pain behaviours resulting from peripheral nerve injury, and a core neuron-microglia-neuron signaling pathway has been elucidated. Within the dorsal horn, microglia suppress neuronal inhibition by a cascade involving activation of microglial P2X4 receptors causing the release of brain derived neurotrophic factor (BDNF). BDNF acts on trkB receptors leading to a rise in intracellular Cl^- concentration in dorsal horn nociceptive output neurons, transforming the response properties of these neurons. In addition to suppressing inhibition, peripheral nerve injury causes activity-dependent potentiation at dorsal horn glutamatergic synapses which enhances nociceptive transmission. BDNF mediates the enhancement of synaptic NMDAR responses through activation of TrkB and the Src-family kinase, Fyn. We have discovered that microglia-to-neuron signaling is not only critical for pain hypersensitivity after nerve injury but also for the paradoxical hyperalgesic effect of morphine and other opioids. This core signaling pathway has been extensively characterized, in studies using male mice. We have recently discovered that in female mice, however, pain hypersensitivity depends upon the adaptive immune system, likely upon T cells. Despite this profound difference in cellular mechanisms, pain hypersensitivity in female mice is as robust as that in male mice. Taking into consideration sex differences in the spinal immune-neuronal signaling has important implications ranging from diagnostics, to therapeutics, to prevention of chronic pain. Supported by CIHR, Krembil Fdn, CRC, Anne and Max Tanenbaum Chairs, and Northbridge Chair.

Session 3: The potential of δ-opioid receptors as novel target for alcohol use disorders

Richard M. van Rijn[1,2,3], Terrance Chiang[1], Meridith T. Robins[1,3], Robert J Cassell[1,2,3], Mohamed El-Sayed[1,3], Mark S. Cushman[1,3]; [1]Purdue University; [2]Purdue Institute for Drug Discovery, [3]Purdue Institute for Integrative Neuroscience

Alcohol use disorder (AUD) is defined as a chronic, relapsing brain disease and as such carries a large socioeconomic burden; it is estimated that 17 million adults in the United States suffer from AUD. The delta opioid receptors (DORs) are an exciting new target for treatment of AUD as activation of DORs can not only reduce alcohol intake, but can also reduce anxiety and depression, which are often co-morbid with AUD. We have previously shown that depending on the agonist DOR activation can either decrease or increase alcohol use in mice. We have recently identified that the ability of DOR agonist to modulate alcohol consumption in mice is tightly correlated with a DOR agonist's ability to recruit beta-arrestin2, such that weak beta-arrestin recruiters decrease intake, but strong beta-arrestin recruiters increase alcohol intake. Here we report on our ongoing efforts to pharmacologically characterize DORs for their role in modulating alcohol-related behavior using an approach that runs the gamut from medicinal chemistry, to molecular and cellular pharmacology ending with preclinical testing in mouse models of alcohol use. Specifically, we will provide evidence that implicates the dorsal striatum as important brain region for the DOR-mediated effects and that biased signal transduction strongly impacts the directional response of alcohol use. We will also describe our efforts to synthesize novel DOR-selective, G-protein biased agonists. Finally we will discuss the impact of interspecies differences on drug development of signal-biased DOR agonists.

Session 3: A role of epigenetically regulated dynorphin/K-opioid receptor and Neuropeptide Y system in alcohol tolerance

Subhash C Pandey PhD, Martina Palmisano, Harish Krishnan, Huaibo Zhang
University of Illinois at Chicago & Jesse Brown VA Medical Center Chicago, IL

Alcohol tolerance has been associated with promoting alcohol intake. Evidence suggests that the dynorphin (DYN) /kappa opioid (KOP) receptor and neuropeptide Y (NPY) along with brain-derived neurotrophic factor (BDNF) contributes to the negative reinforcing effects of alcohol. Here, we investigated the role of epigenetic regulation of DYN/KOP, NPY and BDNF systems in rapid ethanol tolerance (RET) to anxiolysis using an animal model. Adult male Sprague-Dawley rats were treated with ethanol (1g/kg intraperitoneal) or n-saline once or twice (24hr apart). Single but not two injections of ethanol exert anxiolysis. Ethanol increases PDYN mRNA levels in the amygdala (AMY) that remain increased after the second injection. Acute ethanol increases NPY and BDNF but not KOP mRNA levels. An increase in KOP receptor expression, but no change in NPY and BDNF expression was observed during RET. The changes in PDYN and KOP receptor gene expression do not seem to be mediated by H3K9/K14 acetylation or H3K9 dimethylation, but may be related to higher occupancy of trimethylated H3K27 and H3K4 at promoter regions. However, changes in NPY and BDNF expression may be regulated by H3K9/14 acetylation during RET. Interestingly, nor-BNI (KOP antagonist) and HDAC inhibitor treatment pretreatment or BDNF infusion into AMY prevented RET. Nor-BNI treatment increased NPY mRNA levels in the AMY of tolerant rats. Together, these data suggest that epigenetic regulation of DYN/KOP system and its interaction with NPY/BDNF in the amygdala could be involved in RET. Supported by NIH-NIAAA grants and by the VA Senior Research Career Scientist award to SCP

Session 3: The OPRM1 A118G SNP in drug reinforcement and reward: modulation of treatment response to naltrexone

Annika Thorsell PhD; Linköpings Universitet, Linköping, Sweden

Therapeutic responses to naltrexone may be moderated by variation at the mu-opioid receptor gene locus (OPRM1: Oprm-A118G). This however remains controversial since human results vary. We have generated “humanized” mice carrying the respective human OPRM1 A118G alleles (i.e.118AA and 118GG). Here, we examine the role of the OPRM1 A118G variation in animal models of drug reinforcement and reward, as well as in treatment response to opioid antagonist treatment (naltrexone and/or nalmefene). For reward, alcohol-induced dopamine-release in the nucleus accumbens was measured, as well as brain stimulation reward thresholds in the right medial forebrain bundle in the lateral hypothalamus. Reward-related behavior for alcohol and opiates was measured using the CPP model. Alcohol intake was measured using both operant and non-operant paradigms. Alcohol lowered brain stimulation reward thresholds in 118GG, but not 118AA, mice, and this was blocked by naltrexone. In addition, brain microdialysis showed greater peak dopamine-response to alcohol in 118GG than in 118AA mice. Furthermore, the A118G SNP modulated reward-related behavior in the CPP-model. In the home cage, 118GG showed increased alcohol intake and naltrexone selectively suppressed alcohol intake in 118GG. Similarly, both naltrexone and nalmefene were more effective in suppressing operant alcohol self-administration in 118GG mice. Therefore, OPRM1 A118G variation is a genetic determinant of dopamine responses to alcohol, a mechanism by which it likely modulates alcohol reward, and robustly moderates effects of opioid antagonism on alcohol reward and consumption. These

findings strongly support a personalized medicine approach to alcoholism treatment that takes into account OPRM1 genotype.

Session 3: The novel nociceptin receptor antagonist LY2940094 reduces ethanol-seeking and self-administration in animal models and significantly reduces heavy drinking in alcohol-dependent subjects

Linda Rorick-Kehn PhD; Lilly Research Laboratories, Indianapolis, IN

Nociceptin is a 17 amino acid peptide that produces physiological effects through activation of the opioid-receptor-like (ORL1, or NOP) receptor. The NOP receptor is localized in the mesolimbic reward pathway and has been suggested to play a role in feeding, mood, stress, and addiction. We discovered a novel NOP receptor antagonist (LY2940094) and found it to have high binding affinity (Ki = 0.11 nM), antagonist potency (Kb = 0.17 nM) and selectivity for NOP receptors. LY2940094 is orally bioavailable, CNS penetrant and potently occupies NOP receptors in vivo. Herein, we characterize LY2940094 in several rodent models of ethanol consumption. After Phase 1 safety studies, we conducted an 8-week proof-of-concept double-blind, placebo-controlled clinical study to evaluate the efficacy of LY2940094 in reducing alcohol consumption in heavy drinking subjects with alcohol dependence. In preclinical experiments, LY2940094 dose-dependently reduced homecage ethanol self-administration in rats, without affecting food/ water intake or locomotor activity. LY2940094 attenuated progressive ratio operant responding and breakpoints for ethanol in rats, as well as stress-induced reinstatement of ethanol-seeking. Furthermore, LY2940094 blocked ethanol-stimulated dopamine release in response to ethanol challenge (1.1 g/kg, IP). In the clinical study, LY2940094 increased abstinence rates in alcohol-dependent subjects and reduced both heavy drinking days and plasma GGT levels. Our findings demonstrate for the first time that blockade of NOP receptors attenuates ethanol self-administration and ethanol-motivated behaviors in rodents and may help alcohol-dependent individuals initiate and maintain abstinence from alcohol drinking. Results suggest that LY2940094 may have potential therapeutic utility in treating alcohol addiction.

Session 4: Protein kinase mechanisms in opioid-induced hyperalgesia

Zaijie Jim Wang; University of Illinois at Chicago

In addition to the development of drug addiction and tolerance, chronic treatment with opioid drug(s) also produces paradoxical opioid-induced hyperalgesia (OIH) in humans. OIH can also be established in preclinical models where opioid-induced hypersensitivity to cold (cold allodynia), heat (heat hyperalgsia), and mechanical stimuli (mechanical allodynia or hyperalgesia) can be demonstrated. Intriguingly, animals with OIH did not show condition place preference in an attempt to determine spontaneous pain. I will present evidence for potential roles of protein kinase in OIH especially those of CaMKII-alpha and protein kinase C isoforms. Elucidating the protein kinase-mediated cellular signaling pathways may help to understand the mechanisms of OIH and potentially offer new targets for interventions.

Session 4: Circuits and synaptic mechanisms for pain control by endogenous and exogenous opioids

Gregory Scherrer PhD; Stanford University, Stanford, CA, USA

Opioids represent the mainstay treatment for the management of severe pain; however, the mechanisms underlying their analgesic properties remain unclear. Elucidating the functional organization of the opioid system in pain neural circuits is urgently needed to develop innovative analgesic therapies with limited side effects. We use mouse genetics, neuroanatomy, electrophysiology, electrophysiology, opto/ chemogenetics and *in vivo* calcium imaging to localize opioid peptides and receptors along pain neural circuits and determine how opioids modulate the different components of pain experience. We found that mu opioid receptors (MORs) expressed by primary afferent nociceptors initiate analgesic tolerance and OIH development. Deletion of MORs specifically in TRPV1+ nociceptors eliminated morphine tolerance, hyperalgesia, and pronociceptive synaptic long-term potentiation, without altering antinociception. Furthermore, co-administration of methylnaltrexone bromide, a peripherally restricted MOR antagonist, is sufficient to abrogate morphine tolerance and in perioperative and chronic pain models. We also identified the spinal neurons expressing MOR, delta opioid receptors (DORs), and both receptors. In the dorsal horn, MORs and DORs are present in largely separate neural populations, with limited co-expression in subclasses of excitatory lamina II interneurons and NK1R+ lamina I projection neurons of the anterolateral tract. Conditional knockout experiments revealed that DOR agonists act on DORs expressed by somatostatin+ dorsal horn interneurons to reduce mechanical pain, but can also non-selectively activate spinal MORs to diminish heat pain. Unexpectedly, we report that the majority of spinal neurons co-expressing DOR and MOR correspond to ventral horn V1 inhibitory interneurons that regulate the activity of motoneurons.

Session 4: Therapeutic recovery from diminished morphine analgesia in chronic pain state

Hiroshi Ueda1, Hiroyuki Neyama; Nagasaki University Graduate School of Biomedical Sciences 1-14, Bunkyo-machi, Nagasaki 852-8521, Japan

We have often experienced the cases of less sensitivity or resistance of various types of pain to opioid treatments in clinic. In relation to such clinical evidence, our group has studied the diminished morphine analgesia and underlying mechanisms in various experimental models in mice, such as 1) morphine analgesic tolerance against acute pain following repeated treatments through an anti-opioid NMDA receptor mechanism (J Neurosci, 23:6529-6536, 2003), 2) decreased peripheral morphine analgesic activity in a model of neuropathic pain following partial sciatic nerve ligation/pSNL through an epigenetic gene silencing of MOPr (J Neurosci, 30: 4805-4814, 2010)) and 3) loss of central morphine analgesia in models of experimental fibromyalgia-like pain model following intermittent cold or psychological (empathy) stress in mice (Neurosci Lett, 472: 184-187, 2010; Neurobiol Pain, in press). In the present study we will introduce some successful examples to recover the diminished morphine analgesia in models of pSNL-type neuropathic pain and intermittent stress-induced fibromyalgia-like pain, by using novel inhibitors of interaction between repressor and co-repressor or NMDA receptor antagonists.

Session 4: Targeting RGSz1 complexes to optimize the actions of opioid analgesics

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Regulators of G protein signalling z1 (RGSz1) is a member of the RGS family of proteins, present in several networks expressing mu opioid receptors (MOPR). Using genetic mouse models for global or brain region-targeted manipulations of RGSz1 expression we demonstrate that prevention of RGSz1 action increases the analgesic efficacy of MOPR agonists in male and female groups of mice. Importantly, prevention of RGSz1 action delays the development of morphine tolerance, but decreases sensitivity to the rewarding and locomotor activating effects of morphine. We used information from next generation RNA Sequencing (RNA-Seq) analysis and applied a number of molecular and biochemical studies to understand the detailed signal transduction mechanisms by which RGSz1/Gaz complexes affect functional responses of MOPR in addiction and analgesia networks. Our studies reveal that RGS complexes promote the development of morphine tolerance by controlling the availability of Gaz protein to effector molecules, including components of the Wnt/beta-catenin pathway. Interventions in RGSz1-controlled signal transduction may be used to optimize the analgesic actions of opioids and decrease the risk of dependence or addiction.

Founder's Lecture: Opioid Research: Past achievements and future challenges

John Traynor PhD; Department of Pharmacology, University of Michigan, Ann Arbor, Michigan

Opiates and opioids have long fascinated scientists by the complexity of their chemistry, cell biology and physiology. It is easy to understand how the Founders of INRC come to introduce this conference as a forum to meet and discuss their latest research.

A reoccurring theme of the Founders lecture has been "How did we get here and where are we going next". In this spirit I will review previous research that has inspired my work, concentrating on the receptors, their ligands and signaling pathways. I will discuss findings on alternative approaches to traditional opioid therapies aimed to increase the activity of the endogenous opioid peptides. This includes enhancement of the activity of the receptors with allosteric modulators or promoting signaling downstream of the receptors with regulator of G protein signaling proteins.

However, in spite of all the outstanding science that has been carried out to understand the control of endogenous opioid systems, how opioid ligands activate receptors, the intricacies of signaling pathways and the mapping of brain circuits modulated by opioids, many challenges still remain if we are to understand the full beauty of the opioid systems.

Session 5: Probing whole-brain connectivity in live mice: receptors and drugs

Brigitte L Kieffer; McGill University, Douglas Research Center

In humans, resting state magnetic resonance neuroimaging (rsfMRI) has opened the era of connectome/imaging genetics, in order to elucidate how genetic factors affect brain organization and connectivity in healthy individuals and disease. Yet the causal impact of a single gene on anatomical, functional and effective connectivity remains largely unknown, and animal research is best suited to this goal. We tested whether rsfMRI in living animals would reveal connectivity alterations upon targeted inactivation of a single gene, the mu opioid receptor (MOR) gene (used here as a proof-of-principle). MOR is broadly distributed throughout the nervous system and mediates the remarkably potent analgesic and addictive properties of opiates like morphine. Hypothesis-free analysis of rsfMRI data revealed pronounced modifications of functional connectivity (FC) in MOR knockout mice, indicating for the first time that loss of one gene produces alterations at whole-brain level, which are detectable non-invasively in live animals. Further, fine-grained rsfMRI mapping captured a unique MOR gene-to-network signature and revealed strongest perturbations in connectional patterns of pain/aversion-related nodes, consistent with current knowledge of MOR function. We also found specific FC alterations in conditional mice lacking MOR in the striatum, as well as a very distinct signature in mice lacking another G protein coupled receptor, and all the latter findings strongly correlate to known behavioral phenotypes of these mice. This pioneering approach, which is fully translatable to human research, opens an entirely new view on functional implications of gene activities on whole-brain networks and how these ultimately drive behavior.

Session 5: Unbiased characterization of intercellular signaling peptides using mass spectrometry. Elena V Romanova PhD; University of Illinois, Urbana-Champaign

Often times there are indications of peptides involvement in the response to drug exposure, but (neuro)peptide identity may not be known. Mass spectrometry measurements referred to as peptidomics allow comprehensive, targeted or exploratory analyses of unknown, endogenous signaling peptides. The advantage of peptidomics is its ability to evaluate and characterize final gene products at the location of action, which provides information most other “omics” technologies cannot. Dynamic peptide level changes potentially associated with illicit drug use, pain or therapeutic response to narcotics can be evaluated. At the NIDA UIUC Neuroproteomics Center, we apply exploratory MS-based approaches to understand peptide changes in various brain areas relevant to effects of illicit drugs, stimulants, and pain medication. For example, using a rodent model of cocaine addiction, we showed that distinct peptide profiles in the mPFC statistically correlate with individuals that exhibit reduced sensitivity to the behavioral effects of acute cocaine administration. With AMPH, we found that chronic exposure leads to significant peptide dynamics in the brain’s reward circuitry, thus suggesting that in addition to the mesolimbic dopamine system concomitant chemical changes in other pathways and in neuronal organization may play a part in the overall effect of chronic AMPH exposure. In the most recent work, we used MS-based quantitation of peptide levels in mouse models of migraine and opioid-induced hyperalgesia to probe the role of neuropeptides in pain phenomena. The combination of peptidomics measurements with behavioral assessment of pharmacological intervention provides unique avenues for peptide discovery, functional analysis and, ideally, intervention and treatment.

Session 5: Human laboratory paradigms to study conditioned cues

Emma Childs PhD; University of Illinois at Chicago

Learned associations between a drug and the cues surrounding drug experiences are considered critically important in the development of addiction. These associations, formed through the processes of drug conditioning, are robust and long-lasting, inducing cravings and relapse long after the user is drug-free. However, few clinical studies have examined the conditioning processes and little is known about how associations are formed and how they influence behavior including drug use. Recently, we have established a human laboratory model of de novo drug conditioning, based on the Conditioned Place Preference (CPP) paradigm used in rodents. Using this model, we have shown that humans, like animals, come to prefer and spend more time in an environment paired with a rewarding drug. Moreover, individuals exhibit altered behavior, drug effects and drug taking in the drug-paired environment. Together the findings demonstrate that we can use this model to better understand how conditioned associations control behavior. This knowledge will help us to design novel approaches to counteract their powerful influences.

Session 5: Preclinical Assessment of opioid analgesia, side effects, and withdrawal

Michael Morgan PhD; Washington State University, WA, USA

A revolution in the preclinical assessment of nociception from pain-evoked to pain-depressed behaviors has occurred over the past decade. Pain-evoked tests such as the hot plate and von Frey tests require a response to a noxious stimulus. Such tests are useful for assessing nociception, but are inadequate for assessment of analgesic effects because of potential motor confounds (e.g., drugs can block responding on the hot plate test because of sedation or paralysis). In contrast, pain-depressed behavioral tests assess reductions in ongoing behaviors caused by pain. These tests are particularly useful for assessment of analgesia because analgesic and motor effects are opposite (e.g., analgesia is defined as a restoration of behavior, whereas a sedative drug reduces behavior). This presentation will describe a series of studies showing that home cage wheel running is a particularly useful pain-depressed behavior for the preclinical assessment of analgesic efficacy. Because home cage wheel running assesses normal daily activity, it is sensitive to a wide range of disruptive stimuli. Data will be presented showing depression of wheel running caused by pain (hindpaw inflammation, migraine), drug side effects (morphine, THC), and spontaneous morphine withdrawal. These findings indicate that home cage wheel running is an objective and clinically relevant method to screen treatments for analgesia, side effects, and withdrawal profile.

Session 5: Probing axonal trafficking with live cell imaging of pHluorin tagged Mu Opioid Receptor

Damien Jullie; University of California San Francisco

Much is known about the synaptic vesicle cycle and its local modulation by presynaptic GPCRs, but little is known about how GPCRs traffic at terminals. The super ecliptic pHluorin (SEP) is a pH sensitive variant of the GFP which is not fluorescent in an acidic environment like the lumen of intracellular compartments. Using a combination of live cell imaging and local perfusion, pHluorin is a useful tool to probe the trafficking of surface receptors. Using such approaches to study the trafficking of the Mu Opioid Receptor (MOR) in axons, we found that receptors can undergo ligand dependent endocytosis and that internalization is essentially happening within presynaptic terminals. After internalization, MOR containing endosomes support local recycling of the receptors back to the axonal surface, unravelling that another endocytic cycle operates within presynaptic terminals.

Plenary 3: Alterations of the opioid system in chronic pain

Catherine Bushnell; National Center for Complementary and Integrative Health, NIH, Bethesda, MD, USA

There is growing evidence that opioid systems may be disrupted in chronic pain. We examined in fibromyalgia patients and healthy controls the consequences of blocking mu-opioid receptors using naloxone on the pleasantness and intensity of skin brushing. At baseline, the healthy subjects preferred slow brushing over fast, and rated fast brushing as more intense. The pain patients showed a blunted distinction between slow and fast brushing for both pleasantness and intensity. Naloxone increased the perceived pleasantness of skin brushing for healthy subjects, but not for pain patients. In contrast, naloxone decreased touch intensity ratings only for patients. These findings revealed that opioids are involved in touch pleasantness, but this involvement is altered in chronic pain.

We also investigated, using a rodent model, the effect of chronic pain on opioid receptor availability. Studies have shown altered opioid receptor availability in pain patients, but it is not known if this is caused by the pain or related to other factors. Thus, we conducted a controlled 3-month longitudinal study, comparing nerve injured rats (spared nerve injury; SNI) to matched controls. Using [18F]-diprenorphine to label unbound opioid receptors and examining their density with PET, we confirmed in rodents the finding from pain patients of reduced opioid receptor availability in multiple brain areas, including the insula and caudate-putamen. These findings provide new evidence that chronic pain alters the brain opioid system, which might explain the limited effectiveness of opioid therapy in chronic pain patients.

Session 6: History of Prescription Opioid abuse and the Recent Transition to Heroin

Theodore Cicero; Washington University in St. Louis, School of Medicine

Two events late in the last century and early 2000s led to a surge in the use and abuse of prescription opioid drugs: First, a precipitous increase in opioid prescribing rates due to a renewed and perhaps ill-informed focus on treating pain; and, second, the introduction and heavily promoted extended release oxycodone (i.e., OxyContin) which led to diversion of unprecedented amounts of pure oxycodone unadulterated with acetaminophen/NSAIDs. Over the past 15-20 years, admission to treatment programs for opioid use disorder, opioid overdose fatalities and opioid-related emergency room visits have reached epidemic levels. Recently, a number of steps have been taken to reduce the diversion and abuse of these drugs, including the development of abuse-deterrent formulations and efforts to promote better utilization of these products for therapeutic purposes (e.g., closure of "pill mill", prescription monitoring programs to deter "doctor shopping"). Many of these efforts appear to have been successful, but there was a major unanticipated outcome. Faced with a reduced supply of prescription opioids in the drug-seeking culture, and the increased cost of those that were available, many users transitioned to heroin which became a much cheaper and far more accessible alternative. Thus, while the prescription opioid abuse problem shows signs of diminishing in its impact, a resurgence in the abuse of heroin has occurred with all of the complications of such use – overdose deaths and transmission of blood-borne pathogens (e.g., HIV and Hepatitis C).

Session 6: Pharmacological Therapies for Opioid Use Disorder: Implications of Fentanyl Availability on Treatment Effectiveness

Sandra D Comer; Columbia University and NYSPI

Misuse and abuse of opioids is currently a major public health concern. Efforts to address this problem include prescription drug monitoring programs, increased education for prescribers, development of abuse deterrent formulations of opioids, and expansion of access to medication-assisted treatment. Although misuse of prescription opioids appears to be reaching a plateau or declining slightly, heroin use is increasing and the number of overdose deaths attributable to prescription opioids and heroin is continuing to rise. A particularly disturbing new trend is the increase in illicit fentanyl and structurally related analogs that are now being sold on the streets. Fentanyl is either added to or sold as heroin or made to look like prescription drugs such as OxyContin or Xanax. Therefore, in many cases, users are unaware that they are injecting, insufflating, or ingesting fentanyl. Because fentanyl is 50-100x more potent than heroin, the risk of opioid overdose is increased. In addition, its margin of safety is narrow with regard to analgesic effects versus respiratory depression and it has high efficacy at mu opioid receptors. During this presentation, both preclinical and clinical research on fentanyl and structurally similar agonists will be shown in order to highlight the concerns about the risks of fentanyl abuse compared to other mu opioid agonists and how its pharmacology may make it more difficult to treat with the medications that are currently available for managing opioid use disorder.

Session 6: Prescription opioids and migraine: the basic and clinical science of a major public health issue

Andrew Charles; Headache Research and Training Program, UCLA, CA, USA

Migraine is one of the most prevalent of all disorders worldwide, and represents a common path to overuse of prescription opioids. The most commonly prescribed opioids are primarily mu receptor agonists. Despite the fact that the majority of patients report that they are not particularly effective in relieving migraine headache, these medications are nonetheless widely prescribed for migraine in the United States. There is now substantial evidence that use of currently available opioids for migraine is associated with worsening of the disorder, including greater frequency and severity of attacks, and reduced responsiveness to migraine-specific therapies. Underlying mechanisms for worsening of migraine by mu opioid agonists may be similar to those that cause opioid-induced hyperalgesia. Overuse of a prescription opioids by migraine patients is a significant public health issue. Potential approaches to dealing with this issue for migraine as well as for other pain disorders will be discussed.

Session 6: Blocking Pannexin-1 Channels on Microglia Alleviates Opioid Withdrawal

Tuan Trang; University of Calgary

The mechanisms involved in opiate withdrawal are poorly understood, and the limited clinical strategies for treating withdrawal are ineffective. Here, we identified the pannexin-1 (Panx1) channel as a novel therapeutic target for treating morphine withdrawal. We discovered that morphine treatment induces synaptic plasticity in spinal lamina I/II neurons, which manifests as long-term synaptic facilitation upon naloxone-precipitated morphine withdrawal. This synaptic facilitation is critically gated by activation of Panx1 channels expressed on microglia. Pharmacologically blocking Panx1, or genetically ablating this channel specifically from microglia, blocked spinal synaptic facilitation and alleviated the behavioural sequelae of morphine withdrawal. Targeting Panx1 represents a potential novel therapeutic approach for treating the symptoms of opiate withdrawal.

Young Investigator Award: Structural dynamics in G protein-coupled receptor signaling

Aashish Manglik MD PhD; Stanford University, Stanford, CA, USA

The recent determination of X-ray crystal structures of the four major opioid receptor subtypes has revealed key facets of opioid ligand recognition. These inactive-state structures, combined with a structure of the active μOR, have provided a high-resolution molecular description of opioid action. Using these structural insights, we have embarked on new computational discovery campaigns to identify novel opioid analgesics. An early success of uncovering new chemistry has been the discovery of a G_i biased opioid agonist, called PZM21, with unique signaling properties and *in vivo* efficacy. The remarkable efficacy of PZM21 and other novel biased opioids highlights the relevance of G protein-coupled receptor (GPCR) structural plasticity in opioid signaling and physiology. I will describe the approaches and tools that my newly-launched independent laboratory will employ to dissect the biophysical basis of opioid receptor signaling complexity. These approaches may ultimately enable rationally designed control of opioid receptor signaling and may portend exciting new ways to manipulate the vast physiology coordinated by the broader GPCR family.

Session 7: Kratom derived natural products as leads to design analgesics with mu opioid G-biased agonism and delta opioid antagonism

Susruta Majumdar; Memorial Sloan Kettering Cancer Center, NY, USA

Mitragyna speciosa or kratom has been used by Southeast Asian people for hundreds of years both as an analgesic and an opioid antidote. Mitragynine and 7-hydroxy mitragynine are two kratom alkaloids that have been studied in detail and are known to act as opioid receptor modulators. The pharmacology of a related semi-synthetic derivative, mitragynine pseudoindoxyl (MP) has not been well characterized. We have shown that MP is a G-protein biased mu opioid agonist and delta antagonist. It is a potent analgesic in mice, which exhibited no reward, attenuated respiratory depression and constipation when compared with morphine. In addition, MP showed slower development of tolerance and minimal physical dependence. The design, synthesis, and pharmacological evaluation of novel analgesics based on this template will be discussed.

Session 7: Discovery and development of oliceridine, a G protein-biased ligand targeting the mu-opioid receptor

Jonathan Violin PhD; Trevena Inc

Opioids are powerful analgesics and are widely employed for the management of moderate to severe acute pain, but cause many adverse effects, including gastrointestinal dysfunction, abuse and dependence liability, and potentially fatal respiratory depression. In the hospital and similar settings, injectable opioids remain necessary for many patients, but opioid-related adverse events (ORAEs) increase hospital costs and can threaten patient safety. Morphine pharmacology in β-arrestin2 knockout mice suggested that a ligand that promotes coupling of the μ-opioid receptor (MOR) to G proteins, but not β-arrestins, might result in higher analgesic efficacy, less gastrointestinal dysfunction, and less respiratory depression than conventional opioids. Using cell based assays and iterative medicinal chemistry, we sought and discovered oliceridine (TRV130) – a G protein biased MOR ligand that largely avoids the β-arrestin pathway. Oliceridine demonstrated a wider therapeutic window than morphine in rodents, showing less respiratory and gastrointestinal dysfunction at equianalgesic doses. Intravenous oliceridine has been compared to intravenous morphine in Phase 1, Phase 2, and Phase 3 clinical trials. Evidence from these studies, using multiple measures of efficacy, safety, and tolerability, suggest that oliceridine may offer patients who require intravenous opioids the pain relief associated with conventional opioids with potential for less post-operative nausea and vomiting, and reduced risk of opioid-induced respiratory depression. This presentation will review the discovery and development of oliceridine, with an emphasis on recent Phase 3 results. Oliceridine is an investigational agent not approved by the FDA.

Poster Abstracts

1 - Political Priority for Tobacco Control among Adolescents in Local Governments Areas, Osun State, Nigeria

Ademola Adelekan; University of Ibadan

Supervisory Counsellors for Health (SCH) have great responsibility to play for Tobacco Control (TC) as political head of health administration in Local Government (LG). This study therefore designed to determine the political priority for TC among adolescents in LG areas in Osun State, Nigeria. The study was a descriptive qualitative study. All consenting 27 out of 30 SCH in Osun State were interviewed using in-depth interview guide. Interviews took place at the participant's office at the time convenient for them. Data was recorded with tape recorder and analysis was done using thematic method. Majority of the respondents were aware that cigarette smoking among adolescents is high in Nigeria but did not know the significant of this extent. All the respondents were aware of TC policy in Nigeria but only few had ever read through this policy. Almost all the respondents were not aware of any international policy on Tobacco control. Many reported knowing at least five adolescents in their community who smoke cigarette and had a good knowledge of health consequences of tobacco smoking. Only few reported to have TC unit in their LG and among these, almost all reported that no specific programme in place for educating young people. Local Government SCH awareness of tobacco magnitude and political priority for its control among adolescents was low. Tobacco education programme should be intensified for political leaders to develop adequate measures on this issue and making necessary funds available.

2 - Effectiveness of Provider Initiative Approach for Smoking Cessation among Pregnant Women in Osun State, Nigeria

Ademola Adelekan; University of Ibadan

Women who smoke during pregnancy need assistance in quitting, and obstetric health care providers are in a unique position to help them. This study therefore determined the effectiveness of Provider Initiated Approach (PIA) for smoking cessation among pregnant women receiving Antenatal Care (ANC) services in selected primary health care facilities in Osun State, Nigeria. A total of 150 frontline health workers who are ANC providers were trained on PIA to help their patients quit smoking. They were equipped with smoking knowledge and skills on how to integrate smoking cessation with ANC services. Smoking cessation messages were discussed with women who reported smoking through micro-teaching, IEC materials and as well as client provider interaction. A total of 22,845 pregnant women were screened for smoking from March-September, 2014 and data were analysed using descriptive statistics and t-test. A total of 1426 (6.2%) respondents' were identified to be currently smoking with mean age of 27.5 ± 10.1 years. Other reported substances currently using among respondents were alcohol (98.6%) and marijuana (1.6%). A total of 12.9%, 56.9% and 32.7% reported to cease smoking complete within one, two and three months of this intervention respectively. Reasons adduced for smoking included social integration (76.4%), peer pressure (56.3%) and to reduce stress (45.8%). The prevalence use of alcohol and marijuana also decreased to 12.9% and 0.0% respectively. A significant reduction in smoking associated with this intervention was observed. More health care providers should be trained on using this approach.

3 - Ring Substitution in the Macrocyclic Opioid Peptide CJ-15,208 Alters the Opioid Activity Profile in vivo

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The macrocyclic peptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) exhibits mixed agonist/kappa opioid receptor (KOR) antagonist activity and prevents stress-induced reinstatement of drug-seeking behavior after oral administration. As part of our exploration of the structure-activity relationships (SAR) of this peptide, we incorporated different substitutions in the aromatic residues. Analogs were examined for opioid receptor affinity in radioligand binding assays and for both opioid agonist and antagonist activity *in vivo* after intracerebroventricular (i.c.v.) administration using the mouse 55 ^{o}C warm-water tail-withdrawal assay. Selected analogs were further evaluated for their ability to prevent stress- and cocaine-induced reinstatement of extinguished cocaine-conditioned place preference (CPP) responses. Incorporation of a substitution on one of the phenyl rings of CJ-15,208 was generally well tolerated by KOR, with variable effects on the affinity for mu opioid receptors (MOR). All of the analogs exhibited antinociception *in vivo* at a dose of 30 nmol i.c.v. The most potent of these analogs produced MOR- and KOR-mediated antinociception with an ED_{50} (and 95% C.I.) value of 5.95 (3.91-8.77) nmol i.c.v. and subsequent KOR antagonism. The identity of the substituent affected antagonist activity, with fluorination of either phenylalanine residue abolishing KOR antagonism *in vivo*. One analog exhibited antagonism of MOR and delta opioid receptors as well as KOR and prevented stress-, but not cocaine-induced, reinstatement of extinguished cocaine-CPP. In addition to advancing our understanding of their SAR, these results further support exploring the potential application of such macrocyclic peptides for the treatment of drug abuse.

This research supported by NIDA grant R01 DA023924.

4 - Identification of a novel interaction site of the mu-delta opioid receptor heterodimers

Doungkamol Alongkronrusmee, Hamed T. Ghomi, Shiqi Tang, Markus A. Lill, Richard M. van Rijn; Purdue University, West Lafayette, IN, USA

Mu opioid receptor (MOR) agonists remain highly effective for the treatment of pain, and would represent ideal analgesics if their side effects could be prevented. Evidence suggests that MORs can interact with delta opioid receptors (DORs) to form MOR-DOR heterodimers which may contribute to certain adverse effects of mu opioids. Our goal is to develop drug-like compounds that disrupt the heterodimers to lessen these side effects. Yet, a lack of tools to investigate the role of MOR-DOR heterodimers in preclinical and clinical models is stifling our ability to target the heterodimers with drugs. To aid in the development of such tools, it is important to understand the MOR-DOR interaction by determining which amino acids in the heterodimer interface play major roles in the MOR-DOR formation. We constructed DOR mutants to probe for destabilization of the MOR-DOR heterodimers. We used a multipronged approach including calcium signaling assay, bimolecular fluorescence complementation and co-immunoprecipitation to investigate the impact of mutations on the stability and function of the heterodimers. We confirmed that amino acids 242, 243 and 244 located in the intracellular loop, as previously reported by others, were crucial in stabilizing the heterodimers. Intriguingly, we identified novel amino acids 208, 209 and 288 to be necessary for the heterodimer formation. These amino acids are located on the extracellular regions, thereby providing a promising new target for the heterodimer disruption. These findings will move us closer to our goal to develop small-molecule drugs that can be used with opioids to prevent their side effects.

5 - Low Abuse Liability of the Endomorphin Analog ZH853 is Supported by Reduced Psychomotor Activation and Withdrawal.

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Opioids are highly effective analgesics, but their use is limited by adverse side effects. We previously demonstrated that an endomorphin analog (ZH853), a selective agonist of the mu-opioid receptor, provides potent analgesia with reduced or absent side effects, including reduced reward, respiratory depression, motor impairment, tolerance, hyperalgesia, and glial activation as compared to morphine (1). Here we further characterize the potential of this analog for reduced abuse liability. The progression from casual drug use to addiction is thought to be initially motivated by positive reinforcing properties of the drug, mediated by activation of mesolimbic dopamine signaling. Later, continued drug use is driven by negatively reinforcing effects, including physical dependence (2). Our previous study showed that ZH853 failed to induce conditioned place preference or self-administration, indicating reduced reward (1). Increased locomotion in mice is an indicator of dopamine release, which in turn is associated with reward (3). Acute subcutaneous administration of morphine, but not equi-antinociceptive doses of ZH853, induced locomotor activation in CF1 mice, indicating differential dopaminergic activation. A negatively reinforcing property of abused drugs, withdrawal, was also assessed. Repeated exposure to ZH853 failed to produce naloxone-precipitated jumping or weight loss, significant signs of physical dependence. These data suggest ZH853 may provide effective long-term treatment with low risk of abuse compared to currently-used opioid medications.

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6 - Morphine influences cell proliferation, migration and apoptosis of tumor cell lines endogenously expressing mu opioid receptors

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Opioids are widely used to treat acute, chronic, post-operative cancer pain. Mu opioid receptor (MOR) is expressed in several human malignancies but opioid-mediated effects on uncontrolled cancer cell growth, invasion of normal tissues, resistance to apoptosis and tumor recurrence aren't still fully understood. We aimed at characterizing any morphine-mediated effect on cell proliferation, migration, apoptosis of different tumor cell lines expressing MOR. MOR mRNA and protein levels were preliminarily measured in each cell line; [^{3}H]thymidine incorporation, wound-healing and annexin assays were employed to evaluate cell proliferation, migration and apoptosis, respectively. HeLa cervix carcinoma cells displayed the highest MOR mRNA levels, followed by DAOY medulloblastoma, U87-MG astrocytoma, HT-29 colon-carcinoma. MOR protein expression, evaluated by receptor binding assay, did not mirror that of mRNA, as DAOY cells displayed the highest expression, followed by HeLa, U87-MG, HT-29 cells. Morphine tripled HeLa and HT-29 cell proliferation after 24h and 48h, respectively; HeLa migration after 18h, and only moderately increased apoptosis in both cell lines after 12h; these effects were sensitive to the MOR antagonist beta-funaltrexamine.Morphine increased U87-MG viability, migration and proliferation with peaks at 12h, 18h, 48h, respectively; beta-funaltrexamine and naloxone significantly reduced U87-MG and HeLa basal migration, possibly unmasking a contribution of endogenous opioids to cell migration.DAOY cells express the highest levels of MOR; however, morphine did not influence cell proliferation, migration or apoptosis, nor antagonized forskolin-induced cAMP accumulation. Sequencing MOR in DAOY cells and investigations on other medulloblastoma cell lines are currently ongoing and will be presented at the conference.

7 - OREX-1019; a small molecule with potential for addiction maintenance treatment and relapse prevention

Barbara Belli PhD[1], Charles P. France PhD[2,3], David R. Maguire PhD[2], Lisa R. Gerak PhD[2], Monica Bennett PhD[4], Gerta Cami-Kobeci PhD[5], Stephen M. Husbands PhD[5], Peter Flynn PhD[1]
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In the US, over 16 million people have used prescription opioids or heroin for non-medical purposes, resulting in death rate of nearly 9 per 100,000 population in 2016. OREX-1019 is a novel small molecule in development for addiction maintenance. Binding assays across the human opioid receptor family revealed an affinity for all receptors less than 10 nM; similar receptor binding affinities were obtained for buprenorphine. Agonist activity of compounds was evaluated by measuring the enhancement of [^{35}S] GTPgS binding and antagonism was evaluated by measuring inhibition of control agonist-induced [^{35}S] GTPgS binding. Agonist/antagonist activity (% effect) values for OREX-1019 were: mu (MOP) 21/69; kappa (KOP) 27/81; delta (DOP) 49/65; and nociception/orphaninFQ peptide (NOP) 24/34. Agonist/antagonist values for buprenorphine were: MOP 79/39; KOP 32/93, DOP 25/125 and NOP 0/100, respectively. OREX-1019 was tested in nonhuman primates (NHP) for its ability to maintain self-administration responding (i.v.) and its ability to block self-administration of remifentanil. Up to a dose of 1.0 mg/kg, OREX-1019 failed to maintain self-administration responding that was greater than vehicle. At doses of 0.1 or 0.3 mg/kg s.c., OREX-1019 reduced the average number of daily infusions of remifentanil (0.32 µg/kg/infusion) from 25-28 to 5-15. Collectively these results suggest that OREX-1019 might be effective for managing opioid addiction and have less abuse liability compared with currently available medications.

8 - OREX-1038; a potent analgesic molecule with potential for reduced abuse liability

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OREX-1038 is a novel opioid-based small molecule in development for acute and chronic pain. Binding assays across the opioid receptor family revealed potency at the human mu-opioid receptor (MOP) and the nociception/orphaninFQ peptide (NOP) receptor with pIC_{50} values of 10.04 and 8.3 nM, respectively. Agonist and antagonist activity was determined and OREX-1038 was demonstrated to have mixed agonist/antagonist activity at the human mu-opioid receptor (MOP, 50/46% effect) and agonist activity at the nociception/orphaninFQ peptide (NOP) receptor (40/0% effect). Following subcutaneous (s.c.) administration, OREX-1038 (20 mg/kg) exhibited potent antinociceptive effects in the Brennan rat model of incisional pain comparable to morphine (5 mg/kg s.c.). The duration of the analgesic effect was longer, 24 hr versus 6 hr for morphine. OREX-1038 did not affect motor coordination in the rat rotarod assay and did not reduce GI transit time. In the non-human primate (NHP) warm water tail withdrawal assay OREX-1038 (0.03 mg/kg s.c.) treatment increased tail withdrawal latency, between 40-60% of the maximum possible effect 2-3 hours after administration and in some experiments maintained the antinociceptive effect for up to 24 hrs. In a NHP self-administration study, OREX-1038 maintained responding that was greater than saline but less than remifentanil with some monkeys receiving 20 or more infusions of 1.0 µg/kg/infusion (30 times the maximally effective dose for antinociception). Self-administration of OREX-1038 decreased over consecutive sessions. These data suggest that OREX-1038 may be a potent, novel analgesic with significantly reduced potential for abuse.

9 - Rewarding effects of opioidergic projections from the ventral pallidum to substantia nigra

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Opioid addiction has become an epidemic, but the role of endogenous opioid circuits is still poorly understood. The ventral pallidum (VP) is one the strongest projections to the midbrain dopamine neurons and expresses enkephalin at high levels. We hypothesized that stimulation of VP enkephalin projections to the substantia nigra pars compacta (SNc) would be rewarding and mediated by enkephalin. We used Penk-IRES-Cre mice for optogenetic, chemogenetic and fiber photometry interrogations of this circuit. Optogenetic stimulation of VP enkephalin terminals in the SNc was reinforcing in real time place preference (RTTP) and operant intracranial self-stimulation paradigms. Chemogenetic inhibition of these neurons was aversive in a conditioned place aversion paradigm, demonstrating that the enkephalinergic VP-SNc circuit bi-directionally modulates reinforcement behaviors. To demonstrate that the evoked rewarding behaviors is due to signaling at the level of the SNc rather than antidromic activation of VP projections to other regions, we performed lidocaine microinfusions in the VP and photostimulation of VP terminals in the SNc, which failed to abolish the self-stimulation or real time preference behavior. To understand the dynamic circuit properties of the VP-SNc enkephalin neurons during reward-seeking behaviors, we measured GCAMP6s calcium transients of VP terminals in the SNc through fiber photometry in a Pavlovian paradigm with two cues, one that predicted sucrose reward availability and one that didn't. We found that VP-SNc differentially encoded both reward-predicting and nonreward-predicting cues with less than 200 ms latency. Together, our results implicate the enkephalin VP-SNc circuit as an important bidirectional modulator of reward and aversion.

10 - Opioid receptors modulate skin ageing and melanocytic disorders

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Typical signs of skin ageing are changes in skin thickness, pigmentation and delayed wound healing. On cellular level ageing affects differentiation, proliferation, migration of various cell types including keratinocytes, melanocytes and fibroblasts. Previously we have shown that the d- opioid receptor system (DOPr) affects differentiation and migration capabilities of the epithelial keratinocytes resulting in wound healing delay and significant alterations of the important skin differentiation upon DOPr expression level changes.

Further investigation of opioid receptor system in skin ageing, a multidecade study was performed on Chinese population comparing sun-exposed and unexposed skin. Small excisional skin biopsies were taken from 30 healthy Chinese women at different age from the anterior surface of the upper arm (sun-protected) and the posterior surface of the forearm (sun-exposed). Various parameters such as epidermal thickness, pigmentation and m- (MOPr) and d- (DOPr) expression in epidermis were evaluated in these different age group. Interestingly, the expression of DOPr (but not MOPr) decreased significantly with age. This was independent from photoageing and sign for biological ageing in skin. In addition the reduction of epidermal DOPr expression correlated significantly with an increase of epidermal melanin content with age. Consequently, we performed in-vitro experiments using monolayer 2D cultures of human pigmented cells and observe a significant modulation of melanin production in these cultures by various opioid ligands. Similar results were obtained by using 3D organotypic co-culture models.

This has implications on benign skin pigmentation in aged skin and other dyspigmentations in malignant melanotic cells with potential clinical applications.

11 - μ-Opioid receptor trafficking in human keratinocytes

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Opioids receptors are mostly studied for their function in the CNS, but less known for their peripheral effects. Our group discovered opioid receptors in skin and the epidermis of the skin, which shares the same ectoderm origin as the nervous system, is most affected by the regulatory function of the opioid receptors. This receptor system is not only involved in modulating peripheral sensation, but also in skin homeostasis, wound healing and skin ageing.

Topical treatment with the opioid antagonist naltrexone in chronic pruritus relieves itching by modifying the localization of the epidermal μ-opioid receptor (μ-OR), but the precise molecular mechanisms of μ-OR modulation are unclear. Here, we evaluated the real-time behavior of μ-OR–ligand complexes in the presence of OR agonist and antagonists. We conjugated the μ-OR ligand endomorphine-1 (EM) to the fluorescent dye to directly visualize OR dynamics in cultured keratinocytes. By live-cell imaging we identified fast trafficking of the endogenous fluorophore conjugated μ-OR complex. Furthermore, acute and chronic treatment of keratinocytes with naltrexone plus EM increased fluorophore conjugated μ-OR complex binding to the μ-OR. Overall, competition for the μ-OR with OR agonist (EM), or antagonists elicited canonical and non-canonical effects on basal and differentiated keratinocytes. Our approach has helped uncover the complexity of skin-specific μ-OR–ligand dynamics and the mechanistic consequences of antagonists and agonists on the μ-OR. Such studies will facilitate the development of novel treatment strategies for peripheral sensory disorders and could be used for predicting interindividual differences in OR behaviour and trafficking in the CNS.

12 - Opioid modulation of synaptic transmission in affective pain circuitry

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Affective/ motivational pain processing involves the medial thalamus, anterior cingulate cortex (ACC) and striatum. Opioid analgesics are known to modulate this circuitry and relieve the negative emotional effects of pain. However, the identity of the opioid receptors, their cellular locations, and their physiological effects on this affective circuitry remain uncharacterized. In the present study, synaptic connections between the three brain regions; thalamus, striatum, and ACC, were characterized based on the effect of opioid agonists to modulate glutamatergic transmission between these regions. Using optogenetic and electrophysiological approaches, it was determined that single striatal projection neurons in the dorsomedial striatum received direct input from both thalamic and cortical afferents. It was found that opioid receptors differentially modulated thalamic and cortical glutamate release in striatum. Additionally, μ and *∂*-opioid receptors were found to play distinct roles in modulating thalamo-cortical and intracortical synaptic transmission in the ACC. These data identify opioid receptor subtype-dependent inhibition of specific pathways within the thalmao-cortico-stiatal circuitry, providing insights into how opioid analgesics modulate affective pain at the synaptic and circuit level.

13 - Antidepressant activity of the buprenorphine analog BU10119

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Buprenorphine, a potent kappa opioid receptor (KOR) antagonist and mu opioid receptor (MOR) partial agonist, rapidly and effectively alleviates symptoms in treatment-resistant major depressed patients. Previously, our laboratory demonstrated that low-dose buprenorphine reversed behavioral deficits induced in two rodent models of depression, chronic mild stress and chronic social defeat. Moreover, we determined that at 24 h KOR antagonism mediates buprenorphine's antidepressant effects, whereas blockade of MORs facilitates buprenorphine's anxiolytic action. Development of buprenorphine as a treatment modality for depression may be complicated by its MOR agonist activity and consequently its potential abuse liability in a vulnerable patient population. Here we evaluated the behavioral effects of a novel buprenorphine analog, BU10119, which was modified to reduce efficacy at MORs while retaining the beneficial profile at KORs. BU10119 was screened in male C57BL/6J mice using the forced swimming test (FST) at 1 and 24 h post administration and in novelty induced hypophagia (NIH) 24 h post treatment and compared with buprenorphine, the selective KOR antagonist CERC-501 and the selective MOR antagonist cyprodime. BU10119 was active both at 1 and 24 h post admisntration, producing a similar inverted U-shape dose reponse curve to that of buprenorphine, reducing immobility in the FST and latency scores in the NIH, in the absence of MOR agonist induced hyperactivity. Cyprodime was only effective at 1 h. CERC-501 reduced immobility at both timepoints, but reduced latency scores only at the highest dose at 24 h. Overall, these data show that BU10119 retains the beneficial behavioral profile of buprenorphine.

14 - Peripherally restricted opioid combination therapy synergizes in multiple pain states

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The adverse effects of opioids are largely mediated by mu-opioid receptors (MORs) in the CNS. However, opioid receptors also mediate analgesia on the peripheral terminals of primary sensory afferents. Despite decades of research targeting peripheral MORs, no peripherally directed opioid has provided effective pain control without the risk for addiction and diversion. Because recordings from spinal cord slices had demonstrated presynaptic synergy between the MOR agonist loperamide (Lo) and the DOR agonist oxymorphindole (OMI) in the central terminals of nociceptors in spinal cord, we tested for anti-hyperalgesic synergy in the periphery. Using models of inflammatory, nerve injury, post-operative, or tumor-induced pain and intraplantar, systemic, or topical application showed that when combined in a 1:1 dose ratio OMI-Lo produces a robust, 50- to 100-fold anti-hyperalgesic synergy. This synergy was blocked by the peripherally restricted opioid antagonist, naloxone methiodide, reinforcing the peripheral localization of the effect. The synergistic interaction is also completely ablated when G protein-coupled, inwardly rectifying potassium channels (GIRKs) are blocked or knocked out. Although repeated dosing with supra-therapeutic doses induces tolerance, therapeutic doses do not. Initial self-administration studies also indicate a significantly reduced abuse liability compared to prescription opioids such as oxycodone. We conclude that MOR agonists significantly synergize with DOR agonists at peripheral sites of action, providing strong evidence in support of peripherally restricted combination opioid therapy. The systemic and topical efficacy of the combination, along with loperamide's extremely low abuse liability, suggests that this combination might be therapeutically useful to control multiple pain modalities in the clinic.

15 - Characterization of opioid receptor subtypes and biased signalling as factors for modulation of alcohol use in mice.

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The non-subtype selective opioid receptor antagonist naltrexone is one of the few FDA-approved drug treatments for alcohol use disorder. However, opioid receptor subtypes may each uniquely modulate alcohol use, exemplified by opposing alcohol consumption patterns in mu (MOR) and delta (DOR) opioid receptor knockout mice. Additionally, we have recently shown that biased signalling at DORs is correlated with alcohol intake (Chiang et al., 2016). We hypothesize that Gi-protein signalling is important for DOR selective agonists to reduce alcohol intake, but that other pharmacological mechanisms may determine how non-DOR selective agonists control alcohol use in mice. To test our hypothesis we characterized DOR and MOR agonists with different degrees of subtype selectivity and signalling bias in cellular assays and for their ability to modulate alcohol intake in C57BL/6 mice. We find that G-protein biased mixed agonists that activate DOR and MOR are capable of reducing alcohol drinking behavior in mice within volitional alcohol self-administration assays and that their effects are only partly abolished in DOR knockout mice. Interestingly the G-protein biased mixed MOR/KOR agonist TRV130 has no effect on alcohol use. Currently our data is suggestive of at least two distinct mechanisms by which opioid receptor agonists are able to modulate drinking behavior in rodents. We have synthesized DOR-selective G-protein biased agonists that could help further elucidate the mechanism by which DORs can reduce alcohol intake. The goal is to identify specific pharmacological properties of opioid receptor agonists that can be utilized to develop new drugs for the treatment of AUD.

16 - Nucleus accumbens mu-opioid receptors are recruited and necessary for the enhancement of motivated behaviors

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Mu opioid receptors (MORs) are involved in motivation for natural and drug rewards. One site for MOR action is nucleus accumbens medial shell (mNAc). MOR stimulation in this mesocorticolimbic hub enhances appetitive behaviors such as food intake and social interactions. By contrast, disruption of MOR signaling has yielded ambiguous results. Here, we sought to determine when mu opioid receptors are recruited to mediate motivated behaviors by disrupting MOR signaling during baseline or enhanced motivated states (i.e., ad libitum versus food deprived, social versus isolated housing). To disrupt MOR signaling, we used constitutive $MOR^{-/-}$ knockout (KO), conditional knockout (*Oprm1*$^{fl/fl}$), and pharmacological approaches. KOs showed normal baseline behaviors compared to wildtype controls, but failed to show enhanced motivation after food deprivation or social isolation. Targeted viral deletion of MORs in mNAc of *Oprm1*$^{fl/fl}$ mice showed a similar pattern, suggesting that MORs are recruited to enhance, not generate motivated behaviors. Next, to determine whether MORs are dynamically recruited, we microinjected CTAP (MOR antagonist) into mNAc. MOR blockade only reduced motivated behaviors when animals were in heightened motivated states. Finally, to pinpoint which neuronal populations are important in mNAc for MOR-mediated enhancements, we crossed *Oprm1*$^{fl/fl}$ mice with dynorphin- or enkephalin-cre mice to delete MORs from those neuronal populations. Results suggested that MORs act primarily via D1/dynorphin neurons, but not D2/enkephalin neurons. Future work will test MOR sufficiency to enhance behaviors by either selectively rescuing MORs in knockout mice or optically stimulating novel photosensitive MOR chimeras. This work was supported by grants T32NS007205 and R01DA042499.

17 - Characterization of Sigma 1 Receptor (S1R) antagonist CM-304 and a related analog, AZ-66: Evaluating novel therapeutics for allodynia and induced pain

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Sigma-1 receptors (S1R) are associated with activity-induced spinal sensitization and pain, particularly with respect to nerve injury. The purpose of this study was to investigate the in vivo analgesic and anti-allodynic activity of two novel sigma receptor antagonists, the S1R selective CM-304 and a related analog, the non-selective S1R/Sigma-2 receptor(S2R) antagonist AZ-66, in mouse assays of thermal, chemical, induced inflammatory pain, as well as the chronic nerve constriction injury (CCI) model of neuropathic pain. Both compounds produced time- and dose-dependent analgesia and anti-allodynic effects in each respective pain model. Interestingly, while less potent than morphine in a 55ºC warm-water tail-withdrawal assay, both CM-304 and AZ-66 produced antinociception in the writhing test (0.48 (0.09-1.82) and 2.31 (1.02-4.81) mg/kg, i.p., respectively), equivalent to morphine (1.75 (0.31-7.55) mg/kg, i.p.). Likewise, pretreatment (10-30 mg/kg, i.p.) with either sigma-receptor antagonist dose-dependently produced antinociception in the formalin paw assay. While the selective S1R antagonist CM-304 dose-dependently (10-45 mg/kg, i.p.) reduced allodynia in the CCI neuropathic pain model equivalent to the control analgesic gabapentin (50 mg/kg, i.p.), AZ-66 demonstrated a much longer duration of action. Place conditioning with either sigma-receptor antagonist with doses up to 45 mg/kg, i.p. produced neither rewarding nor aversive effects, and neither compound produced hyperlocomotion as observed with morphine (10 mg/kg, i.p.). Although each compound exhibited modest respiratory depression at high doses (30 mg/kg, i.p.), this seemed likely due to modest sedative effects as observed in the rotorod assay. Overall, this data demonstrates sigma receptor-selective antagonists produce antinociception with fewer liabilities.

18 - Oxycodone-induced conditioned place preference and locomotor activity in male and female Oprm1 A112G mice

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A common single nucleotide polymorphism (SNP) in the human mu-opioid receptor (MOR) gene (*OPRM1*), A118G, has been demonstrated to alter mu-opioid receptor function in healthy humans, and is associated with opioid addiction. Mice bearing an equivalent SNP, A112G, in the mouse MOR gene were generated, characterized, and studied (Mague *et al*., 2009). Male and female mice homozygous for the 112G allele show genotype-dependent reductions in morphine-stimulated locomotor activity and genotype-dependent, sex-specific reductions in morphine-induced conditioned place preference (Mague et al., 2009). GG mice show enhanced heroin self-administration (Zhang *et al*., 2015). Female mice bearing one or two copies of the 112G allele show reduced buprenorphine-stimulated locomotor activity, blunted buprenorphine-induced analgesia, diminished sensitivity to buprenorphine-mediated reduction of novelty-induced hypophagia, and similar depressive-like behavior in the forced-swim test (Browne et al., 2017). We have previously characterized conditioned place behavior and locomotor responses to oxycodone in male and female wild-type mice, but we have not yet investigated these effects in A112G mice. We found that oxycodone (1 mg/kg) produced a similar conditioned place preference in AA and GG mice regardless of sex. Oxycodone (1 mg/kg) produced a significant increase in locomotor activity during conditioning sessions in A112 males only; there was no such increase in GG males or females regardless of genotype. These data suggest that differences in opioid-mediated behavior in GG mice may be drug- and sex-specific.

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19 - A neural network for abstracting nociceptive information into an aversive pain perception

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Pain is an unpleasant experience that drives motivated behaviors to limit exposure to noxious stimuli. However, how activity in affective neural networks generates the aversive quality of pain remains unexplored. Here, we utilized recurrent *in vivo* calcium imaging and manipulation of activity in nociceptive ensembles to determine the neural basis of pain aversion. We identified a discrete ensemble in the basolateral amygdala, distinctive from salience and positive valence networks, in which painful stimuli are innately represented through combinatorial coding. Silencing of this ensemble selectively alleviated pain affective-motivational behaviors, without affecting detection of noxious stimuli, withdrawal reflexes, anxiety, or reward. Furthermore, innocuous stimuli engage this nociceptive ensemble in neuropathic pain state, to facilitate the pathological dysfunctions characterizing chronic pain. Collectively, these results identify the neural representation of nociception in the amygdala, necessary for the instantiation of the innate negative qualities of acute and chronic pain experiences.

20 - On Target effects of Mu opioid receptor activation on brain activity and connectivity identified by mouse fMRI

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Success in developing effective drugs has been limited. Although drug-induced molecular processes are well understood at the cellular level, and behavioral effects have been characterized in animal models, the overall impact of target activation and inhibition on neural network connectivity remains largely unknown. Our goal is to develop a new platform to identify and screen novel drugs, using functional Magnetic Resonance Imaging (fMRI) in living mice. Here we present a proof-of-principle study based on Mu opioid Receptor (MOR) responses to morphine. MORs are expressed in brain areas belonging to pain and addiction and, using gene knockout (KO) in mice, our laboratory demonstrated that MOR mediates both the remarkably analgesic and addictive properties of morphine. MOR is a prime target in the development of novel potent analgesics, possibly devoid of adverse effects, and also new treatments for substance use and mood disorders.

The approach measures morphine-induced modifications of Blood-oxygen-level-dependent signal in the brain of live WT and MOR KO animals. Images are acquired using 7 Tesla Scanner and functional data are preprocessed using standard pipeline. Activation maps are then established, functional connectivity and information flow are characterized. Comparing WT and MOR KO data will allow subtracting off-target effects, and establish the on-target signature of MOR-mediated activation of networks. Altogether, the characterization of activation and connectivity effects induced by the prototypic opiate drug provides a reference dataset to further test other mu opiates used in the clinic and under development, and constitutes a unique platform to characterize drugs effects on the functional-connectome.

21 - Differential regulation of delta opioid receptor-mediated behaviors by arrestin2 in mice

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The delta opioid receptor (DOR) has been shown to produce multiple active conformations that differentially engage downstream signaling effectors, including both G protein- and arrestin-mediated pathways. However, the *in vivo* consequences of this phenomenon, known as functional selectivity or biased agonism, is not readily understood. To investigate the potential *in vivo* consequences of acute arrestin-mediated signaling at DOR, we evaluated the behavioral effects of the DOR agonist SNC80 in arrestin2 and arrestin3 knockout mice. Antihyperalgesia was evaluated using a nitroglycerin-induced thermal hyperalgesia assay. Antidepressant-like effects were measured using the forced swim test. Mice were also observed continuously for convulsive activity following SNC80 administration. In both arrestin2 and arrestin3 knockout mice, the acute antihyperalgesic and antidepressant-like effects of SNC80 were not significantly altered. There was also no change in SNC80-induced convulsions in arrestin3 knockout mice. In arrestin2 knockout mice, the potency of SNC80 to produce convulsions was enhanced, as demonstrated by a leftward shift in the dose response curve. Furthermore, these mice often exhibited multiple convulsions in response to a single dose of SNC80. Loss of arrestin2 also slowed the development of tolerance to the convulsive effects of SNC80 following repeated daily treatments of SNC80. Arrestin2 knockout mice did not convulse following treatment with KNT-127, a DOR agonist purported to not produce convulsions. Taken together, these data suggest that arrestin2 is an important negative regulator of SNC80-stimulated convulsions. Future studies will further evaluate the *in vivo* consequences of functional selectivity at DOR.

22 - Distinct MOR agonist profiles revealed by visualizing Mu opioid receptor trafficking with MOR-Venus mice

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G protein-coupled receptors (GPCRs) are cell membrane receptors which bind to stimuli like neurotransmitters or hormones, to activate cell signaling responses that govern cellular changes which in turn influence behavior and physiology. Thus, GPCRs are highly exploitable targets in biomedicine. The mu opioid receptor (MOR) is activated by morphine, a widely prescribed powerful pain reliever encumbered by aversive aspects including constipation, respiratory depression, tolerance and addictive euphoria. The “Holy Grail” pain reliever therefore looks like morphine in respect to pain relief but lacks aversive effects (Kieffer, 2016). Two distinct signaling pathways may distinguish these behaviors: the former occurring via G proteins while the latter due to β-arrestin recruitment (Bohn et al., 1999; Bohn et al., 2000). Here, we generated a functional fluorescent MOR knock-in mouse, MOR-*Venus*, in a manner previously described (Scherrer et al., 2006; Erbs et al., 2015) and found morphine induced analgesic responses in hotplate and tail-immersion tests were comparable to wild-type. Next, we mapped the receptor in the mouse brain and prepared primary neurons and DRGs from these mice to examine MOR agonist-induced trafficking to screen compounds based on the redistribution of the receptor. We tested mu agonist, DAMGO, and found internalization of MOR in primary neurons. We are now testing several other compounds to demonstrate how these analyses reveal a unique method to evaluate MOR agonist-induced MOR trafficking profiles which may be useful to predict the clinical behavior of an agonist.

23 - Percutaneous Electrical Nerve Stimulation (Electro-Acupuncture) elevates Agmatine in Spinal Cords of Nerve-Injured Rats.

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Decarboxylated L-arginine (Agmatine) prevents or reduces the development of opioid tolerance and self-administration, as well as manifestations of neuropathic pain in rodents. Since Agmatine is an endogenous moiety, we hypothesized that Agmatine released in response to percutaneous electrical nerve stimulation (electro-acupuncture) may contribute to alleviation of neuropathic pain in rodents. We report here that Agmatine is elevated in spinal microdialysate collected from nerve-injured rats during and following electro-acupuncture stimulation. Male Sprague Dawley rats were nerve-injured and allowed to recover for one week before implantation of transverse spinal cord microdialysis fibers (AN69 Hospal). Dialysate was perfused at a flow rate of 5 µL/min and collected in 15-minute bins. Baseline samples were collected for 1 hour before and during a 30-minute electro-acupuncture stimulation (4 Hz, Zsusanli acupoint), which took place under isoflurane anesthesia. Control subjects were anesthetized, but did not receive stimulation. Samples were collected for 3 hours following cessation of stimulation and anesthesia. Rats were then assessed via von Frey stimulation for anti-allodynic effects of the treatment. Samples were derivatized and assessed for Agmatine content via HPLC fluorescence detection. Spinal Agmatine was elevated in microdialysate collected from electro-acupuncture stimulated subjects, but not anesthetized controls. von Frey thresholds were elevated in electro-acupuncture stimulated, but not anesthetized controls, at 3 hours post-stimulation (following conclusion of microdialysate sample collection). Percutaneous electrical nerve stimulation (electro-acupuncture) mediates release of endogenous Agmatine, which may contribute to alleviation of neuropathic pain responses in rats.

24 - Evaluation of opioid-nociceptin bifunctional ligands by biochemical, pharmacological and structural modeling tools

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Our novel synthesized opioid-nociceptin hybrid peptides H-YGGFGGGRYYRIK-NH_2, H-YGGFRYYRIK-NH_2 and Ac-RYYRIKGGGYGGFL-OH were designed to target opioid and NOP receptors as bifunctional ligands. Two of these chimeric molecules are composed of the minimum opioid tetrapeptide structure YGGF targeting the opioid receptors, and a NOP receptor specific synthetic sequence Ac-RYYRIK-NH_2 isolated from combinatorial chemical libraries. The pharmacophores were connected directly or with a triglycine spacer. These chimeric pharmacophores were studied with circular dichroism spectroscopy, molecular dynamics calculations, radioligand competition, ligand-stimulated [^{35}S]GTPgS binding assays, and electrically stimulated mouse *vas deferens* (MVD) bioassays, to study their solution structures, receptor binding affinities, potencies and efficacies, respectively. In circular dichroism spectroscopy, more a-helical periodicity was present in the structure of H-YGGFRYYRIK-NH_2, while H-YGGFGGGRYYRIK-NH_2 showed a tendency of having either unordered or b-sheet structures, and Ac-RYYRIKGGGYGGFL-OH had the most b-sheet structures. In molecular dynamics calculations, the amount of turns in the peptides was proportional to the activity. In contrast, the presence of helices showed a reverse effect, as the most helical H-YGGFRYYRIK-NH_2 proved to be inactive on NOPr. In radioligand competition receptor binding assays H-YGGFGGGRYYRIK-NH_2 showed a preference for NOPr and KOPr only, while H-YGGFRYYRIK-NH_2 and Ac-RYYRIKGGGYGGFL-OH displayed DOPr affinity as well. In [^{35}S]GTPgS binding assays, H-YGGFRYYRIK-NH_2 and H-YGGFGGGRYYRIK-NH_2 exhibited highly efficacious agonist actions. In MVD bioassay Ac-RYYRIKGGGYGGFL-OH was the most potent compound.

25 - How did the Opioid System Evolve?

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Endogenous opioid systems (enkephalin, endorphin, dynorphin and nociceptin/orphanin FQ) comprise at least four genetically homologous precursor-proteins and four genetically homologous G-protein coupled receptors in every vertebrate species thus far examined. However, little is known about how opioid systems might have evolved from invertebrates. We recently leveraged de-novo transcriptome and low-coverage whole-genome assembly in the Pacific hagfish to identify and characterize the first full-length coding sequence for a functional opioid receptor in a cyclostome. Additionally, we defined two novel endogenous opioid precursors in this species that predict several novel opioid peptides (submitted for publication). Bioinformatic analysis shows no closely related opioid receptor genes in invertebrates with regard either to the genomic organization or to conserved opioid receptor- specific sequences that are common in all vertebrates. Furthermore, no proteins analogous to vertebrate opioid precursors could be identified in genomic searches despite previous claims of protein or RNA-derived sequences in several invertebrate species. The presence of an orthologous receptor and opioid precursors in hagfish indicates that a functional opioid system arose from a common ancestor of cyclostomes and gnathostomes some 550 Mya, earlier than all previous authenticated accounts, and probably in the common ancestor to all extant vertebrates. The premise that opioid systems evolved from invertebrate systems concerned with antimicrobial defense systems is a likely scenario and we speculate that the high concentrations of opioid precursors in tissues such as the testes, gut, and activated immune cells are key remnants of this evolutionary role.

26 - The role of striatal mu opioid receptor populations in reward-related behaviors.

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Mu opioid receptors are densely expressed in the different neuronal populations of the striatum yet the role of these populations in regulating reward behaviors is relatively unknown. So as to examine how these receptor populations modulate reward, we bred a genetically engineered mouse in which exons 2 and 3 of the mu opioid receptor gene are flanked by LoxP cassettes [1] with different cre driver lines[2]. We first verified the mu receptor deletion from D1 and D2 neurons. We then assessed the initial hyperlocomotor response and subsequent sensitization of this response to oxycodone or morphine. Mice lacking mu receptors on D1 neurons showed an initial response to these opioids but no sensitization thereafter, whereas those lacking mu receptors on D2 neurons showed reduced exploratory behavior and an enhanced sensitization to repeated opioids. We also generated a profile of contingent opioid self-administration, assessing establishment of this behavior under a short-access FR1 schedule over 9 days. This was followed by 3 days of an extinction profile of behavior in which all cues except the drug were presented in a contingent manner under an FR1 schedule. There was no effect of the deletion during the initial establishment phase but mice lacking mu receptors on D1 and D2 neurons showed a different profile of extinction behaviors. These findings demonstrate the divergent roles of striatal mu receptor populations in different behavioral paradigms of reward.

1.Weibel, R. *et al. PLoS One* **8**, e74706, doi:10.1371/journal.pone.0074706 (2013).2. Gong, S. *et al.* . *J Neurosci* **27**, 9817-9823, doi:10.1523/JNEUROSCI.2707-07.2007 (2007).

27 - The role of the deorphanized receptor, GPR83, in stress and reward.

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Anxiety and drug dependence are prevalent disorders that often occur in a comorbid state therefore therapeutics that target common mechanisms are highly desirable. The recently deorphanized G-protein coupled receptor, GPR83, is highly expressed in the nucleus accumbens, medial prefrontal cortex and central nucleus of the amygdala therefore GPR83 is a potential regulator of reward and anxiety related behaviors. Preliminary studies demonstrate that morphine conditioning induces an increase in GPR83 expression in the nucleus accumbens and that GPR83 downregulation attenuates preference for morphine. Analysis of genes that regulate the dopamine and opiate system in GPR83 knockout mice reveal potential increases in expression for the dopamine transporter, delta opioid and kappa opioid receptors in the nucleus accumbens. GPR83 has also been shown to be regulated by glucocorticoids indicating that this receptor system is involved the stress and anxiety. Our preliminary data show that global GPR83 deletion reduces anxiety-related behaviors, especially in female mice. Overall, these data suggest that blocking GPR83 function is a potential therapeutic target for anxiety and substance abuse disorders however more details about the GPR83 system need to be understood. Future studies are to determine in which brain regions GPR83 is necessary and sufficient for anxiety and reward behaviors and whether GPR83 is a critical regulator of stress induced drug relapse.

28 - Assessment of ZH853, a novel endomorphin analog, versus morphine in acute and long term dosing for chronic pain.

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Chronic pain affects 100 million Americans, many of whom are inadequately treated. While opioids are effective against many types of chronic pain, significant side effects limit patient and physician willingness to use them for these conditions. We have developed a mu-opioid receptor selective endomorphin (EM) analog (ZH853) with reduced or absent side effects of traditionally prescribed opioids including: abuse liability, respiratory depression, motor impairment, tolerance, and glial activation (1). In the current study, we evaluated ZH853 and morphine in chronic pain states after both acute and chronic dosing. Acutely, both intravenous and intrathecal administration of ZH853 produced a long lasting reversal of hypersensitivity. Additionally, in ongoing experiments, we are evaluating whether chronic, moderate doses of ZH853 prolong pain like morphine does (2). Classical pain tests (e.g. von Frey) are used to determine pain sensitivity, while functional recovery is assessed with a multitude of gait parameters from the CatWalk XT system. Using these tests, we will determine whether functional and pain recovery correlate, and whether ZH853 or morphine alter the time-course of recovery.

(1) Zadina, JE et al. (2016). Neuropharmacology (**105**): 215-227.

(2) Grace, PM et al. (2016). Proc Natl Acad Sci (**113**): E3441-50.

29 - Structural Insights into Opioid Peptidomimetics with Bifunctional Mu-Opioid Agonism and Delta-Opioid Antagonism

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Opioid analgesics exert both their beneficial and unwanted effects by activation of the mu-opioid receptor (MOPR). Furthermore, repeated administration of opioid analgesics and subsequent chronic MOPR activation promotes opioid tolerance and physical dependence. It has been proposed that activation of MOPR with concurrent antagonism of the delta-opioid receptor (DOPR) can ameliorate the development of opioid tolerance and dependence. Here we report on compounds containing a tetrahydroquinoline core (THQ) that are bifunctional in that they activate MOPR and inhibit DOPR. Compounds were evaluated by two *in vitro* assays in membrane preparations of cloned cell lines expressing either MOPR, DOPR, or kappa-opioid receptors (KOPR): 1) [^{35}S]-GTPγS binding assays to measure maximal G protein activation; full agonists at each opioid receptor (DAMGO, DPDPE, and U69,593, respectively) were used as standards. Maximum binding and potency (EC_{50}) values were determined from concentration-effect curves. 2) Competition binding assays using [^{3}H]-diprenorphine to determine affinity (K_i) values. Modification of the THQ core led to changes in G protein activation and binding affinity at MOPR and DOPR. In addition, sulfone side chain substitutions revealed greater receptor activation at MOPR and KOPR while leaving DOPR inactive. The observed structure-activity relationships will guide further chemical modification and drug development of analgesic opioid compounds devoid of tolerance and dependence liabilities. Supported by DA-03910 and the PREP program at the University of Michigan.

30 - Structural and Dynamic Elements of μ-Opioid Receptor Functional Selectivity

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The wealth of structural information on opioid receptors that has appeared in the literature over the past five years offers a superb opportunity to study these systems at an unprecedented level of molecular detail and to provide novel testable hypotheses about the molecular determinants responsible for opioid receptor function. Since static information from X-ray crystallography is not sufficient to provide a comprehensive, mechanistic understanding of the diverse biological functions mediated by the μ-opioid receptor (MOR) and induced by ligands with different efficacy profiles, we complement this information with dynamic representations using state-of-the-art computational methods. Although the dynamics of MOR could also be studied experimentally using various spectroscopic and imaging techniques, these do not provide the necessary level of atomistic detail that one would need to identify the molecular determinants that are directly responsible for the receptor's functional selectivity. Molecular dynamics (MD) simulations are a particularly valuable tool to deduce these details for sparsely-populated conformational states of the receptor that are impossible or difficult to retrieve experimentally, especially when these computations are conducted alongside experiments. Coupled with state-of-the-art algorithms for statistical analyses of MD simulation data, these simulations provide a very powerful approach to identify new and potentially druggable metastable states that have not been (and may never be) observed by crystallography, as well as the slowest and therefore most important conformational degrees of freedom underlying opioid receptor functional selectivity.

31 - Characterizing the biochemical and synaptic properties of CNIH3, a novel component in individual risk for opioid dependence

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In the last decade, the CDC reported a drastic increase in opioid use across socioeconomic status, age group, and sex. In a recent genome-wide association study (GWAS) by Nelson et al., the presence of single nucleotide polymorphisms (SNPs) in cornichon family AMPAR auxiliary protein 3 (CNIH3) was the strongest correlating factor for protection against addiction despite drug misuse (*Mol Psychiatry,* 2016). Previous studies have either focused exclusively on its homolog CNIH2, or used dual CNIH2/3 knock-out mouse models. However, CNIH2 had no significant impact on opioid dependence in the GWAS. Although more information on the unique function of neuronal CNIH3 is needed, CNIH3 is suspected to aid in the trafficking of AMPA receptors (AMPARs) to the post-synaptic density (PSD). CNIH3 is highly expressed in the hippocampus, a key area for AMPAR mediated opioid-associated memory and learning. This study aims to establish the molecular-level foundation for CNIH3 activity in the hippocampus using a CNIH3 knock-out mouse model as a prelude to future opioid studies. Biochemically, we found that CNIH3 plays a critical role in maintaining both GluA1 and GluA2 AMPAR subunits at the PSD. After measuring the levels of PSD synaptic scaffolding proteins, synaptic spines, and PSD morphology, we found that CNIH3 may also regulate the synaptic properties of hippocampal neurons. This study sets the stage for future investigations into the biochemical, physiological, and behavioral relevance of hippocampal CNIH3 in opioid-induced plasticity and conditioned behavior.

32 - Mechanistic insights into opioid-induced chemoresistance in breast cancer cells: DOR stimulation increases P-glycoprotein abundance by exploiting EphA2/c-src signaling

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Opioids were recently shown to promote the development of drug resistance in breast cancer cells towards cytotoxic chemotherapeutics. The signaling mechanisms of the phenomenon, however, have not been identified so far. As the development of drug resistance is often related to an up-regulation of the drug efflux transporter P-glycoprotein (P-gp), we investigated here whether and how opioids may modulate P-gp abundance in triple negative breast cancer cells. For the purpose, delta-opioid receptor (DOR) expressing 4T1 and MDA-MB-231 breast cancer cells were incubated with [D-Ala^2, D-Leu^5]Enkephalin (DADLE) and tested for P-gp abundance by Western blot analysis. In both cell lines, DADLE treatment resulted in a significant, time- and DOR-dependent increase of P-gp abundance. The increase of P-gp abundance was associated with a reduced activation of caspase-3 by the P-gp substrate Doxorubicin. The finding implies that DOR stimulation increases the expression of functionally active P-gp and protects breast cancer cells from Doxorubicin-induced apoptosis. Further analysis revealed that DADLE exposure leads to an activation of the Ephrin receptor tyrosine kinases EphA2 in 4T1 and MDA-MB-231 cells. Inhibition of EphA2 activity by Dasatinib diminished DADLE-mediated P-gp up-regulation. In addition, P-gp regulation was also abolished by an inhibition of the EphA2 down-stream substrate c-src kinase. Together the findings let us suggest that stimulation of DORs in breast cancer cells may trigger Doxorubicin resistance by increasing P-gp abundance via stimulating EphA2/c-src kinase signaling pathway.

33 - Investigating delta opioid receptors in a new conditional knockin mouse

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Delta opioid receptors (DOPrs) represent a promising target for the treatment of chronic pain and emotional disorders. In order to further investigate the role of this receptor, we have generated a genetically-engineered mouse in which a Flag epitope has been introduced between the first and second amino acids of DOPr, and a floxed translational stop cassette introduced upstream of the initiation codon. In the absence of recombination, these mice (STOP::Flag-DOPr) behave as DOPr knockout mice. By breeding STOP::Flag-DOPr mice with Zp3-Cre mice, we generated a mouse line expressing a Flag-tagged DOPr in place of the endogenous receptor (Flag-DOPr knockin). In the present study, we thoroughly characterized these mice using pharmacological and behavioral approaches. We found that the Flagged receptor is expressed at similar levels and in the same areas as its endogenous counterpart and that DOPr agonists produce similar behavioral effects than in wildtype animals. Most importantly, we succeeded in immunoprecipitating Flag-DOPr endogenously expressed in the brain of these mice. Not only the Flag-DOPr knockin mice are a useful complementary tool to existing genetic mouse models (ex. DOPr-KO and cKO-, DOPr-eGFP knockin), they also represent a unique opportunity to better understand the role and the regulation of DOPr, *in vivo*, in specific brain regions or in a subpopulation of neurons.

This work was supported by the Quebec Pain Research Network (QPRN), the Canadian Institutes for Health Research (MOP-123399), and the Fonds de Recherche Québec-Santé.

34 - Oxycodone conditioned place preference alters hippocampal mossy fiber leu-enkephalin levels in a sex-dependent manner

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In the rat hippocampus, females with elevated estrogen states (proestrus) compared to males contain twice the levels of leu-enkephalin (LENK)-immunoreactivity (ir) in mossy fibers and three times as many delta opioid receptor (DOR)-labeled CA3 dendritic spines contacted by mossy fibers. We examined the effects of repeated exposure to the opioid agonist oxycodone (3mg/kg, I.P.) during a 10-day conditioned place preference (CPP) task on LENK mossy fiber levels and in the subcellular distribution of DORs in CA3 dendritic spines contacted by mossy fibers of female and male rats. All female rats were in proestrus/estrus as assessed by vaginal smear cytology. Hippocampal sections were processed for quantitative light microscopic localization of LENK-ir using immunoperoxidase and electron microscopic localization of DOR using silver-intensified gold (SIG). In females only, oxycodone CPP resulted in decreased LENK-ir levels in two subregions of the mossy fiber pathway: stratum lucidum of CA2/CA3a and the central hilus of the dentate gyrus. In both females and males, oxycodone CPP did not alter the number of DOR SIG particles in CA3 pyramidal cell dendritic spines contacted by mossy fibers. These results suggest that in females oxycodone CPP results in the selective depletion of LENK in mossy fibers that overlap with pyramidal cell dendrites known to be enriched in phosphorylated DORs and with GABAergic hilar interneurons known to contain DORs. Moreover, they also suggest that following oxycodone-CPP DORs remain positioned in CA3 neuron dendritic spines where they could promote learning processes in females.

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35 - Delta-opioid receptor (DOR) antagonism/inverse agonism reduces the development of mu-opioid receptor (MOR) tolerance

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Opioid analgesics are prescribed to combat moderate to severe pain yet there are many problems associated with the use of these medications, including constipation, respiratory depression, and physical dependence. Previous studies have reported that elimination of the delta-opioid receptor (DOR) or co-administration of naltrindole, a DOR antagonist, with the mu-opioid receptor (MOR) agonist morphine reduces the development of analgesic tolerance and also lessens dependence. As such we and others have designed and synthesized opioid ligands with a MOR agonist-DOR antagonist profile that show antinociception in rodent models but with reduced tolerance liability. To probe the mechanism behind the action of these so called "mixed efficacy" compounds we have employed SH-SY5Y neuroblastoma cells, which endogenously express both human opioid receptor types. MOR tolerance at the level of the G-protein, determined as a decrease in GTPγ35S binding, was induced by overnight incubation with high concentrations of the selective MOR agonist DAMGO. Tolerance was observed as a loss of the maximal effect and potency of DAMGO and morphine. Co-treatment of the cells with the DOR antagonist naltrindole or the DOR inverse agonist ICI-174864 at DOR-selective concentrations reduced the development of tolerance. These findings provide support for the hypothesis that DOR inhibition is advantageous for improved MOR agonist activity and that at least one contributing mechanism involves receptors in a single cell. Overall, these data support the development of "mixed-efficacy" MOR agonist-DOR antagonist compounds. Funded by DA-03910 and NIGMS-GM007767.

36 - Selective Inhibition of M5 Muscarinic Acetylcholine Receptors Attenuates Remifentanil Self-Administration without Blocking Morphine-induced Analgesia in Rats

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Opioid use disorder (OUD) has reached epidemic proportions in the United States; yet current treatment options remain limited and are associated with abuse potential and other adverse side effects. Accumulating evidence suggests that selective inhibition of the M_5 muscarinic acetylcholine receptor (mAChR) subtype may provide a novel approach for the treatment of OUD. Recently, we reported the successful discovery and optimization of a novel series of highly selective M_5 negative allosteric modulators (NAMs), represented by the M_5 NAM tool compound ML375. Since our previous studies have demonstrated that ML375 can reduce cocaine self-administration in rodents, we set out to determine if ML375 could also attenuate opioid self-administration in rats without blocking opioid-induced analgesia.

In the present study, male Sprague-Dawley rats were trained to self-administer remifentanil under a fixed ratio schedule of reinforcement. The ability of ML375 to attenuate the reinforcing effects of remifentanil at each unit dose was then determined. The effects of ML375 were also determined when rats responded under a progressive ratio (PR) schedule for remifentanil alone and in combination with cocaine. Finally, the potential analgesic effects of ML375 alone and in combination with morphine were assessed using a hot plate apparatus.

ML375 reduced remifentanil self-administration under the FR 10 schedule and under the PR schedule. Moreover, preliminary data indicate that ML375 has no effect on morphine-induced analgesia. Taken together, these results suggest that selective negative allosteric modulation of the M_5 receptor may represent a promising novel pharmacotherapy for the treatment of OUD.

37 - Functional consequences of OPRM1 A118G (MOR N40D) on human neurotransmission

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Functional genetic variation at the mu opioid receptor (MOR), particularly rs1799971 (ORPM1 A118G, MOR N40D), has been linked to addiction and other reward-related behaviors. To investigate the functional consequences of this SNP in a human neuronal context, we derived human induced neuronal (iN) cells from induced pluripotent stem (iPS) cell lines generated from human subjects carrying either homozygous N40 or D40 alleles. Our data obtained from induced human neuronal cells exposed to DAMGO reveal two new fundamental insights into the functional consequences of this SNP: **1)** D40 MOR exhibits stronger inhibition of synaptic release, suggesting enhanced acute signaling and **2)** D40 MOR iN cells exhibit defective signaling after chronic (7 days) DAMGO exposure compared to N40 MOR iNs, suggesting a possible impaired receptor re-sensitization is associated with the D40 MORs. Additionally, in order to rigorously control for background genetic variation among human subjects that may confound disease phenotypes, we used CRISPR/Cas9 mediated gene targeting to create two isogenic pairs of human stem cell lines from which we recapitulated differences in DAMGO sensitivity observed in subject derived neurons, providing strong additional evidence to support our new data. Our study provides important insight into how functional genetic variation in OPRM1 may modify synaptic transmission in a human neuronal context.

38 - Crucial role of the κ-opioid receptor system in tumor angiogenesis

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Opioids are effective analgesics for the management of moderate to severe cancer pain. However, little is known about the role of opioid systems in tumor angiogenesis. The tumor angiogenesis is required for tumor progression with the highly expression of several activators such as vascular endothelial growth factor (VEGF). In the previous study, we demonstrated that the κ opioid receptor (KOR) ligand could act as a novel endogenous angiogenesis inhibitor in vascular development. In the present study, we investigated whether KOR system could inhibit tumor angiogenesis in the process of tumor growth. We confirmed that treatment with KOR agonists, U50,488H and nalfurafine (TRK-820), significantly inhibited human umbilical vein endothelial cell (HUVEC) migration and tube formation by suppressing VEGFR2 expression. In contrast, treatment with a μ opioid receptor agonist DAMGO or a δ opioid receptor agonist SNC80 failed to prevent angiogenesis in HUVECs. Furthermore, Lewis lung carcinoma or B16 melanoma grafted in KOR knockout mice and prodynorphin (PDYN) knockout mice increased proliferation and remarkably enhanced tumor angiogenesis. Repeated intraperitoneal injection of nalfurafine (0.1–10 mg/kg, b.i.d.) significantly inhibited tumor growth by suppressing tumor angiogenesis. Finally, treatment with nalfurafine enhanced the survival advantage induced by treatment with gemcitabine in pancreatic cancer-bearing mice. These findings indicate that KOR system could act as an inhibitory modulator of tumor angiogenesis via suppressing VEGF signaling. This knowledge could lead to a novel strategy for cancer therapy.

39 - CaMKIIα controls the biogenesis of let-7 microRNAs in opioid tolerance

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Emerging evidence suggests that microRNAs (miRNAs) – mediated cellular adaptations are critical for drug addiction. We previously reported that let-7 family miRNAs contribute to the development of opioid tolerance by targeting the μ opioid receptor. Chronic morphine treatment induced a marked increase of let-7 expression, which functionally correlated with the development of opioid tolerance. The aim of this study was to understand the mechanisms how let-7 is regulated by opioids. We first determined the transcription status of let-7 and found that the expression of primary let-7 (pri-let-7) remained unchanged in SH-SY5Y cells that were treated with morphine (1 μM, for 48 h). In agreement with the *in vitro* observation, chronic morphine tolerance (one 75 mg morphine pellet/mouse, *s.c.*) did not alter the level of pri-let-7 in mouse brain front cortex region. These findings suggested that the robust elevation of let-7 occurred at the post-transcriptional level. In the presence of KN93, inhibitor of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), chronic morphine treatment failed to generate let-7 up-regulation in SH-SY5Y cells. We further determined whether inactivation of CaMKIIα by T286A point mutation would affect let-7 expression and opioid tolerance. Indeed, antinociceptive tolerance was absent in $CaMKII\alpha^{T286A}$ mutant mice. Meanwhile, the level of let-7 in $CaMKII\alpha^{T286A}$ mutant mice was much lower than that in wild-type mice, and was resistant to chronic morphine stimulation. Taken together, these data suggested that the activity of CaMKIIα was essential for the biogenesis of let-7 in opioid tolerance.

40 - The G-protein Biased Opioid Agonist PZM21 Does Indeed Depress Respiration

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PZM21 is a novel opioid agonist reported to produce G-protein biased signalling at the MOPr. PZM21 was also reported to have decreased side effects, including a lack of significant respiratory depression [1]. In the current investigation we sought to replicate those results.

Bioluminescence resonance energy transfer (BRET) was used to investigate the relative activation of G-protein signalling or arrestin-3 recruitment by PZM21. Respiration was measured by whole body plethysmography in male mice (CD-1) breathing either 95% air/5% CO_2 or 100% air.

PZM21 (0.01-30 μM) showed little to no recruitment of arrestin-3 in the BRET assay compared to significant activation of the G-protein signalling pathway (EC50=0.27 μM). For G-protein activation PZM21 had higher efficacy than morphine but lower efficacy than DAMGO. PZM21 thus displayed significant bias for G-protein activation over arrestin recruitment at MOPr.

Acute administration of morphine (10 mg/kg i.p.) rapidly depressed respiration in both 100% air and 95% air/5% CO_2. Saline did not decrease respiration under either condition. Contrary to the previous report [1] acute administration of an equi-analgesic dose [1] of PZM21 (40 mg/kg i.p.) produced significant respiratory depression under both conditions that was not significantly different from that observed following morphine. This resulted from a decrease in respiratory rate, not tidal volume. PZM21 induced respiratory depression was completely inhibited by naloxone (1 mg/kg).

These data demonstrate that PZM21 is indeed a G-protein biased MOPr agonist. However, despite this biased signalling profile, PZM21 retains the undesired side effect of respiratory depression.

41 - Inflammatory pain alters the vulnerabilitiy to alcohol relapse without impacting the intensity of the relapse in rats.

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Clinical research has highlighted the relationship between pain and drug use disorders, especially opiates and ethanol. Moreover, a recent clinical study showed that the correct management of pain in patients with a previous history of alcohol use disorder decreases the risk of relapsing, revealing that pain may increase the vulnerability to alcohol relapse. Recent data demonstrated that, in rats, inflammatory pain desensitizes the mu opioid receptors located in the mesocorticolimbic dopamine system and consequently increases heroin voluntary intake at high doses. It is well known that alcohol reinforcing effects and alcohol relapse are also mediated through the activation of mu opioid receptors in the mesolimbic pathway. However, the role of inflammatory pain in the consumption or relapse into ethanol has not been explored yet. In this study we evaluated the effect of inflammatory pain on the alcohol deprivation effect (ADE) in long-term ethanol-experienced rats using a non-operant paradigm. To this end, rats were exposed to four free ethanol intake periods followed by four forced abstinence periods. The complete Freund's adjuvant was used to induce pain during the fourth period of imposed ethanol deprivation. In the group of rats presenting ADE in all periods of reintroduction, the presence of pain did not induce changes in the ADE. Noteworthy in the rats that never showed ADE, the development of an inflammatory pain condition increased the amount of rats that spontaneously presented ADE phenomenon. Our results suggest that pain may affect the vulnerability to relapse without altering the intensity of the ADE phenomenon.

42 - Simultaneous administration of a chemokine receptor antagonist with morphine enhances the analgesic effect of morphine on incisional pain in rats

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Cross-desensitization between opioid and chemokine receptors is well established. It has also been shown that chemokines are involved in pain modulation. We investigated if simultaneous administration of a chemokine receptor antagonist with morphine would enhance the analgesic effect of morphine on incisional pain in rats. Dose-response curves for morphine (1-10 mg/kg) and maraviroc (0.5-5 mg/kg), a CCR5 antagonist, alone were established using male rats. Animals underwent incisional surgery on the left hind paw. Pain responses were evaluated using von Frey filaments at various times post-surgery 15 to 360 minutes and 24 to 96 hours. Morphine, maraviroc, or their vehicles were injected s.c. at 25 minutes post-surgery. While morphine significantly reduced pain in a time- and dose-dependent manner, maraviroc had no effect. Mean % reversal of mechanical allodynia was established at 60 min post-surgery with 5 (56 ± 14%) and 10 (86 ± 5%) mg/kg of morphine. Another group was given both maraviroc (2.5 mg/kg) and morphine (5 mg/kg) at different s.c. sites (t = 25). Mean % reversal of mechanical allodynia at 60 min was significantly increased (100 ± 0%). The data show that combining morphine with maraviroc significantly enhanced the analgesic effect of morphine. This result indicates that the same analgesic effect of morphine alone can be achieved with lower doses of morphine when combined with maraviroc, which could have clinical treatment implications for some types of pain. Immune changes induced by the incision and the drugs were measured.

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43 - Dermatolgical manifestations in substance abuse and how the community responses in developing country

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Introduction: It has been estimated that there are around 60000 drug abusers in Nepal. Some of the herbal by products are used as a cultural, medicinal and traditional exchange from generation to generation such as marijuana, alcohol, cannabis. Later on, other products such as pharmaceutical drugs either by import or for export in this globalization era.

Methods: It was a study 2015-2016, of patients who came for treatment with dermatologic related disorders in the clinical dermatology practice. 163 cases which were taken in-depth interview and counseling.

Results/Conclusion:: Most of the cases are adolescent group (16-35) , M:F;60:3. 65% of the cases have complained of dryness of mouth and skin. 12% having erythema and swelling of Injecting site and 4% having an infection for which they had taken broad spectrum antibiotics. Keloid, abscess formation and post Inflammatory-pigmentation also seen in 6% of the cases. *Staphylococcus aureus* , streptococcal species, and E.coli found in the culture and sensitivity test. Those who are taking marijuana regularly 45% had Common skin manifestations include premature aging such as periorbital darkening, hair loss and graying of hair.

30% of the cases those were chronic alcoholic have had features of CNS depressant, impairing motor function and coordination, decision-making and judgment, and memory.

In a resource poor setting areas, dermatologist plays a vital role for the treatment, management with a multidisciplinary team. Early diagnosis, treatment, and rehabilitation are important to improve the quality of life of people in developing country

44 - Opioid receptors reveal a parallel cycle of regulated presynaptic membrane trafficking

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Much is known about how presynaptic GPCRs regulate the synaptic vesicle (SV) cycle, but little is known about GPCRs trafficking at the presynapse. We show that mu opioid neuropeptide receptors (MORs), GPCRs that mediate inhibition of SV cycling, localize diffusely along the axonal plasma membrane when not activated and rapidly concentrate at presynaptic terminals in response to ligand-induced activation. This dynamic accumulation process occurs through ligand-induced endocytosis of MORs into a population of endosomes that accumulate in boutons separately from SV membranes and are specifically marked by retromer. These endosomes, in turn, locally source vesicular insertion of receptors into the presynaptic membrane. The GPCR cycle is mechanistically distinct from the SV cycle because it is stimulated, rather than inhibited, by ligand-induced activation of MORs and operates in a fully calcium-independent manner. These findings reveal a discrete cycle of presynaptic membrane trafficking that dynamically accumulates neuromodulator receptors at the presynapse.

45 - Probe Dependence of High and Low Affinity Agonist Binding to the Delta Opioid Receptor is Allosterically Regulated by BMS-986187 and Na+ Ions

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BMS-986187 is a positive allosteric modulator (PAM) for the delta opioid receptor (DOPr) that enhances the binding affinity and/or the efficacy of orthosteric agonists. The probe dependence of BMS-986187, or the differential cooperativity between orthosteric ligand types and BMS-986187, was examined by measuring the effect of the PAM on DOPr binding affinity of a variety of ligands. Competition binding was performed using the non-selective ligand 3H-diprenorphine in CHO-hDOPr membrane fractions in the presence of 100mM NaCl and 10uM GTPγS to promote inactive (R) receptor states. The results demonstrated that BMS-986187 increased agonist affinity. The affinities of the endogenous opioid peptides β-endorphin, dynorphin A (1-17), dynorphin B, leu-enkephalin, and met-enkephalin were enhanced by approximately 140, 50, 11, 30, and 40-fold respectively. The small molecule agonist SNC80 shifted 14-fold whereas the shift for both TAN67 and BW373U86 was 3-fold. These values were compared to the binding affinities of the ligands in the absence of NaCl and GTPγS, representing a high affinity (R*) form of the receptor. The increase in affinity in the presence of BMS-986187 closely correlated with binding affinities obtained in the absence of NaCl and GTPγS. BMS-986187 likely modulates the DOPr in part by destabilizing the Na+ binding site to promote agonist binding and receptor activation. This work was supported by R01 DA033396 and T32GM008597.

46 - Allosteric Agonism at the Delta Opioid Receptor: Potential Mechanism for Functional Selectivity

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The delta opioid receptor (DOPr) has gained attention in recent years as a pharmacological target. DOPr agonists have been shown to promote analgesia and relieve depression. Additionally, evidence suggests that targeting DOPr results in less respiratory depression and abuse liability than the commonly targeted mu-opioid receptor. Unfortunately, the potential use of DOPr ligands is limited by their rapid development of tolerance and induction of convulsions. Evidence suggests that biased ligands may be capable of activation of downstream effectors associated with the positive effects while mitigating the negative ones. BMS 986187 is an allosteric modulator of the DOPr with apparent agonist properties, thus acting as an “Ago-PAM”. Since Ago-PAMs act at a site other than the orthosteric site it can be hypothesized that they may show unique signaling properties. This study set out to validate BMS 986187 as an Ago-PAM and to determine the downstream signaling complement associated with its activation of DOPr in the absence of orthosteric ligand. We confirmed that BMS 986187 is an Ago-PAM at the DOPr. Additionally, BMS 986187 shows biased agonism favoring G protein activation over β-arrestin relevant pathways including receptor phosphorylation and internalization. This is the first evidence of an opioid Ago-PAM showing bias and suggests that targeting the DOPr through this allosteric site may be a unique way to potentially mitigate side effects. Funded by DA 033397 and 5T32GM007767-38.

47 - Allosteric activation of delta opioid receptors in vivo: promising behavioral profile

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Positive allosteric modulators (PAMs) bind to receptor sites distinct from the orthosteric binding site, can modify the affinity and activity of orthosteric ligands, and may have activity on their own. It has been proposed that PAMs could have distinct advantages as compared with orthosteric agonists, such as increased receptor selectivity, improved margins of safety, and selective augmentation of endogenous tone. Activation of delta-opioid receptors (DORs) at the orthosteric binding site produces analgesic and antidepressant effects in animal models; however, prototypical DOR agonists, such as SNC80, also produce adverse convulsive effects. Therefore, this study aimed to evaluate the in vivo effects of the DOR PAM BMS-986187, alone and in combination with endogenous and exogenous agonists, to determine its profile of activity. We evaluated potential pain-relieving effects in the acetic acid-induced stretch assay and in the nitroglycerin-induced hyperalgesia assay in mice. We measured antidepressant-like effects in the mouse forced swim and tail suspension tests and convulsions by continuous observation. Alone, BMS-986187 produced only antidepressant-like effects that were attenuated by the DOR antagonist naltrindole and absent in the DOR knockout mice. BMS-986187, but not the orthosteric agonist SNC80, enhanced the antidepressant-like effects of the enkephalinase inhibitor RB101. BMS-986187 increased the potency and/or efficacy of the DOR agonists SNC80 and KNT127 to produce antihyperalgesia and antidepressant-like effects but did not alter their convulsive effects. These findings suggest that the DOR PAM has a promising profile of behavioral effects, likely by enhancing endogenous tone to alter mood without seizurogenic activity.

48 - Analysis of medication overuse headache after repeated morphine or THC administration using home cage wheel running

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Medication overuse headache (MOH) is characterized by more frequent and intense headaches upon repeated use of anti-migraine treatments. Thus, emerging anti-migraine treatments must be tested for MOH in preclinical studies. We have shown that activation of TRPA1 receptors on dural afferents via microinjection of allyl isothiocyanate (AITC) mimics migraine-like pain as indicated by depression of home cage wheel running. Although opioids can reduce migraine pain, they are not recommended because of the potential for MOH. The objective of this study was to assess morphine-induced MOH in rats and determine whether MOH also occurs with repeated administration of Δ^9-tetrahydrocannabinol (THC). Rats had access to a running wheel in their home cage 23 hrs a day for one week. Baseline running was assessed on the last day. Rats were injected intraperitoneally twice-daily for 2.5 days with either THC (1.0 mg/kg), morphine (3.2 mg/kg), or vehicle. Migraine-like pain was induced 24 hrs following the last injection via dural microinjection of AITC (10%; 10 µL). Immediately after, rats received either THC (0.1, 0.32 mg/kg), morphine (0.32, 1.0 mg/kg), or vehicle intraperitoneally. Dural AITC depressed running for 3 hours. Administration of 0.32 mg/kg THC prevented AITC-induced depression of wheel running in THC- and vehicle-pretreated animals. Administration of 1.0 mg/kg of morphine prevented depressed wheel running for one hour in saline-pretreated animals, but was ineffective in morphine-pretreated animals. AITC-depressed wheel running was prolonged in morphine-pretreated animals compared to THC- and vehicle-pretreated animals. These data indicate that repeated morphine, but not repeated THC, administration elicits MOH

49 - Effect of opioids on the cellular responses to anti-cancer drugs

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Cancer patients routinely receive opioids during surgery and for the management of severe cancer-related pain. In preclinical studies, morphine has been found to drive breast cancer progression (1-3). In patient cohorts, polymorphism of the mu-opioid receptor (MOR)-coding gene *OPRM1* has been proposed to be linked with the onset and prognosis of breast cancer (4,5) and oesophageal squamous cell carcinoma (6). Specifically, the A118G polymorphism may correlate with the better survival of breast cancer patients (5, our unpublished results). Morphine has even been suggested to promote stemness and chemoresistance in breast cancer cells (7). Chemotherapy is the main line of treatment in the most severe breast cancer cases that lack targeted treatments. Thus, the role of opioids in chemoresistance might play a role in their prognosis.

In this study, we address the effects of different commonly used opioid MOR-agonist analgesics and one opioid antagonist on breast cancer cells in vitro, in combination with cytostatics. We study whether morphine, oxycodone or fentanyl affect proliferation, migration and invasion of breast cancer cells in vitro, and whether opioids have a role in chemoresistence. Our first results suggest that morphine may enhance proliferation of cells. Currently, we are studying the combinations with chemotherapeutics.

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50 - Adolescent social isolation increases kappa opioid receptor function in the nucleus accumbens and basolateral amygdala of rats.

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Adverse social experiences during adolescence increase the risk of developing alcohol use disorder during adulthood in humans. Similarly, rats exposed to adolescent social isolation show greater ethanol intake in adulthood compared to group housed controls. Acute stress elevates dynorphin levels, a kappa opioid receptor (KOR) ligand, which regulates dopamine. Activation of KORs inhibits dopamine release in the NAc and the BLA. The NAc and BLA are interconnected and play integral roles in the neurobiology of stress, anxiety, and reward-seeking behavior. In order to investigate early-life stress-induced changes in KORs, we housed the rats individually (social isolation, aSI; 1 rat/cage) or in groups (group housed, aGH; 4 rats/cage) for six weeks (PD 28-74). Baseline levels of dopamine were significantly lower in the NAc and BLA of aSI compared to aGH rats. KOR inhibition increased baseline dopamine levels in both NAc and BLA. The aSI rats showed increased dopamine responses to ethanol (2 g/kg) in both NAc (200% of baseline) and BLA (280% of baseline). Ethanol augmented dopamine responses in the NAc of aSI rats pre-treated with norBNI, but attenuated responses in the BLA. The inhibitory effects of U50,488 on dopamine release were enhanced in the NAc of aSI rats suggesting that chronic stress increases KOR function. It is possible that KOR differences may explain in part the effects of ethanol on behaviors related to specific brain regions, for example, augmentation of DA in the NAc results in increased reinforcement, whereas augmentation in DA in the BLA may contribute to decreased anxiety.

51 - Norbuprenorphine pharmacokinetics and pharmacodynamics: First in man evaluation

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(Bup) is a partial μ agonist, δ and κ antagonist, and nociceptin receptor agonist, with complex pharmacology. It is extensively metabolized, primarily to norbuprenorphine (norBup); both Bup and norBup undergo glucuronidation. Metabolite exposure can exceed that of Bup. NorBup, Bup-glucuronide and norBup-glucuronide are pharmacologically active. Mechanisms of Bup pharmacology in humans remain undefined. We conducted a series of first-in-man studies to evaluate norBup clinical pharmacology. The first was a dose-escalation study (0.005-1 mg) with venous plasma sampling. The second evaluated a 1 hr infusion of 0.3 mg, with arterial and venous sampling, and measurement of pupil diameter, respiratory rate, end-expired CO_2, and response to thermal stimulus. NorBup was well-tolerated, without adverse effects. Plasma norBup-glucuronide exceeded norBup concentrations. NorBup and norBup-glucuronide plasma AUCs, and urine excretion, were proportional to dose ($r>0.9$) throughout the entire dose range. NorBup-glucuronide was formation-rate limited. NorBup (0.3 mg) caused mild miosis (maximum pupil diameter change 1.3 ± 0.8 mm), minimal respiratory depression (respiratory rate decreased from 16 ± 1 to 14 ± 3 and end-expired CO_2 increased from 39 ± 2 to 41 ± 2 mmHg), and was slightly anti-analgesic (maximum tolerated temperature decreased from 49 ± 1 to 48 ± 1 °C and NRS pain ratings to a preset temperature increased to 113 ± 13% of baseline). Drug effects were negligible after 24 hr. Effect (miosis) vs concentration showed hysteresis. NorBup is pharmacologically active in man, shows both μ agonist and κ antagonist properties and may contribute to the pharmacologic effects of parent Bup.

52 - Serum and urine concentrations of morphine and morphine metabolites in patients with advanced cancer receiving continuous intravenous morphine: an observational study

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The feasibility and clinical implication of drug monitoring of morphine, morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G) need further investigation. This study aimed to determine what predicts serum concentrations of morphine in cancer patients receiving continuously intravenous morphine, the relationships between serum concentration of morphine/its metabolites and urinary concentrations, and the relation between morphine concentrations and with clinical outcomes. We collected serum and urine samples from 24 patients with advanced cancer undergoing continuously intravenous morphine therapy. Serum samples were obtained at day one. Spot urine samples were collected once daily on three consecutive days. Pain and adverse drug events were assessed using the Korean version of MD Anderson Symptom Inventory. A total of 96 samples (72 urine and 24 serum samples) were collected. Median dose of morphine was 82.0mg/24hours. In a multivariate analysis, total daily morphine dose was the most significant predictors of both serum and urine concentration of morphine. Morphine, M6G, and M3G in serum and urine were statistical significantly correlated (correlation coefficient = 0.81, 0.44, 0.56; p values < 0.01, 0.03, 0.01, respectively). There was no significant correlation between clinical symptoms and morphine/metabolite concentrations. Spot urine concentrations of morphine and its metabolites were highly correlated to those of serum. Total dose of daily morphine was related to both serum and urine concentration of morphine and its metabolites.

53 - Evaluation of the dual kappa and delta opioid receptor agonist MP1104 in rat models of cocaine self-administration, analgesia and behavioural side-effects

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Activation of kappa-opioid receptors (KOPr) have demonstrated anti-cocaine effects and analgesic properties and have therapeutic potential as non-addictive pain medications and anti-addiction pharmacotherapies. However, side-effects limit their clinical development. We hypothesise that mixed agonists activating both delta-opioid receptors (DOPr) and KOPr, such as MP1104, will have desired analgesic and anti-cocaine effects with reduced side-effects. To evaluate this, male Sprague Dawley rats were administered MP1104 (0.3 and 0.6 mg/kg/i.p.) and analgesic effects evaluated in the tail-withdrawal assay. MP1104 showed analgesia at 1 h, with peak effects at 3 h and a duration of 8 h. To evaluate the anti-cocaine effects of MP1104 rats were trained to self-administer cocaine (0.5 mg/kg/iv), followed by extinction and then subject to cocaine-prime reinstatement-tests. We show that MP1104 dose-dependently decreased reinstatement (0.3 and 1 mg/kg/i.p.) ($p<0.01$ & $p<0.001$ respectively) in a KOPr dependent, but not DOPr dependent manner. In cocaine dose-response experiments, acute administration of MP1103 (0.3 ($p<0.01$) & 0.6 mg/kg ($p<0.0001$)) caused a downwards shift in the cocaine dose-response curve, demonstrating potent anti-cocaine effects. Evaluation of side-effects, including aversion in Conditioned Place Aversion tests, depression, in the Forced Swim Test and anxiety in the Elevated Plus Maze showed no significant effects compared to vehicle treated controls following MP1104 at 0.3 or 0.6 mg/kg doses. However, MP1104 at 1 mg/kg ($p<0.01$) but not 0.3 or 0.6 mg/kg doses showed sedative effects in spontaneous locomotor tests. MP1104 shows potent analgesic and anti-cocaine effects with few side-effects to the selective KOPr agonist U50,488.

54 - Regulation of morphine sensitivity and adenylate cyclase sensitization by carboxyl-terminus of V1b vasopressin receptors

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Chronic morphine exposure accelerates adenylate cyclase (AC) signaling and reduces the analgesic efficacy, a condition known as opioid tolerance. The mechanism of how non-opioid neurotransmitters modulate AC responsiveness during chronic morphine exposure has not been fully clarified. In tail-flick and hot plate tests, we show that morphine sensitivity was increased in mice lacking vasopressin V1b receptors (V1bRs), but not in mice lacking V1a receptors. Analgesic tolerance to morphine developed with significant delay in V1bR-deficient mice and in mice administered a V1bR antagonist into lateral ventricle. More specifically, administrations of a V1bR antagonist to rostral ventromedial medulla (RVM), where V1bRs and μ-type opioid receptors (MORs) were co-expressed, delayed tolerance development. In HEK cell model, the carboxyl-terminus of the V1bR was constitutively phosphorylated and associated with β-arrestin 2. Complex formation between constitutive V1b-β-arrestin 2 and MOR was detected in bioluminescence resonance energy transfer experiments. V1bR-mediated association between β-arrestin 2 and MOR was necessary for vasopressin-mediated accelerations of ERK phosphorylation and AC sensitization. Inhibitor of ERK signaling to the RVM alleviated morphine tolerance. Genome editing and deletion of the V1bR carboxyl-terminus, which was necessary for β-arrestin 2 binding, increased morphine analgesia in mice and suppressed morphine-induced AC sensitization in the RVM. These findings indicated that inhibition of V1bR that is functionally associated with MOR provides a novel approach to enhance morphine analgesia without accelerating analgesic tolerance.

55 - Generation and characterization analysis of human iPS cell derived-sensory neurons as a useful research tool for pain and opioid systems

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Chronic pain is a major challenge to clinical practice and basic science. The lack of access to sensory neuron has limited our understanding of its development and the physiology of pain in humans. Induced pluripotent stem cell (iPSC) technology allows generation of cells from patients, and can recapitulate disease pathology. Such cells can be used in place of human tissue or animal models for disease modeling, drug screening, and even cell replacement therapies. In the present study, we generated sensory neuron-derived from human iPS cells following a small molecule differentiation protocol, which results in the differentiation of human iPS to sensory neurons of a nociceptor phenotype. This protocol involves dual SMAD inhibition, which efficiently induces neuro-ectoderm formation from human embryonic stem cells. This is followed by inhibition of GSK-3, γ-secretase, and vascular endothelial growth factor receptor/fibroblast growth factor receptor, which enables fate specification toward a sensory phenotype followed by maturation of the neurons with growth factors. Under these conditions, we found the cells expressing markers of sensory neurons such as *TAC1* (substance P), *SLC17A6* (VGLUT2), *NTRK1* (trkA) and *TRPV1* at 15 days after differentiation. Furthermore, the μ-opioid receptor expression was clearly found in sensory neurons derived from human iPS cells with the progress of cell differentiation. These findings suggest that human iPS cells derived sensory neuron is useful tool for development and functional analysis of sensory neuron and opioid system. In near future, we intend to apply this technique to pain and opioid research.

56 - Factors associated with ketamine use in pancreatic cancer patient in a single hospice center

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Up to 90% of pancreatic cancer patient suffer from neuropathic pain. In palliative care setting, pain control in pancreatic cancer patient is one of the major goals. Ketamine is a NMDA receptor antagonist effective in neuropathic pain. Also there have been studies about opioid sparing effect of ketamine. This study was held in palliative care unit among pancreatic cancer patients to find out the factors related to ketamine use and the opioid sparing effect.

Medical records of pancreatic cancer patients admitted to St.Mary’s hospital palliative care unit from 2013.1 to 2014.12 were reviewed. Patients were divided in to 2 categories according to ketamine use. Also opioid use before and after ketamine use was compared in ketamine group.

Compared to non ketamine use group, patients in ketamine group required higher dose of opioid. Total opioid dose, daily opioid dose, number of daily rescue medication, daily average rescue dose were statistically significantly higher in ketamine group. Opioid requirement was increased after ketamine administration.

In this study, ketamine group required more opioid. Ketamine is frequently considered in patients with severe pain, requiring high amount of opioid. Also ketamine did not have a opioid sparing effect. Future studies about palliative use of ketamine in larger number of patients are required.

57 - Sex differences in opioid signaling

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To further understand opioid desensitization and its effect on the clinical population, it is important to investigate the presence of sex-based differences in our rodent models. We focused on the densely packed, homogenous population of Locus Coeruleus (LC) neurons. These neurons express mu-opioid receptors that are coupled to G-coupled inwardly rectifying potassium channels (GIRKs) and have been previously shown to undergo opioid desensitization. Using whole-cell voltage-clamp recordings from LC neurons in acute brain slices from opioid naïve Sprague-Dawley rats, concentration response curves to morphine and [Met^5] enkephalin (ME) were constructed in slices from male and female rats. The morphine and ME currents were normalized to the current induced by a saturating concentration of the α_2-adrenergic agonist UK. Interestingly, while there was no significant difference in the ME concentration response curves, the morphine concentration response obtained in slices from female rats had a larger maximum current induced by morphine than experiments in slices from male rats. Intracellular recordings were used to elucidate sex differences in ME desensitization. There was no significant difference between males and females in the degree of desensitization during ME application at a saturating concentration, but females showed a reduction in the recovery from desensitization. The potential differences in tolerance induced by chronic morphine treatment will be investigated.

58 - Genetic deletion of mu opioid receptors from Kölliker-Fuse neurons reduces morphine-induced respiratory depression

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Activation of mu opioid receptors in the pontine Kölliker-Fuse (KF) causes a reduction in respiratory rate in anesthetized rats. We have previously shown that mu opioid agonists hyperpolarize a population of KF neurons (Levitt et al., *J Physiol*, 2015). The goal of these experiments was to determine the contribution of the opioid-sensitive KF neurons to the reduction in respiratory rate caused by systemic administration of the opioid agonist morphine. Targeted deletion of mu opioid receptors was accomplished by viral expression of Cre recombinase in the KF of mice with floxed mu opioid receptors. Experiments were performed 4-5 weeks later. In whole-cell recordings from brain slices, infected KF neurons were no longer hyperpolarized by opioid agonist, confirming deletion of mu opioid receptors. Head-out plethysmography was used to measure respiratory rate. Morphine (10 mg/kg, ip) caused a significantly smaller reduction in respiratory rate in mice with bilateral deletion of mu opioid receptors from KF, compared to uninjected or GFP injected controls. The time-course for the effect of morphine was also delayed in mice lacking mu receptors in KF neurons. Thus, opioid-sensitive KF neurons contribute to, but are not entirely responsible for, systemic morphine-induced respiratory depression.

59 - Cannabinoid CB2 receptor plasticity in rostral ventromedial medulla in inflammatory pain

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Treatment of chronic pain is still challenging. Emerging data indicate that drugs that target endocannabinoid systems produce analgesia with fewer side effects than opioids. The analgesic activity of cannabinoids is mediated by activation of CB receptors (CB1R and CB2R). However, the benefits of CB1R analgesia are limited by its psychotropic effects and the development of tolerance. We have recently demonstrated that the function and expression of CB1R was reduced in the rostral ventromedial medulla (RVM) in persistent inflammation induced by complete Freund's adjuvant (CFA). Using whole-cell patch-clamp recordings from adult RVM slices, the effects of CB2 agonists on GABAergic neurotransmission were studied. CB2 agonist inhibition of GABAergic miniature IPSC (mIPSC) frequency is increased in RVM neurons from CFA-treated rats. CB2 antagonists increased mIPSC frequency only in CFA-treated RVM neurons indicating an endocannabinoid tone in inflamed rats. Systemic administration of a CB2 agonist (AM1241, 3mg/kg, i.p.) reverses mechanical hypersensitivity in CFA-treated rats. Finally, we find that administration of minocycline, a microglia inhibitor, blocks the development of CB2 plasticity in the RVM during inflammation. Our data provide that CB2 receptor function emerges in the RVM in persistent inflammation and that selective CB2 receptor agonists may be useful for treatment of persistent inflammatory pain.

60 - The CB2 cannabinoid receptor agonist LY2828360 suppresses chemotherapy-induced neuropathic pain with sustained efficacy and attenuates morphine tolerance and dependence

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Cannabinoid CB2 receptors represent an attractive target for pharmacotherapies relative to CB1 receptors due to the lack of psychotropic effects following activation of this receptor. The CB2 agonist LY2828360 lacked both toxicity and efficacy in a clinical trial for osteoarthritis. However, whether LY2828360 shows efficacy in neuropathic pain models and/or enhances efficacy of narcotic analgesics has not been reported. We evaluated anti-allodynic efficacy of chronic treatments with LY2828360 and the opioid analgesic morphine in paclitaxel-treated wild-type (WT) mice and CB2 knockout (CB2KO) mice. The impact of LY2828360 treatment on naloxone-precipitated morphine withdrawal was also assessed. LY2828360 suppressed paclitaxel-induced allodynia without producing tolerance in WT mice. Anti-allodynic efficacy of LY2828360 was absent in CB2KO mice. Morphine tolerance developed in CB2KO mice but not WT mice with a history of LY2828360 treatment. LY2828360-induced anti-allodynic efficacy was preserved in WT mice previously rendered tolerant to morphine but absent in morphine-tolerant CB2KO mice. Coadministration of LY2828350 with morphine blocked morphine tolerance in WT but not CB2KO mice. WT mice that received coadministration of LY2828360 and morphine also exhibited a trend towards lower numbers of naloxone-precipitated jumps compared to CB2KO mice. In conclusion, the CB2 agonist LY2828360 attenuates chemotherapy-induced neuropathic pain without producing tolerance, and may prolong effective analgesia while reducing opioid dependence. Our studies suggest that the CB2 agonist LY2828360 may be useful as a first line treatment in chemotherapy-induced neuropathic pain and may be highly efficacious in neuropathic pain states that are refractive to opioid analgesics.

61 - Kappa opioid receptor up-regulation contributes to mood and reward dysregulation in neuropathic pain

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Many patients with chronic pain also present with mood disorders such as anxiety or depression, which negatively impact their quality of life. The kappa opioid receptor (KOR) is known to regulate mood, motivation and reward pathways in the brain by modulating dopamine neurotransmission. Our study aimed to determine whether KOR activity is increased in chronic neuropathic (NP) pain and whether antagonism of this receptor alleviates the tonic aversive component of pain and restores reward.

We induced NP pain in adult male C57/BL6 mice and Long Evans rats via polyethylene cuff implantation around their left hind limb sciatic nerve. We discovered that KOR signaling was sensitized in NP pain mice where pain animals exhibited exacerbated conditioned place aversion to KOR agonist U50488. Using qRT-PCR and [^{35}S]-GTPγS autoradiography, we also found that NP pain mice exhibited significant increases in KOR gene expression and protein activation in the nucleus accumbens (NAc) and ventral tegmental area (VTA). Furthermore, fluorescent *in situ* hybridization of the VTA confirmed the majority of this KOR up-regulation occurred within dopaminergic neurons. NP pain led to a blunting of reward because intra-VTA injection of MOR agonist DAMGO failed to produce a conditioned place preference (CPP), an effect that could be reversed with pretreatment of KOR-selective antagonist JDTic. The mechanism of reward disruption was determined using microdialysis to be KOR-mediated inhibition of dopamine release at the NAc. These results highlight the impact of KOR activation on NP pain, which has negative consequences on mood and reward.

62 - The role of endogenous dynorphins in amphetamine sensitization and depression-like behaviors in mice

Abdul Hamid, Patrick Chan, Kabirullah Lutfy

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Endogenous dynorphin has been implicated in the action of amphetamine as well as in depression-like behaviors in rodents. In the present study, using male and female mice lacking the preprodynorphin (ppDYN) gene and thier wild-type littermates/controls, we determined the role of endogenous dynorphin in amphetamine-induced hyperlocomotion and sensitization as well as in depression-like behaviors in naive as well as following short-term amphetamine withdrawal. Our results showed that amphetamine increased locomotor activity in both male and female mice, the magnitude of which was greater in wild-type compared to ppDYN knockout mice. Sensitization developed to the action of amphetamine in male mice but there was no difference between mice of the two genotypes. On the other hand, sensitization develped in female mice lacking the ppDYN gene but not in their wild-type controls. The immobility time was not different between wild-type and knockout mice of either sex, showing that male and female mice of the two genotypes exhibited comparable depression-like behaviors. Similarly, the magnitude of depression-like behaviors following amphetamine cessation was not different between mice lacking the ppDYN gene and their wild-type controls. On the other hand, male mice lacking the ppDYN gene exposed to the FST and then tested for locomotor activity exhibited sensitized response to an amphetamine challenge dose compared to their respective non-stressed naive mice. In contrast, amphetamine-induced hyperlocomotion was comparable between wild-type mice exposed to the FST and their non-stressed controls. Taken together, these results suggest that dynorphin does not play any functional role in depression-like behaviors in mice.

63 - AT-328, a selective NOP agonist, reduces the rewarding action of ethanol and cocaine

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The NOP receptor has been characterized as a potential target to reduce the rewarding action of addictive drugs. In the present study, we characterized the selectivity and affinity of a novel small-molecule NOP agonist AT-328 toward NOP compared to classical opioid receptors, and tested its effects on the rewarding actions of ethanol and cocaine using the conditioned place preference (CPP) paradigm. Mice lacking the NOP receptor and their wild-type littermates/controls were tested for baseline place preference on day 1, in which each mouse was placed in the neutral chamber of the CPP apparatus and allowed to freely explore the chambers for 15 min. The amount of time that mice spent in each chamber was recorded. On days 2-4, mice received twice daily conditioning with the NOP ligand (3 mg/kg) or its vehicle 5 min prior to ethanol (2 g/kg) or cocaine (15 mg/kg) or vehicle followed by saline in a counterbalance manner. On day 5, animals were tested for preference toward the conditioning chambers, as described for day 1. Our results showed that ethanol and cocaine each induced a significant CPP response in both wild-type and knockout mice; however, the magnitude of this response was not different between mice of the two genotypes. AT-328 blocked the development of CPP induced by either drug in wild-type but not knockout mice, suggesting that the novel NOP agonist reduced the rewarding action of ethanol and cocaine via the NOP receptor. Thus, NOP agonists may represent potential pharmacotherapy to treat drug addiction.

64 - The role of mu opioid receptors in hyperlocomotion and sensitization induced by amphetamine

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The endogenous opioid system has been implicated in hyperlocomotion and sensitization induced by a number of addictive drugs including amphetamine. For example, naltrexone the non-selective opioid receptor antagonist, has been shown to attenuate the motor stimulatory action of amphetamine. In the present study, we determined the role of mu opioid receptor in amphetamine-induced hyperlocomotion and sensitization in female mice. Mice lacking the mu opioid receptor and their wild-type littermates/controls were habituated to the locomotor activity chambers for one hour, injected with amphetamine (1 or 3 mg/kg) and motor activity was recorded for another hour. Mice in the high-dose amphetamine-treated groups were given the same treatment for 3 consecutive days and then tested for sensitization following a challenge dose of amphetamine (1 mg/kg). We also tested these mice for amphetamine-induced reward in the conditioned place preference (CPP) paradigm. Mice were tested for baseline place preference on day 1, received conditioning with saline/amphetamine (3 mg/kg) or amphetamine/saline in a counterbalance manner on days 2 and 3, and then tested for CPP on day 4. On each test day, the amount of time that mice spent in each CPP chamber was recorded for 15 min. Our results showed that the motor stimulatory action of amphetamine was not altered in mice lacking the mu opioid receptor compared to their wild-type controls. However, amphetamine-induced locomotor sensitization was attenuated in knockout mice. Likewise, this single-conditioning paradigm induced CPP in wild-type but not knockout mice, suggest that the mu opioid receptor may be involved in amphetamine-induced sensitization.

65 - Male but not female mice lacking nociceptin exhibit higher anxiety and enhanced reward following exposure to the elevated plus maze test

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We determined the role of endogenous nociceptin in anxiety-like behaviors and assessed if a subsequent test for ethanol-induced conditioned place preference (CPP) would be altered in these compared to non-exposed mice. Male and female mice lacking N/OFQ and wild-type mice were tested for anxiety-like behaviors in the elevated plus maze (EPM) test on days 1 and 2 and in the presence of ethanol (0.5 mg/kg) on day 5 followed by a test for CPP a month later. In the EPM test, mice were placed in the center of the maze facing one of the open arms and the amount of time that mice spent on each arm was recorded. For the CPP experiments, naive mice (non-exposed) as well as mice exposed to the EPM test (exposed group) were tested for baseline preference on day 1, received twice-daily conditioning on days 2-4 with saline/ethanol (2 g/kg) in a counterbalance manner, and then tested for CPP on day 5. Our results showed that male mice lacking N/OFQ expressed greater anxiety-like behaviors compared to their wild-type controls. However, this response was not observed in female mice. The rewarding action of ethanol was enhanced in exposed male but not female mice lacking N/OFQ compared to wild-type mice. On the other hand, there was no significant difference in ethanol-induced CPP in non-exposed male or exposed female mice of the two genotypes. Together, these results suggest that lack of N/OFQ increases anxiety-like behaviors in males as well as leads to enhanced reward in exposed male mice.

66 - Activation of δOR alleviates κOR-mediated place aversion: MP1104, a dual κOR-δOR agonist is an analgesic and anti-addiction agent with attenuated side effects

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Kappa receptor agonists are known to be agents with analgesic and anti-addictive properties whose therapeutic benefit is limited by dysphoria and sedation. **MP1104**, a morphinan derivative and an analog of IBNtxA is a picomolar binder at δOR and κOR. In functional assays, it is a full agonist at both δOR and κOR while displaying arrestin bias at these receptors. *In vivo*, it is a potent analgesic in mice and exhibits its actions through kappa and delta receptors. **MP1104** also shows no convulsions at doses 30-fold higher than its analgesic ED_{50}. Surprisingly, in spite of its very high affinity for kappa receptors it shows no conditioned place preference or aversion (CPP/CPA) at doses 3-fold higher than its analgesic ED_{50}. Closer evaluation of this compound in the place aversion assays in the presence of delta antagonists shows place aversion. We now report that dual activation of kappa and delta receptors leads to a separation of analgesia and cocaine blockade from aversion. Our preliminary findings suggest that activation of delta receptors alleviates kappa-mediated place aversion and could be an effective strategy to counteract kappa-mediated adverse effects.

67 - Dissecting dopamine pathways altered by pain-induced dysfunction in opioid signaling

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Pain is a complex phenomenon composed of sensory and emotional-affective components. In addition to sensory disturbances, patients experiencing pain report the presence of other negative symptoms such as dysphoria, anhedonia, and depression. These pain-induced psychiatric disorders are commonly linked to a decrease in dopamine (DA) levels within the mesolimbic reward pathway (VTA-NAc). Recent studies from our lab demonstrated that opioid-evoked DA release in the nucleus accumbens (NAc) is attenuated in the presence of pain. This suppression of DA transmission in a condition of pain is likely due to downregulation of mu opioid receptor function in the VTA GABAergic neurons. Moreover, changes in DA transmission within the mesolimbic pathway are associated with impaired motivated responses to natural and drug rewards. Thus, we hypothesize that restoring DA levels by stimulating DA neurons in the ventral tegmental area (VTA) is sufficient to prevent motivational deficits induced by pain. Here, we utilized a chemogenetic approach to determine the role of DA transmission in the pain-induced attenuation of motivation. We demonstrate that activation of DA neurons in the VTA is sufficient to prevent pain-induced decrease in motivation. Furthermore, using an intersectional approach, we assessed whether this effect is mediated by a subset of VTA neurons projecting to the NAc or prefrontal cortex (PFC). Our results indicate that the overall activation of VTA DA neurons (but not solely VTA-NAc or VTA-PFC) is sufficient to prevent motivational deficits during inflammatory pain. Our findings provide mechanistic evidence for the involvement of DA pathways in pain-induced negative emotional states.

68 - Pain Recruits Accumbal Kappa Opioid System And Alters Opioid Consumption.

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The quality of life for patients suffering from chronic pain is impacted by co-morbidities such as prolonged negative affective states. The presence of these severe emotional disturbances might underlie the misuse of drugs of abuse reported in 25% of pain patients, and lead to involuntary overdose. Thus, uncovering the mechanisms responsible for pain-induced negative affect represents an important step to tackle the opioid epidemic. In the mesolimbic pathway, the nucleus accumbens (NAc) shell plays a central role in the integration of aversive and rewarding valence of stimuli. Prior work has revealed that the dynorphin-Kappa Opioid Receptor (KOR) system decreases reinforcing properties of rewards and induces dysphoria and aversive behaviors. The abundance of dynorphin neurons and KORs in the NAc shell led us to hypothesize a role for this system in pain-induced negative affect.

In this study we demonstrate that inflammatory pain, induced by Complete Freund's Adjuvant (CFA) injection in the hindpaw, enhances dynorphin A content and dynorphin neurons excitability in the NAc Shell. A PET scan imaging revealed that CFA-induced inflammation decreases radiotracer binding potential, an indirect measurement of KORs binding pockets occupancy. Lastly, we silenced dynorphin neurons, using a chemo-genetic approach, in inflamed versus controls rats to demonstrate the role of dynorphin release in the pain-induced misuse of fentanyl self-administration.

Our results confirm the recruitment of dynorphin-KORs system in the NAc Shell to drive pain-induced negative affect. This work provides a novel path for future studies aiming to prevent the pain-induced opioid misuse and involuntary overdoses.

69 - Design and Synthesis of Collybolide Probes for Kappa-Opioid Receptor

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Studies suggest that selective kappa-opioid receptor (KOR) agonists biased towards G-protein signaling could be novel therapeutics to treat itch and pain with reduced side effects. Deciphering the structural requirements essential for KOR selectivity through novel chemical probes is the first step in developing selective- and biased-KOR ligands. In this vein, we have identified collybolide, a non-nitrogenous sesquiterpene natural product extracted from the mushroom *Collybia maculata as* a highly selective KOR agonist having *in vivo* analgesic and antipruritic activity. Collybolide has a furyl-*δ*-lactone core similar to that of salvinorin A, however differs from the latter in exhibiting biased agonistic activity. *Collybolide shows no conditioned place aversion unlike classical kappa agonists at doses at which it blocks non-histamine-mediated itch, suggesting the possibility of separating kappa mediated liabilities from its ability to block itch with this novel natural product based template.* However, there are liabilities associated with use of collybolide as a drug candidate such as aqueous solubility, efficacy, and metabolic stability. These liabilities can be overcome by rational structural diversification of collybolide, as supported by the preliminary studies. The goal is to comprehensively examine the SAR of collybolide, which will yield a set of selective- and biased-KOR ligands with the potential of becoming lead drug candidates for itch and pain with reduced side effects**.** Further, high-throughput screening at the NIMH-PDSP against 50 CNS receptors could find new hits, which could be optimized further to explore new therapeutic opportunities.

70 - Trafficking and Signaling of Endogenous Mu-Delta Heteromers

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Heteromerization of Mu-Delta opioid receptors was postulated more than a decade ago using heterologous expression systems. Accordingly, signaling and trafficking properties of Mu-Delta heteromers have been extensively studied in transfected cells. However, little is known about the functional impact of endogenous Mu-Delta heteromerization and, until recently, evidence for in vivo Mu-Delta physical proximity remained limited. Using double knock-in mice co-expressing functional fluorescent Mu and Delta receptors, we previously revealed strong Mu-Delta neuronal co-localization in pathways associated with nociception and opiate withdrawal and showed close physical proximity of the two receptors, which strongly supports the existence of Mu-Delta heteromers in the brain (Erbs *et al.* 2015 Brain Structure Function). Using primary neuronal cultures from double fluorescent knock-in mice (Delta-eGFP/Mu-mcherry), we have now examined whether Mu-Delta heteromerization induces bias in pharmacologically-induced receptor trafficking and/or signaling compared to neurons only expressing one receptor type. Pharmacological stimulation included the use of selective mu or delta agonist alone or in combination with an antagonist for the other receptor, administration of the Mu-Delta agonist CYM51010 or stimulation by endogenous opioid peptides. Modifications in receptor trafficking resulting from Mu-Delta co-expression were identified by immunocytochemistry in primary neuronal cultures. In parallel, Erk1/2 phosphorylation analyzed by Western blot to address G protein and beta-arrestin signaling also revealed Mu-Delta specificities upon stimulation with the different agonists.

71 - Measurement of Heroin Antibody Binding Affinity Using Microscale Thermophoresis

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An effective heroin vaccine needs to induce high affinity antibodies that bind heroin and its metabolites. We developed a microscale thermophoresis (MST)-based method that accurately measures affinity of serum antibodies to heroin and other structurally related drugs. MST exploits the movement of molecules in microscopic temperature gradients to measure binding interactions. The thermophoresis is monitored by measuring the changes in a fluorescence signal. The technique measures the competition of a fluorescently-tagged hapten tracer with opioids. This MST assay can be used with small molecule/protein interactions in serum. The fluorescent tracer (MorHap-Cy5) was used as a probe in MST measurements. The binding affinities (K_i) of a monoclonal antibody to morphine (Ab1060) and hapten-induced polyclonal antibodies (6-AmHap antibodies) to heroin and other opioids were calculated by comparing the antibody K_d against tracer to the IC_{50} of the drug competitor against an antibody:tracer complex. The K_i of ab1060 measured by MST was consistent with the reported K_d value of 2 nM. The 6-AmHap antibodies had high affinities to heroin and its metabolites (6-acetylmorphine, morphine, morphine-3-β-glucuronide, morphine-6-β-glucuronide and normorphine). The TT-6-AmHap vaccine induced antibodies that cross-reacted with heroin and other opioids. The K_d of 6-AmHap antibodies were consistent with the facial recognition hypothesis, which holds that antibodies are generated to the face of the hapten opposite the carrier attachment site. The antibodies cross-reacted with the same face on other opioids. This study demonstrates that MST is a valuable technique for the measurement of the K_i of opioid with proteins.

72 - Neuroinflammatory effects of HIV-1 Tat protein correlate with elevated brain dopamine levels and increased morphine consumption and conditioned place preference (CPP), and are prevented by indomethacin treatment

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The HIV-1 Transactivator of Transcription (Tat) protein activates brain microglia and increases brain temperature, both markers of neuroinflammation. Exposure to Tat protein also increases the rewarding effects of cocaine and ethanol. We hypothesized that mice exposed to Tat protein would demonstrate elevated dopamine levels concordant with the potentiation of morphine-CPP and increased morphine consumption in a two bottle choice (TBC) assay. Western blot analysis confirmed the expression of Tat protein in GT-tg bigenic (iTat) mice, in which brain-selective Tat expression is induced by activation of a doxycycline (Dox) promotor for 7 days. HPLC analysis of iTat mouse brains demonstrated increased dopamine content in prefrontal cortex and Nucleus accumbens by 92% and 37%, respectively. Consistent with these neuroadaptive changes in the dopamine system, a 7-day Tat exposure significantly potentiated morphine-CPP 3-fold over saline-treated littermates. The magnitude of potentiation depended on the degree of Tat exposure. iTat mice treated 7d with Dox significantly increased voluntary consumption of morphine in the TBC, effects that lasted up to a week after Tat induction. Confirming the contribution of neuroinflammation to these effects, daily pretreatment with the anti-inflammatory indomethacin (10 mg/kg/d, i.p.) during the 7-d induction prevented Tat-induced changes in morphine-CPP and TBC. Overall, these data suggest that expression of HIV-1 Tat protein potentiated the rewarding effects of morphine through the elevation of neuroinflammation and dopamine in reward systems, suggesting a biological basis by which HIV infection may increase the vulnerability to opioid abuse. Supported by R01-MH085607 and R01-DA039044 (JPM and MK) and R21-DA041932 (Zhu).

73 - Agonist functional selectivity determined by rgs proteins

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RGS proteins are GTPase accelerating proteins that accelerate the hydrolysis of Galpha-GTP at GPCRs. Using a transgenic knock-in mouse that expresses Galphao proteins insensitive to RGS proteins, we show that GPCR coupling to effectors in the periaqueductal gray (PAG) is altered by the mutation. We compared mu opioid receptor (MOR), nociception/orphanin FQ and GABAB receptor agonist inhibition of evoked GABA release and activation of postsynaptic GIRK channels. We also confirmed the role of Gao proteins in the different receptor-mediated signaling pathways using myristoylated-peptide inhibitors of Gao and Gai. MOR agonists met-enkephalin and morphine induced greater inhibition of presynaptic GABA release in the knock-in mice. Maximal DAMGO and fentanyl inhibition of mIPSCs were not altered suggesting that low efficacy agonists are more affected by RGS activity. MOR agonist activation of GIRK currents was reduced for DAMGO and fentanyl but not for met-enkephalin. The GABAB agonist baclofen produced comparable inhibition of GABA release in both genotypes but GIRK currents were significantly reduced in the knock-in mice. Nociceptin-mediated inhibition of GABA release was not altered in the knock-in mice over a range of concentrations (300 nM - 3 μM) and activation of GIRK currents was not reduced. Thus, not all Galphao/i-coupled GPCRs are modulated by the mutation reducing RGS interactions with Galphao.

74 - Single-particle tracking as an effector-independent readout of mu opioid receptor activity after agonist exposure

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Mu opioid receptors (MORs) mediate analgesia and reward by changing excitability and output from neurons expressing them. However, the cellular effects of MOR agonism often diminish with continued agonist exposure as the receptors undergo desensitization, a process that likely contributes to analgesic tolerance. Most current approaches to observe opioid receptor function, such as electrophysiology or fluorescent cyclic AMP indicators, only resolve signaling consequences based on the output of entire MOR populations within a cell. These assays cannot identify the physical properties of individual receptors in different signaling states and how differentially signaling receptors contribute to an overall cellular response to agonist. Here we established single-particle tracking as a method to measure agonist-induced changes in individual receptors' mobilities, which could reflect unique signaling states between receptors. Single particle tracking in AtT-20 cells expressing Flag-tagged MORs (FLAG-MORs) revealed immobile and mobile populations of receptors under basal conditions. When these cells were treated with DAMGO for 10 minutes, a higher fraction of receptors were immobile. Other agonists with varying pharmacological profiles, such as morphine, were also tested due to known differences in internalization, desensitization, and covalent modifications they induce. These results demonstrate heterogeneity of MOR mobility under basal conditions that is shifted, but not eliminated, after activation with an agonist. Further experiments simultaneously tracking known MOR interacting partners could identify physical receptor properties that correspond to specific mobilities, and tracking in neurons from transgenic FLAG-MOR mice would allow endogenous pharmacological dynamics to be better understood both within entire cells and distinct cellular compartments.

75 - The delta opioid receptor as an emerging therapy for mTBI-induced headaches

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Post-traumatic headache (PTH) is a migraine-like headache disorder secondary to mild traumatic brain injury (mTBI). Up to 90% of mTBI patients develop PTH, which often progresses as a chronic condition. Many PTH patients are highly dependent on anti-migraine medications, as there are no PTH-specific pharmacotherapies. Delta opioid receptor (DOR) agonists may be an emerging therapy for these mTBI-induced headaches because they are uniquely anti-nociceptive in chronic pain and migraine-associated states. DOR agonists, which have anxiolytic and antidepressant effects, may also combat post-traumatic stress disorder which is often co-morbid with PTH. In this study, we characterized a novel mouse model of PTH, and determined the effectiveness of DOR as a therapeutic target. The PTH model combines the closed-head weight-drop method and the nitroglycerin (NTG) chronic migraine model. To induce mTBI, a 30-gram weight was dropped on the intact crania of anesthetized C57Bl6/J adult male mice. After recovery, mice were chronically treated with saline, a subthreshold dose (0.1 mg/kg), or a high dose of NTG (10 mg/kg) over 5 test days. Basal and post-treatment mechanical thresholds were assessed using von Frey hair stimulation. Only the mTBI group developed a progressive and sustained basal hypersensitivity to the subthreshold dose of NTG. Additionally, sumatriptan and topiramate, commonly used migraine therapies, were partially effective in reversing PTH-associated pain. SNC80, a hallmark DOR agonist, reversed acute hyperalgesia, and had a protective effect on basal hyperalgesia. Overall, mTBI increases sensitivity to developing migraine-associated pain, and DOR activation may be effective in alleviating PTH-associated pain.

76 - Identifying the role of peripheral delta opioid receptors in chronic migraine

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Chronic migraine is a debilitating disorder with limited therapeutic options. We have previously shown that delta opioid receptor (DOR) agonists are effective in models of migraine-associated pain, aura, and negative affect. The aim of this study was to further characterize the effectiveness of DOR agonists in other models of headache associated pain, and to determine the specific role of peripheral DORs in migraine. We initially tested the effect of SNC80 in a model of medication overuse headache (MOH) and opioid induced hyperalgesia (OIH). We also tested conditional knockout mice, in which DORs were deleted in $Na_v1.8$-expressing ganglia, within the nitroglycerin (NTG) migraine model; and we started a preliminary characterization in trigeminal ganglia of DOR relative to the pro-migraine neuropeptide, CGRP. C57BL6 mice were treated chronically with sumatriptan or morphine which produced significant mechanical sensitivity – a hyperalgesia that was inhibited by SNC80. $DOR^{Nav1.8}$ and floxed counterparts were treated with vehicle or NTG (10 mg/kg, ip) every other day for 9 days. SNC80 inhibited migraine-associated pain in floxed controls, an effect that was partially blunted in $DOR^{Nav1.8}$ cKOs. Furthermore, we also observed that in DOR-eGFP mice there was a small amount of co-expression between DOR-eGFP and CGRP in trigeminal ganglia of naïve/pain-free animals. Future studies will examine the effect of NTG on DOR-eGFP and CRGP expression. Overall, our results show that DOR activation can inhibit other types of pain that contribute to migraine, and that peripheral/trigeminal ganglia are an important site of action for the anti-migraine effects of DOR agonists.

77 - Ethanol disrupts synaptic specific mu opioid receptor-mediated long term depression in dorsal striatum

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Background: One of the current treatments for alcohol use disorders (AUDs) is the use of the opioid receptor antagonist naltrexone, indicating that the μ opioid receptor (MOPr) has a role in AUD. MOPr activation in dorsal striatum produces long-term synaptic depression (LTD) of specific striatal synapses. Interestingly, the dorsal striatum (DS) is a brain region involved in action selection, control of movement and motivation, and habit development all of which are affected in AUD. Therefore, it's possible that MOPr function in DS can be related to regulation of synaptic plasticity in AUDs.

Results: We found that MOPr activation by DAMGO (0.3 μM) produces static LTD of striatal glutamatergic transmission on to medium spiny neurons in mouse brain slices. Using optogenetics to probe specific dorsal striatal inputs, we found that MOR activation at thalamic glutamatergic inputs produces short term depression (STD), but MOR activation at cortical glutamatergic input produces a static LTD. We determined that a single in vivo exposure to 2.0 g/kg (i.p.) of ethanol (EtOH) disrupts corticostriatal-mediated LTD. Similarly, experiments using a mouse model of binge drinking, drinking-in-the-dark, show that LTD was disrupted at 72 hrs after the last exposure of binge drinking. In addition, we determined that the MOR LTD at specific cortical afferents was disrupted by in vivo EtOH exposure.

Conclusion: The study shows that MOPr activation leads to specific neuroplastic changes at cortical inputs to the DS and plays an important role in alcohol's effects on synaptic regulation possibly regulates dorsal striatal-dependent habitual alcohol abuse.

78 - Free fatty acid receptor hypothalamic GPR40/FFAR1 regulate β-endorphin release via prohormone convertase 2 protein expression

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Previous studies have shown that the administration of docosahexaenoic acid (DHA) or GW9508, a GPR40/FFAR1 (free fatty acid receptor) agonist, facilitates β-endorphin release in the arcuate nucleus of the hypothalamus in mice. However, the mechanisms mediating β-endorphin release induced by GPR40/FFAR1 agonists remain unknown. In this study, we focused on the changes in expression of hypothalamic prohormone convertase (PC) 2, which is a calcium-dependent subtilisin-related proteolytic enzyme. The intracerebroventricular injection of DHA or GW9508 significantly increased PC2 protein expression in the hypothalamus. This increase in PC2 expression was inhibited by pretreatment with GW1100, a GPR40/FFAR1 antagonist. Furthermore, PC2 protein expression gradually increased over time after complete Freund's adjuvant. These increase in PC2 expression were inhibited by pretreatment with GW1100. However, GW1100 by itself had no effect on PC2 levels. Taken together, our findings suggest that activation of the hypothalamic GPR40/FFAR1 signaling pathway may regulate β-endorphin release via PC2, and regulate the endogenous pain control system.

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79 - Opiate Use Disorder in Pregnancy in Crawford County, Ohio: A Case Study

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Opiate Use Disorder (OUD), characterized by opiate addiction and misuse, has increased in the U.S. in recent years. This rise in OUD has affected pregnant women and their infants. An increasing number of infants are diagnosed with neonatal abstinence syndrome (NAS), requiring treatment for withdrawal from opiates postpartum. Furthermore, heavy opiate use during pregnancy is associated with lower birthweight, smaller head circumference, and lower test scores later in a child's life. This case study utilizes a meta-analysis of 26 relevant research studies, birth records from Crawford County's largest maternity ward, and interviews with Crawford County healthcare officials and outside OUD experts. Additionally, this study includes anecdotes from a local woman who became pregnant during treatment for her heroin addiction. According to Crawford County's birth records, the prevalence of opiate use in pregnancy in 2015 was 6.7%; opiates were the most used drug during pregnancy, after marijuana. Opiate-positive infants accounted for 5% of all births in that year. The effect of increasing opiate use during pregnancy is apparent: Crawford County's NAS rate is the highest in any surrounding county, with 12.1 per 1,000 live births. Furthermore, half of the infants transferred from Crawford County to surrounding neonatal intensive care units in 2015 were due to complications of NAS. The data indicate that although all local healthcare officials acknowledge the opioid epidemic, biases regarding treatment strategy have resulted in medical inaction. Unbiased approaches to treating OUD in pregnancy will prevent further harm to addicted mothers and their infants.

80 - The effects of commonly used opioids in cell cultures

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Introduction. Accumulating evidence suggest that long-term opioid consumption are associated with cognitive dysfunctions such as reduced memory and learning. The non-medical use of opioids as well as the number of opioid prescriptions is increasing, even in young adults. The observed cognitive decline can be a cause of increased neuronal cell death, reduced neurogenesis, and morphological changes in the brain. Taken together, these toxic effects associated with opioid consumption must be further evaluated. The aim for present study was therefore to investigate the neurotoxic effects of commonly used opioids using neuronal cell cultures. This study is part of a larger project were the toxic effects of the opioids methadone, morphine, ketobemidone, fentanyl, oxycodone, hydromorphone, buprenorphine, as well as the opioid antagonist naloxone are evaluated.

Methods. Cells were exposed to opioids for 4, 24 or 72h and the neurotoxic effects were measured using a battery of cell viability assays.

Results. There was a varying degree of toxicity between the different opioids and time points. At 4h exposure, toxicity occurred with some opioids while at 24h or longer a more pronounce toxicity was observed with most of the opioids used.

Conclusion. This study reveals that commonly used opioids may induce toxic effects in neuronal cell cultures.

81 - Metformin acting through the mammalian target of rapamycin complex 1 (mTORC1) attenuates morphine efficacy in a mouse model of neuropathic pain.

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There is a pressing need for the identification of new strategies to improve efficacy of opioid-based treatments. Here, by extending upon studies on the mammalian target of rapamycin complex 1 (mTORC1) in chronic pain, for the first time we explored the effect of relatively safe and widely used anti-diabetic drug metformin that inhibits mTORC1 through activation of the 5' adenosine monophosphate-activated protein kinase (AMPK) on the modulation of morphine analgesic efficacy in neuropathic mice. The originality of our study showed that chronic metformin administration (200 mg/kg i.p., 24 h before each morphine) blocked the development and maintenance of morphine tolerance (40 mg/kg, i.p., twice daily for up to 10 consecutive days) in adult male C57BL/6J mice (n=6-8) subjected to spared nerve injury model (SNI) and assessed by the tail-flick, von Frey and acetone tests. Also, a single metformin dose injected in morphine tolerant SNI mice fully restored the analgesic effect of morphine. In addition, when metformin was injected in combination with morphine (3, 10, 20 mg/kg, i.p.) in SNI mice it potentiated dose-dependently analgesic effect of morphine. The involvement of mTORC1 in these behavioural effects was confirmed by our parallel studies using the direct mTORC1 inhibitor CCI-779 (25 mg/kg, i.p.), and by western blotting and immunohistochemistry showing inhibition of mTORC1 downstream targets P-p70 S6 kinase and P-S6 ribosomal protein in the dorsal spinal cord after metformin treatment. Together, our results define the role of mTORC1 in opioid analgesia and thus support a novel approach for the improvement of opioid therapy.

82 - Development of a Selective Antagonist for the Mu-Delta Heterodimer

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Mu and delta opioid receptor (MOR, DOR) agonists induce anti-nociception, tolerance, and withdrawal *in vivo*; however, selective MOR and DOR ligands modulate the other receptor's activity. For example, morphine mediated side effects – including tolerance and withdrawal – are attenuated by DOR selective antagonists. Whether these synergies arise from the formation of MOR and DOR heterodimers (MDOR) remains unclear. Current tools using immunochemical, genetic, or chemical methods show MDOR upregulation after chronic morphine treatment in mice, in addition to potential roles in tolerance, dependence, and drug seeking behavior. However, directly assessing MDOR-mediated behaviors is extremely difficult because no selective MDOR antagonists are available. To address this need, we created a novel series of bivalent MDOR antagonists by connecting low affinity MOR (H-Tyr-Pro-Phe-D1Nal-NH_2) and moderate affinity DOR (H-Tyr-Tic-OH) pharmacophores with variable length polyamide spacers (15-41 atoms). We tested the *in vitro* selectivity of this series using radioligand binding and [^{35}S]-GTPγS coupling in MOR, DOR, and MDOR expressing cell lines. *In vitro* screening of MDOR antagonism and affinity show a clear length dependence in MDOR but not MOR or DOR cell lines. The lead compound – D24M with a 24-atom spacer – displayed high potency (IC_{50MDOR} = 0.84 nM) with 91-fold selectivity for MDOR:DOR and >1:1,000 MDOR:MOR selectivity. Preliminary tail-flick anti-nociceptive assays indicate D24M blocks CYM51010 mediated anti-nociception – a compound previously reported to mediate antinociception through MDOR. After completing *in vivo* characterization, this first-in-class MDOR antagonist will be used to probe how MDOR influences opioid behaviors including withdrawal and tolerance.

83 - Nociceptin receptor trafficking in midbrain dopamine neurons

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Agonists that activate the nociceptin/orphanin FQ receptor (NOPR) have been found to inhibit the rewarding effects of commonly abused drugs including cocaine, morphine, amphetamine, and alcohol in the conditioned place preference (CPP) paradigm in rodents. The Bruchas Lab has obtained recent data demonstrating that the site of NOPR action for inhibiting natural reward seeking (sucrose), as well as cocaine CPP is through its action in dopaminergic (DA) neurons of the ventral tegmental area (VTA). However, little is known about NOPR signaling, or its regulation by receptor trafficking, in these neurons. Here we used primary cultures of ventral midbrain neurons from NOPR-YFP knock-in mice to examine NOPR trafficking at (or very near) endogenous receptor expression levels. We also examined NOPR trafficking selectively in DA neurons in primary cultures using a Cre-dependent AAV construct to express NOPR-YFP only in DA neurons. Using live cell imaging, we found that NOPR is primarily localized to the plasma membrane, and that activation by nociceptin causes pronounced receptor internalization within 10 minutes. This system will be used to identify molecular mechanisms controlling NOPR trafficking in midbrain DA neurons, and to determine if synthetic NOPR ligands can differentially control its trafficking. This work is funded by the NIH through NIDA award K01-DA042219.

84 - Spinal pain regulation of the N/OFQ-NOP receptor system in a spinal nerve injury mouse model

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The endogenous opioid system regulates pain sensitivity and is targeted by both endogenous and exogenous opioid reagents in order to manage pathological pain. The NOP receptor system plays a significant role in the regulation of pain, and interestingly functions differently in the spinal cord and supraspinal tissues. Yet, the mechanism by which NOP receptor agonists regulate pain transmission is still understudied due to lack of appropriate tools to interpret the development of NOP receptor-based analgesics. Here we examined changes in the peripheral and spinal NOP receptor distribution mediated by spinal nerve ligation (SNL) using NOP-eGFP mice, as well as the antihyperalgesic and antiallodynic effects of intrathecally (i.t) administrated N/OFQ in SNL mice. The immunoreactivity of NOP-eGFP receptors in the spinal superficial lamina that is generally responsible for noxious heat stimulation was significantly decreased in SNL mice. In contrast, immunoreactivity was unchanged in the ventral border of lamina II inner, a region essential for injury-induced allodynia. NOP-eGFP expression was also significantly decreased in a large number of primary afferents in the L4 DRG of SNL mice, especially in small DRG neurons. However, SNL mice showed increased sensitivity, compared to sham animals, to the effects of i.t administered N/OFQ with respect to mechanical as well as thermal stimuli. These results suggest the possibility that the spinal NOP receptor system directly down-regulates the neurons intrinsic to the spinal cord but not the excitability of DRG neurons to attenuate injury-induced hyperalgesia under certain chronic pain conditions.

85 - A novel non-canonical role of RGS4 in δ-opioid receptor mediated neuronal outgrowth and differentiation

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The Regulator of G-protein signaling 4 (RGS4) has been found to be a negative regulator of μ, δ and κ-opioid receptor signaling that confers selectivity for G protein coupling to these receptors by directly interacting with them (Georgoussi et al., 2006, 2012; Leontiadis et al. 2009; Papakonstantinou et al., 2015). It was also shown that δ-opioid receptor (δ-OR) forms a multicomponent signaling complex, consisting of Gi/Go proteins and the Signal Transducer and Activator of Transcription STAT5B, that leads to neurite outgrowth and neuronal differentiation via a phosphoryalted-STAT5B-Gαi/o pathway (Georganta et al., 2010, 2013). Knowing that RGS4 is a multitask protein we questioned whether RGS4 could be implicated in neuronal differentiation and neurite outgrowth upon δ-OR activation. We demonstrate that RGS4 directly interacts with STAT5B *in vitro* and in living cells independently of δ-OR presence. This interaction involves the N-terminal portion of RGS4 and the DNA-binding-SH3 domain of STAT5B. Expression of RGS4 in HEK293 cells expressing δ-OR and/or erythropoietin receptor results in inhibition of DSLET and erythropoietin-dependent STAT5B phosphorylation and transcriptional activation. Measurements of DSLET-dependent neurite outgrowth of neuroblastoma cells is also blocked by RGS4, whereas primary cortical cultures of *RGS4*$^{-/-}$ mice exhibit enhanced neuronal sprouting after δ-OR activation. Finally, neural progenitor cells from *RGS4*$^{-/-}$ mice exhibit enhanced proliferation with concomitant increases of the mRNA levels of the *Bcl2* and *Bcl-xl* STAT5B target genes. These observations suggest that RGS4 plays a significant regulatory role in opioid dependent neuronal differentiation and neurite outgrowth via a "non-canonical" signaling pathway involving STAT5B-directed responses.

86 - Genetic dissociation of morphine analgesia from hyperalgesia in mice

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Morphine is the prototypic mu opioid, producing its analgesic actions through traditional 7 transmembrane domain (7TM) G-protein coupled receptors generated by the mu opioid receptor gene (*Oprm1*). However, the *Oprm1* gene undergoes extensive alternative splicing to yield three structurally distinct sets of splice variants. In addition to the full length 7TM receptors, it produces a set of truncated variants comprised of only 6 transmembrane domains (6TM). This study explored the relative contributions of 7TM and 6TM variants in a range of morphine actions. Loss of the 6TM variants in an exon 11 knockout (E11 KO) mouse did not affect morphine analgesia, reward or respiratory depression. However, E11 KO mice lacking 6TM variants failed to show morphine-induced hyperalgesia, developed tolerance more slowly than wildtype mice and did not display hyperlocomotion. Together, our findings confirm the established role of 7TM mu receptor variants in morphine analgesia, reward and respiratory depression, but reveal an unexpected obligatory role for 6TM variants in morphine-induced hyperalgesia and a modulatory role in morphine tolerance and dependence.

87 - Analgesic and Anti-Inflammatory Effects of Novel Kappa Opioid Receptor Agonist 16-Ethynyl Salvinorin A

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Current pain medications are highly addictive. As an alternative, kappa opioid receptor (KOR) agonists have proven analgesic effects without rewarding properties. 16-ethynyl Salvinorin A (Ethynyl SalA) is a potent analogue of Salvinorin A (SalA) and has been shown to attenuate cocaine-prime induced drug-seeking behaviour in rats without causing sedative, anxiogenic, aversive or pro-depressive side effects. Here, we investigated the ability of Ethynyl SalA to modulate pain behaviours in preclinical models of nociceptive, inflammatory and neuropathic pain. The analgesic effects were evaluated in C57BL/6 mice using the 2% intradermal formalin and warm-water tail-withdrawal assays. Ethynyl SalA (2 mg/kg i.p.) showed a significant analgesic effect in the tail-withdrawal assay and a longer duration of action in than SalA. At 2 mg/kg (i.p.) Ethynyl SalA significantly reduced phase one nociceptive ($p<0.0001$) and phase two inflammatory ($p<0.0001$) pain in the formalin assay and reduced the accompanying paw oedema ($p=0.0011$), which was reversed with the KOR antagonist nor-binaltorphimine (10 mg/kg). The paclitaxel-induced neuropathic pain model was used to assess the cumulative dose response effects of Ethynyl SalA, morphine and traditional KOR agonist U50,488, on mechanical and cold allodynia. Paclitaxel-induced neuropathy was evaluated at baseline and every second consecutive day, with dose-response effects evaluated on day 15. Non-linear regression analysis revealed that Ethynyl SalA was more potent at reversing paclitaxel-induced mechanical and cold allodynia than either SalA, U50,488 or morphine. These findings demonstrate that Ethynyl SalA significantly reduces nociceptive, inflammatory and neuropathic pain without the risk of abuse.

88 - Morphine-dosing affects development of antinociceptive tolerance

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Clinical development of antinociceptive tolerance after repeated administration morphine limits its chronic use. Despite growing knowledge about the molecular mechanisms of morphine tolerance, we know little about the influence of dosage regimen in its development. We hypothesized that the morphine dose, as well as the increments of dose after tolerance, contribute to tolerance development and can affect total antinociception levels. Four groups of male Sprague Dawley rats received different daily doses of intermittent subcutaneous morphine for 14 days. After the development of antinociceptive tolerance, different increments of morphine doses were administered until tolerance redeveloped (Group A: 2.5(b.i.d)→5→10 mg/kg/day, Group B: 5(b.i.d.)→10 mg/kg/day, Group C: 5(b.i.d.)→15 mg/kg/day and Group D: 10(b.i.d.)→20 mg/kg/day). Antinociceptive responses were measured daily by tail-flick and hot-plate assays pre-treatment and at various post-treatment time-points. Total antinociception was calculated as AUC over the duration of treatment. Animals treated with smaller morphine starting-doses developed antinociceptive tolerance faster than those started on higher doses. Higher starting-doses and higher dose-increments after tolerance development resulted in more sustained antinociception and delayed the redevelopment of tolerance. Hyperalgesia was absent from all groups, shown by the insignificant variation in basal antinociception levels. Our observations were replicated by both assays and were therefore not assay-specific. These results suggest that morphine dosing regimen in rats significantly influence the manifestation of antinociceptive tolerance and the total antinociception produced. Our results also highlight the need for standardized and validated testing protocols to compare different studies as indispensable to translate pre-clinical results into the clinic.

89 - Role of mu opioid receptor A112G SNP in the mesolimbic neurocircuitry

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Mu opioid receptors (MORs) are highly enriched in the reward circuitry and can be activated by endogenous ligands as well as drugs of abuse. Individuals with the A118G single nucleotide polymorphism (SNP) in OPRM1 exhibit increased risk for drug addiction and require higher morphine doses to achieve adequate analgesia. Genetically engineered mice carrying A112G SNPs (homologous to human A118G) display less total MOR mRNA and protein expression and reduced analgesic response to morphine. In this study, we are investigating how A112G in MOR affects opioid regulation of synaptic transmission in the ventral tegmental (VTA). Previously, it has been proposed that opioids excite dopamine (DA) neurons in the VTA through inhibition of local interneurons; however, recent reports show significant diversity in DA neurons response to MOR. We addressed role of A118G polymorphism in MOR considering VTA heterogeneity, focusing on the mesolimbic pathway. Using slice electrophysiology, we found that both inhibitory and excitatory inputs to VTA DA neurons projecting to the nucleus accumbens medial shell (mAcb sh) were highly inhibited by application of MOR agonist DAMGO, leading to a moderate increase of DA neurons firing. Interestingly, mice carrying minor allele (i.e. GG112) exhibited no change in synaptic transmission compared to their littermate major allele controls (i.e. AA112), but had overall lower DAMGO sensitivity. Additionally, no significant differences in DA neurons firing were found between genotypes. Our current focus is to study the underlying molecular and cellular mechanisms of the A118G SNP which contribute to altered sensitivity of VTA DA neurons to opioids.

90 - Imatinib prevents morphine tolerance without inducing MOR endocytosis.

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Analgesic tolerance limits the use of opioids for the treatment of chronic pain. It was previously hypothesized that sustained signaling by the mu opioid receptor (MOR) could cause morphine tolerance, which could be prevented by promoting MOR endocytosis. However, some studies have challenged this theory. We have shown that Imatinib, by inhibiting PDGFR-β (platelet-derived growth factor receptor-beta), selectively prevents morphine tolerance. In this study, we determine whether inhibition of morphine analgesic tolerance with Imatinib involves endocytosis of the MOR. Rats were injected i.t. with either vehicle, 0.6 nM morphine, 10 µg imatinib or morphine + imatinib. Analgesia was measured daily using tail flick latency (TFL). After either one or five days of treatment, spinal cords were extracted and processed for immunohistochemistry with anti-NeuN (Millipore) and anti-MOR (Abcam) antibodies. Images of the substantia gelatinosa (SG) were analyzed using confocal microscopy. The average number of internalized vesicles containing MOR in neurons was determined utilizing an automated and unbiased software algorithm (Imaris, Bitplane). Co-administration of Imatinib with morphine prevented tolerance without altering morphine's analgesic effect. Interestingly, the levels of MOR internalization in SG neurons were not different between groups. Our results show that imatinib did not prevent tolerance by promoting MOR endocytosis, challenging the hypothesis that the lack of MOR internalization could be the cause of morphine tolerance.

91 - Serotonergic and adrenergic gene variants and vulnerability to non-dependent opioid use and heroin dependence in a Dutch population

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Drug addiction is a chronic relapsing brain disease caused by a combination of genetic, epigenetic, environmental and drug-induced factors. The aim of this case-control association study was to determine if genetic variants in the serotonergic and adrenergic pathways are associated with non-dependent opioid use and heroin dependence in a Dutch Caucasian cohort. Subjects were ascertained in the Netherlands, consisting of the following groups: not opioid dependent (NOD), previous opioid users [n=162]; opioid dependent (OD) patients in methadone maintenance treatment [n=143] or heroin-assisted treatment [n=135]; and healthy controls with no history of heroin use (HC) [n=153]. A total of 152 variants in 19 genes were genotyped using a modified Illumina GoldenGate platform. After filtering for quality, HWE and MAF, 126 SNPs were used in case-control analyses to establish the role of the serotonergic and adrenergic gene variants in (a) non-dependent opioid use, and (b) heroin dependence. The following groups were compared: HC vs. OD; [HC+NOD] vs. OD and HC vs. NOD. In the comparison of HC vs. OD, 4 SNPs in *ADRA1A* and one SNP each in *SLC6A2, SLC18A2*, *S100A10* and *HTR3B* were found significant (p<0.05). In the comparison of [HC+NOD] vs. OD, 3 SNPs in both *SLC18A2* and *ADRA1A* and 1 SNP in *SLC6A2* and *HTR3B* were found significant (p<0.05). Further studies are warranted to confirm and elucidate the potential roles of these variants in the vulnerability to illicit drug use and drug addiction.

92 - Conditioned place aversion to an "extremely" G-protein biased kappa opioid receptor agonist in C57BL6 mice

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Studies of derivatives of *N*-phenylethyl-*N*-3-hydroxyphenylethyl-amine have indicated that select substituents confer preferential binding and GTP-gamma-S stimulation at the kappa opioid receptor (KOR), compared to other opioid receptors or dopamine receptors. We have demonstrated that different N-substituents results in differential G-protein versus arrestin mediated KOR signaling bias. We have determined that *N*-*n*-butyl-*N*-phenylethyl-*N*-3-hydroxyphenylethyl-amine (BPHA) is "extremely" biased towards G-protein mediated KOR signaling, with full agonism in KOR-mediated [^{35}S]GTPγS stimulation, with no stimulation and full antagonism of KOR coupling to β-arrestin 2 . HPHA showed no agonism in mu or delta opioid receptor (MOR, DOR) signaling, although binding inhibition was observed. BPHA showed 29-fold selectivity for KOR versus MOR binding affinity, and 335-fold selectivity for KOR versus DOR binding. *In vivo* in C57BL6 mice, BPHA resulted in increased serum prolactin levels 30 minutes after administration (10-30 mg/kg) , which was blocked by prior administration of KOR antagonist LY2444296. Conversely, 30 mg/kg BPHA had no effect on rotarod incoordination, in contrast to the unbiased agonist U50,488, supporting the hypothesis that beta-arrestin mediated signaling is required for KOR mediated motor incoordination. In a conditioned-place-preference/aversion model in mice, with 4 conditioning sessions, equivalent levels of aversive conditioning were expressed following BPHA as following the unbiased agonist U50,488. This occurred at a dose of BPHA which had no effect on morphine analgesia. Overall, our findings suggest that KOR-mediated aversive behavior is not dependent on KOR-β-arrestin 2 mediated signaling.

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93 - Critical role of Gi-protein signaling in the dorsal striatum for the reduction of voluntary alcohol consumption in C57Bl/6 mice

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Despite the large burden of alcohol abuse at the individual and society level, pharmacological treatment options are limited. Recently, we discovered that systemic administration of Gi-protein biased delta-opioid receptors (DORs) agonists decreased alcohol consumption in a volitional alcohol use mouse model, while β-arrestin-2 biased agonists increased alcohol consumption. We hypothesized that striatal DORs are crucial for modulation of alcohol intake. To test our hypothesis, mice were trained to consume 10% alcohol in a two-bottle, limited-access, drinking-in-the-dark paradigm. We then activated endogenous Gi-protein coupled DORs in the dorsal striatum of cannulated mice by drug microinfusion. Through this approach, we recapitulated our systemic findings in the dorsal striatum: infusion of Gi-protein biased DOR agonist TAN-67 decreased voluntary alcohol consumption ($p < 0.02$), whereas the β-arrestin-2 biased DOR agonist SNC80 increased alcohol intake in the mice ($p < 0.01$). Studies were repeated in β-arrestin-2 knockout mice where SNC80 decreased alcohol consumption ($p < 0.005$) resulting from DOR activation only through Gi-signaling. To study the general role of striatal Gi-signaling on alcohol use, we then utilized a chemogenetic approach to locally express designer muscarinic M4 receptor (hM4Di, DREADD) in neurons of the dorsal striatum using adeno-associated virus. Administration of the DREADD-specific agonist clozapine-N-oxide significantly decreased alcohol consumption in mice expressing the DREADD but not those expressing control GFP. Thus, using both pharmacologic and chemogenetic approaches, we demonstrated that selective Gi-signaling in the dorsal striatum is sufficient to decrease alcohol intake in mice. In contrast, β-arrestin-2 signaling in the dorsal striatum may increase alcohol intake.

94 - Convergent and Divergent Behavioral Changes Caused by Different Patterns of Morphine Exposure in Mice

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Many studies have documented the behavioral changes that develop during chronic opiate exposure in rodents. While analgesic tolerance is widely reported, other behavioral effects (like psychomotor activation and conditioned place preference) can be either enhanced or reduced by prior opiate exposure. This variability is often related to the pattern of chronic opiate administration, with intermittent exposure causing sensitization and continuous exposure causing tolerance. However, few studies have conducted well-controlled comparisons between different patterns of opiate exposure. In an initial series of experiments, we assessed morphine-induced analgesia and psychomotor activation across a range of doses, and compared the effects of intermittent exposure (daily injections) with continuous exposure (via subcutaneously implanted pumps). Both patterns of morphine exposure caused analgesic tolerance, but intermittent exposure caused long-lasting sensitization of psychomotor activation, whereas continuous exposure caused transient psychomotor tolerance. These behavioral differences were apparent after controlling for a variety of factors, including total drug dose, peak analgesic effect, and serum drug level. To determine if these divergent behavioral changes were related to the continuity of morphine exposure, we implanted osmotic minipumps to continuously deliver morphine, and administered daily naloxone injections to interrupt opioid receptor stimulation. This manipulation prevented the initial development of psychomotor tolerance; we are currently evaluating long-term changes in morphine sensitivity. These studies provide a framework for ongoing investigation of the neurobiological consequences of different patterns of opiate exposure. Our results may inform the way opiates are used clinically to treat pain, and suggest new strategies to reduce opiate abuse.

95 - Anxiolytic-like and antidepressant-like effects of a novel delta opioid receptor agonist NC-2800 in rats

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Delta opioid receptor (DOR) agonists have been proposed to be attractive targets for the development of the novel anxiolytic and/or antidepressant. Recently, we succeeded in synthesizing a novel small molecule compound, named NC-2800, containing the morphinan structure. NC-2800 showed DOR agonistic activity in functional cAMP assays using recombinant cells stably expressing opioid mu, delta or kappa receptor, respectively. In this study, we investigated the anxiolytic-like / antidepressant-like effects of NC-2800 in rats. In the elevated plus maze test, which is a screening model for anxiolytics, the single treatment with NC-2800 (1-10 mg/kg, p.o.) significantly increased the time spent on the open arm. In the forced swimming test, which is a screening model for antidepressants, the single treatment with NC-2800 (0.3-10 mg/kg, p.o.) significantly decreased the immobility score and increased the swimming score. In the olfactory bulbectomized rat, which is an animal model of depression, NC-2800 (1 mg/kg/day, s.c. for 14 days) significantly decreased the total score of hyperemotional responses over the entire period for 14 days from day one. In conclusion, it is suggested that NC-2800 is a novel candidate for anxiolytic and/or antidepressant. This study was supported by the Japan Agency for Medical Research and Development (AMED) grant.

96 - Ligand-receptor and structure-function relationship studies on differently substituted diphenethylamines interacting with the κ-opioid receptor

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The κ-opioid receptor (KOR) has an important role in pain modulation and other behavioural responses. Although KOR activation does not produce dependence, euphoria or leads to respiratory suppression, it induces dysphoria, sedation and psychotomimesis. Accumulated evidence indicates that KOR-mediated antinociception results from G protein-mediated signalling events, while alternative signalling pathways (i.e. β-arrestin2) promotes adverse effects. The concept of biased agonism at the KOR has gained significance to drug discovery, with G protein-biased KOR agonists emerging as prospective analgesics with an improved benefit/risk profile. We have designed and pharmacologically investigated a series of structurally-distinct KOR ligands from the class of diphenethylamines. Binding studies showed that these compounds are KOR selective with several analogs demonstrating picomolar affinity for the human KOR. They activate G proteins with high potency as full or partial agonists, while several ligands also display much lower potencies and efficacies in promoting β-arrestin2 recruitment. They induce marked antinociception in the mouse writhing test. Two diphenethylamines HS665 (agonist) and HS666 (partial agonist) were also shown to produce potent centrally KOR-mediated antinociception in the tail-withdrawal assay, associated with reduced sedation and also an absence of conditioned place aversion with HS666. In summary, HS666 displays pharmacological characteristics of a KOR analgesic with diminished aversive liability correlating with its low efficacy for β-arrestin2 recruitment. These results offer valuable structural and functional insights into the design and/or discovery of drugs targeting the KOR with improved pharmacological profiles for the treatment of pain.

97 - Carboxyl-terminal multisite phosphorylation regulates μ-opioid receptor desensitization and tolerance

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Phosphorylation of G protein-coupled receptors (GPCRs,) by GPCR kinases (GRKs) plays an essential role in the regulation of receptor function by promoting interactions of the receptors with interacting proteins. For the mu-opioid receptor (MOR), agonist-induced phosphorylation occurs at a conserved 10-residue sequence, ^{370}TREHPSTANT^{379}, in the carboxyl-terminal cytoplasmic tail. Morphine induces a selective phosphorylation of serine^{375} (S375) in the middle of this sequence that is predominantly catalyzed by GRK5. By contrast, high-efficacy opioids not only induce phosphorylation of S375 but also drive higher-order phosphorylation on the flanking residues threonine^{370} (T370), threonine^{376} (T376), and threonine^{379} (T379) in a hierarchical phosphorylation cascade that specifically requires GRK2/3 isoforms. In vivo GRK3 facilitates MOR desensitization, whereas GRK5 seems to be required for opioid dependence. These findings suggest that agonist-selective recruitment of distinct GRKs can influence different opioid-related behaviors. To further explore the physiological consequences of MOR phosphorylation, we have now generated and characterized a series of different phosphorylation-deficient mice.

98 - Beyond cAMP assays: GIRK channels as ultra-sensitive sensors for the characterization of novel opioids using the voltage-sensitive dye FMP

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All members of the opioid receptor family activate G protein-coupled inwardly rectifying potassium channels (GIRK). Therefore these potassium channels are important effectors of opioid-mediated analgesia. Using GIRK channels as an assay read-out for receptor activation reflects therefore on a naturalistic event of opioid-mediated analgesia. Here, we report on a FLIPR assay system based on the proprietary membrane potential bisoxonole dye FMP that uses HEK293 cells as expression system for both GIRK channel subunits and opioid receptors. As expected, the GIRK1/2 channel selective activator ML297 (VU0456810) induces a decrease of fluorescence signal intensity which is indicative for GIRK channel-mediated membrane hyperpolarization only in the presence of both the GIRK1 and GIRK2 subunits. After co-expression of the GIRK2 subunit and the NOP or the μ receptor we detected a rapid agonist-induced decrease of fluorescence signal intensity induced by Nociceptin/Orphanin FQ or DAMGO respectively. The detected signals were both Pertussis-Toxin (PTX) and Tertiapin-Q (TPN-Q) dependent, yielding EC50 values in the nanomolar range. We tested a panel of selective NOP/μ receptor ligands and multi-opioid receptor agonist. Our findings are in accordance with those made in AtT-20 cells which natively express prototypical neuronal GIRK1/2 channels. We assume that this easy-to-use assay format can be potentially applied 1) to determine the βγ-subunit fraction of G protein signaling, 2) as a general HTS-compatible screening assay for the identification of new opioid ligands, 3) for the identification of GPCR-interacting proteins (GIP) such as GRKs, 4) to identify functional GPCR-GPCR interaction and many other potential applications.

99 - Collybolide, a novel biased agonist to study kappa-opioid receptor pharmacology

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Kappa-opioid receptor (KOR) agonists are promising alternative as therapeutics to mu-opiates with a low risk of dependence and abuse. The use of KOR agonists as analgesics has been limited because of dose dependent neuropsychiatric effects including sedation and dysphoria. Studies with KOR agonists biased to G-protein–mediated signaling (RB64 and triazole 1.1) suggest the possibility of development of novel therapeutics with reduced side effects. In this vein, we have identified collybolide, a non-nitrogenous sesquiterpene natural product extracted from the mushroom *Collybia maculata as* a highly selective and biased KOR agonist with *in vivo* analgesic and antipruritic activity (*Gupta et. al.* PNAS **2016**, *113*, 6041–6046). Collybolide has a furyl-*δ*-lactone core similar to that of salvinorin A, however differs from the latter in exhibiting biased agonistic activity*. Collybolide shows no conditioned place aversion unlike classical kappa agonists at doses at which it blocks non-histamine-mediated itch, suggesting the possibility of separating kappa mediated liabilities from its ability to block itch with this novel natural product based template.* However, there are liabilities associated with use of collybolide as a drug candidate such as aqueous solubility, efficacy, and metabolic stability. These liabilities can be overcome by rational structural diversification of the collybolide template. Design, docking, SAR studies, and pharmacological characterization of collybolide analogues will be discussed. Our hypothesis is to comprehensively examine the SAR of collybolide, which will yield a set of selective- and biased-KOR ligands with the potential of becoming lead drug candidates for itch and pain with reduced side effects**.**

100 - Opioid-Elicited Erk1/2 Phosphorylation, Elk-1 and CREB Activation, and Lipid Raft Localization in HEK 293 Cells Coexpressing μ-Opioid and Nociceptin Receptors

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We aimed to explore the responses of Erk1/2 (p44/42 MAPK), transcription factors Elk-1 and CREB, and lipid rafts elicited by opioids through coexpressed μ-opioid (MOP) and nociceptin/orphanin FQ (NOP) receptors in human embryonic kidney (HEK) 293 cells. Phosphorylation of Erk1/2 was assessed by immunoblotting, Elk-1 and CREB activation was revealed by reporter assays, and receptor colocalization on lipid rafts was detected by immunocytochemistry in HEK 293 cells expressing MOP, NOP, and MOP+NOP receptors after treatment with DAMGO, nociceptin, morphine, methadone, or buprenorphine. We found that coexpressing NOP with MOP compromised the potency of methadone on Erk1/2 activation in MOP-expressing cells. Nociceptin, but not morphine, induced Elk-1 and CREB in MOP+NOP-expressing cells. These results showed that opioids could modulate human Erk1/2, localization of opioid receptors on the lipid rafts, and transcription factors Elk-1 and CREB, in MOP+NOP-expressing cells at the cytosolic, plasma membrane and nuclear levels.

101 - Effect of Magnetic Acupuncture Attachment on Neigun(acupoint P6) on Autonomic Nervous System

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Neigun(acupoint P6) is known to be good acupoint for treating nausea and vomiting occur after surgery and chemotherapy. In addition, it has been reported to stabilize the sympathetic nervous system. The purpose of this study was to investigate the effect of non-invasive magnetic acupuncture attachment on the autonomic nervous system by measuring HRV(Heart Rate Variability). 20 healthy volunteers were recruited through advertisement. Magnetic acupuncture was attached to either Neigun(acupoint P6) or Zu San Li(acupoint ST36) by the randomization table, and HRV was measured before its attachment and 20 minutes after its attachment. One week later, we repeated the procedure at the same time on different acupoint. We measured HRV using SA-2000(Medicare, Korea). Of the 20 subjects who completed the trial, 5 were male and 15 were female, and the mean age of each was 26 and 27. There was no significant difference in HRV indices before attaching the magnetic acupuncture. However, the Neigun(acupoint P6) stimulation showed significant increase in the percent change of SDNN(The Standard deviation of Normal to Normal intervals) when stimulated by magnetic acupuncture(P=0.018) compared to Zu San Li(acupoint ST36) stimulation, but, the percent change of RMSSD(Square root of the mean of the sum of the square of differences between adjacent NN intervals), which mainly reflects parasympathetic activity, was not significant(P=0.069). The result of this study suggest that attaching the magnetic acupuncture on Neigun(acupoint P6) has a positive effect on autonomous function.

102 - TRV0109101, a G protein-biased agonist of the μ-opioid receptor, does not promote opioid-induced mechanical allodynia following chronic administration

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Prescription opioids remain essential for the management of acute moderate to severe pain. However, chronic use leads to adverse events including tolerance and opioid-induced hyperalgesia (OIH). OIH is a process of nociceptive sensitization in which individuals paradoxically experience exaggerated nociceptive responses, instead of pain reduction, when chronically administered opioids. Although the characteristics of OIH have been described in patient populations with high opioid usage, the molecular mechanisms of OIH remain elusive. Here, we describe the pharmacological and pharmacokinetic properties of a G protein-biased μ opioid receptor (MOR) agonist, TRV0109101, a close analog of oliceridine (TRV130). Like oliceridine, TRV0109101 is a potent and selective agonist of MOR-mediated G protein-signaling with reduced β-arrestin2 recruitment compared to morphine. In a model of OIH, continuous infusion of traditional, unbiased opioids in mice promoted significant and sustained hyper-responsiveness to innocuous mechanical stimuli, while continuous infusion of TRV0109101 did not induce mechanical allodynia. Further, after a single dose, TRV0109101 promoted acute reversal of established morphine-induced mechanical allodynia. In subsequent thermal antinociceptive tests, TRV0109101 displayed similar analgesic properties to other conventional opioids with comparable tolerance following repeated dosing or continuous infusion, suggesting the development of OIH is mechanistically distinct from tolerance. Congruent with TRV0109101's pharmacological profile and efficacy, the development of OIH, but not tolerance, is likely dependent on contributions from β-arrestin as oxycodone-induced OIH was absent in β-arrestin1 and β-arrestin2 KO mice. We posit that the use of a MOR G-protein biased agonist may provide robust analgesia with reduced risk of OIH.

103 - Dissecting Mechanisms of Nociceptin Opioid Peptide Receptor Regulation

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The nociceptin opioid peptide receptor (NOPR) is highly expressed in dopaminergic (DA) neurons of the ventral tegmental area (VTA). It is well known that these VTA-DA neurons are associated with reward-related behaviors. Not only does NOPR agonism inhibit the rewarding effects of virtually all drugs of abuse, but nociceptin has been shown to inhibit VTA-DA firing *in slice* and dramatically decrease accumbal dopamine release. Currently, the properties of NOPR regulation and inhibition of VTA-DA neuronal ensembles has yet to be elucidated. We examined NOPR regulation in heterologous cells using cAMP measures and beta-arrestin recruitment (BRET) assays and in acute VTA slices, with endogenous NOPR, using two-photon (2P) imaging. Our preliminary data suggest that NOPR still recruits arrestin3 despite mutated c-terminal phosphorylation sites, but multiple of these sites may be involved in acute desensitization of NOPR. We also demonstrate our ability to use 2P imaging to observe spontaneous and evoked calcium transients in VTA-DA neurons. This method was used to assess the dynamics of NOPR signaling on neuronal activity, including receptor desensitization. We also used real-time 2P-imaging to visualize trafficking of the receptor in brain tissue of a novel NOPR-eYFP mouse line, generated by our lab. We couple these imaging approaches to simultaneously study receptor internalization and desensitization in both heterologous cells and DA neurons. These data provide the groundwork for studies probing the NOPR regulation and signaling in live neuronal populations, which will provide insight on endogenous opioid regulation in neuronal networks and the cellular mechanisms that underline NOPR-mediated behaviors.

104 - Injectable peptide-based hydrogels as efficient matrices for the controlled-delivery of opioid analgesics

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Central goals in chronic pain control aim to provide analgesia of adequate efficacy and duration. Frequent drug administration is commonly required to achieve sustained analgesia. To overcome drawbacks related to repeated opioid administration during the treatment of chronic pain, several controlled-drug delivery systems have been designed. Injectable peptide-based hydrogels represent suitable and attractive controlled-drug delivery matrices, with advantages including protection of the drug against enzymatic degradation by encapsulation in the hydrogel network, while maintaining the therapeutic plasma drug concentration over a long period of time via diffusion from the hydrogel and by degradation of the hydrogel network. Consequently, lower dosage and frequency of administration are possible, and result in an improvement of the drug efficacy, while reducing the risk of side effects. This work reports on the design and investigations of a family of short amphipathic peptide-based hydrogels as controlled-drug delivery systems for opioids. Based on the lead sequence H-FEFQFK-NH_2, distinct peptide hydrogelators were targeted including β^3-homo and D-amino acids. Such hydrogelator sequences show increased proteolytic stability, a feature hypothesized to prolong the in vivo duration of action. The resulting peptide-based hydrogels form thixotropic injectable hydrogels under physiological conditions. Subcutaneous injection of morphine and 14-methoxymetopon (a μ-opioid agonist 500-fold more potent than morphine with an improved side effect profile), co-formulated with different hydrogelators produced prolonged antinociceptive effects up to 96 h in the mouse tail-flick test. These results establish the potential of these peptide-based hydrogels as efficient systems for the controlled-delivery of opioids.

105 - Studies with Nociceptin/Orphanin FQ Peptide (NOP) Receptor knockout rats reveal NOP receptor-dependent sex differences in nociceptive sensitivity, anxiety-like behaviors and serum N/OFQ and CORT levels in response to traumatic stress

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Nociceptin/Orphanin FQ (N/OFQ) modulates pain transmission, learning/memory, stress and anxiety via activation of the N/OFQ peptide (NOP, OPRL1) receptor. We previously reported that single-prolonged stress (SPS), an established animal model of PTSD, produces allodynia and anxiety-like behaviors, elevates N/OFQ and decreases circulating corticosterone in male Sprague-Dawley rats that was reversed by NOP receptor antagonist treatment following SPS. The objective of this study was to further examine the role of the N/OFQ-NOP receptor system with male AND female Wistar Han wildtype (WT) and NOP receptor knockout (*OPRL1*$^{-/-}$) rats randomly divided into control or SPS groups by gender. In male and female WT rats, paw withdrawal thresholds (PWT) to von Frey and paw withdrawal latencies (PWL) to radiant heat stimuli, respectively, decreased within 7 days after SPS, lasting through day 28. Baseline sensitivity did not differ between genotypes. However, while male *OPRL1*$^{-/-}$ rats were protected from SPS-induced nociceptive hypersensitivity, female *OPRL1*$^{-/-}$ rats exhibited exacerbated nociceptive hypersensitivity. Male *OPRL1*$^{-/-}$ rats had a lower anxiety index (AI) than WT, and it was not increased by SPS. In contrast, SPS increased AI in WT and *OPRL1*$^{-/-}$ female rats. SPS increased N/OFQ and decreased circulating corticosterone levels in male WT, but not in male *OPRL1*$^{-/-}$ rats. SPS did not alter serum N/OFQ or CORT levels in either WT or *OPRL1*$^{-/-}$ female rats. Male *OPRL1*$^{-/-}$ rat results are consistent with previous findings with the NOP receptor antagonist, and indicate that endogenous N/OFQ-NOP receptor signaling plays an important, but distinct, role in males and females following traumatic stress.

106 - A pH-sensitive opioid analgesic designed by modeling of pathological receptor-ligand interactions

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Indiscriminate activation of opioid receptors provides pain relief but also severe central and intestinal side effects. We hypothesized that exploiting pathological (rather than physiological) conformation dynamics of opioid receptor-ligand interactions might yield ligands without adverse actions. By computer simulations at low pH, a hallmark of injured tissue, we designed an agonist that, due to its low pKa, selectively activates peripheral mu-opioid receptors at the source of pain generation. Unlike the conventional opioid fentanyl, this agonist showed pH-sensitive binding, G-protein subunit dissociation, and cAMP inhibition *in vitro.* It produced injury-restricted analgesia in rats with different types of inflammatory pain without exhibiting respiratory depression, sedation, constipation or addiction potential.

107 - Hsp90 and PEBP Promote Opioid Anti-Nociception Through the Regulation of Mu Receptor Signal Transduction Cascades

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Recent advances in understanding the signal transduction cascades of the mu opioid receptor (MOR) have suggested new strategies for opioid drug discovery to minimize side effects – such as βarrestin2 biased agonists. One limitation to this approach is that the signaling complex of the MOR is not well understood, including our mechanistic understanding of how signal transduction molecules transduce changes in behavior. To address this limitation, we sought to identify new signaling regulators of the MOR, and to determine their role in MOR-mediated anti-nociception. Through the intracerebroventricular administration of selective small molecule antagonists in mice, we found that the chaperone protein Heat shock protein 90 (Hsp90) and the GRK2/Raf1 sequestering protein phosphatidylethanolamine-binding protein (PEBP) both strongly promote opioid anti-nociception. Hsp90 inhibition completely blocked morphine anti-nociception in HIV neuropathic pain and strongly blocked in post-surgical pain, while only slightly impacting tail-flick anti-nociception. PEBP inhibition by contrast strongly blocked tail-flick anti-nociception. Seeking mechanisms for these changes, we found that Hsp90 inhibition blocked ERK MAPK activation in the brain, and this signaling change could account for all the impact of Hsp90 on opioid anti-nociception. In comparison, we found that PEBP regulates the potency/efficacy of βarrestin2 recruitment in vitro, suggesting a role in regulating arrestin recruitment in the brain with subsequent effects on tail-flick anti-nociception. Through these studies, we have thus identified novel MOR signaling regulators with strong roles in promoting anti-nociception, and begun to illuminate their molecular mechanisms. Future work could utilize this information for the creation of novel drug development strategies.

108 - Modulation of endogenous opioid-mediated antinociception by regulator of G protein signaling (RGS) proteins

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RGS proteins are negative modulators of G protein signaling downstream of G protein-coupled receptors, including the mu-opioid receptor (MOPR) and serve to switch-off signaling. We have shown that Gαo is the predominant Gα protein for mediating MOPR agonist-mediated antinociception in mice. Consequently, 129SvEv mice expressing Gαo that is insensitive to RGS protein activity (RGSi-Gαo) exhibit an increased antinociceptive response to MOPR agonists in the hot-plate test. In contrast, there was a paradoxical inhibition, or no change, of opioid antinociception in the tail-withdrawal test. Here we show, using the hot-plate test as a measure of heat nociception, that endogenous opioid peptide signaling is also enhanced in these RGSi-Gαo mice. The enkephalinase inhibitor RB-101, which increases the half-life of endogenous opioid peptides, administered intracerebroventricular to wild type mice produced antinociception that was reversed by an opioid antagonist, confirming a role for endogenous opioid peptides. This antinociceptive effect was enhanced in RGSi-Gαo mice. A brief swim stress triggered antinociception that was greater in RGSi-Gαo mice compared to their wild-type littermates. This response was also reversed by opioid antagonists. The results show the opioid peptide system is more active in mice expressing RGSi-Gαo and that inhibition of RGS proteins may provide a therapeutic target. On the other hand, the role of RGS proteins in negatively modulating MOPR signaling is not reflected as consistent inhibition of behavioral responses across models suggesting RGS proteins may be affecting other systems and/or acting on different intracellular substrates. Supported by DA035316 and DA035316-03S1.

109 - Label-free mass spectrometry based quantitation of peptides related to chronic migraine and opioid-induced hyperalgesia (OIH) in mice

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Migraine is a pain disorder characterized by recurrent headaches that debilitate around 40 million people annually in the United States. The mechanism that leads to this chronic pain state is currently unknown. Opioid drugs are commonly prescribed medications to treat migraine. However, persistent use of opioids can lead to more severe and increased frequency of migraine. This paradoxical situation is likely related to the known effects of opioid use- opioid-induced hyperalgesia (OIH) - where patients administered with opioids becomes more sensitive to a pain stimulus. Considering that opioids can push episodic migraine into a chronic condition indicates that OIH may share overlapping mechanisms with chronic migraine. Though the same pain signaling pathways and nervous system morphological structures are involved in development and sustaining of both conditions, precise molecular mechanisms driving them are still not well understood. Neuropeptides, which act as cell-to-cell signaling molecules between different regions of the nervous system are believed to play an important modulatory role in controlling these disorders. To identify the peptide complement in the brain regions associated with these disorders, we performed a mass spectrometry based *de novo* sequencing of peptides followed by their label-free quantitation using the open source platform SKYLINE. 10 peptides with significantly changed signals were identified from 6 different prohormones, including pro-CGRP, pro-opiomelanocortin and pro-Enkephalin prohormones. 3 of these 6 prohormones are common to both the conditions. The peptide and prohormone biomarker candidates identified in this study may help elucidate the mechanistic processes leading to OIH and migraine.

110 - Overlapping mechanisms between opioid induced hyperalgesia and chronic migraine

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Opioids are commonly used to treat migraine, but can ultimately result in refractory headache and migraine chronification. This exacerbated pain is likely related to opioid induced hyperalgesia (OIH), a phenomenon in which opioid treatment results in a paradoxical increase in pain. It is currently unknown how mu opioid receptor agonists negatively regulate migraine. The aim of this study was to identify overlapping mechanisms between OIH and chronic migraine. Initially, we determined if morphine could promote chronic migraine-associated pain. We used the known human migraine trigger, nitroglycerin (NTG), which we've shown previously produces a migraine-associated hyperalgesia in mice. Mice were treated daily for 11 days with morphine or vehicle. Following two days of pre-treatment mice were injected with a sub-threshold dose of NTG or vehicle every second day for 9 days. Morphine and NTG treated animals developed a severe chronic hyperalgesia, which was greater than either treatment alone. Further, changes in neuropeptide expression are hallmarks of both chronic migraine and OIH. An additional aim of this study was to perform a peptidomic screen to determine changes in neuropeptides following chronic NTG vs. OIH treatment. Mice were chronically administered a maximal dose of NTG to induce chronic migraine-associated pain; or escalating doses of morphine to induce OIH, along with corresponding vehicle controls. Tissue from multiple pain and reward-processing regions were collected for quantitative mass spectrometry and multiple significantly changed peptides were detected, including a pro-pain/migraine CGRP-related peptide. Our results indicate that opioids may synergistically promote a chronic migraine state.

111 - Influence of tramadol in global cerebral ischemia-induced mechanical allodynia

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Central post-stroke pain (CPSP) is one of the complications of cerebral ischemia and neuropathic pain syndrome. Although CPSP is a serious condition, details pertaining to underlying mechanisms are not well established, making current standard treatments only partially effective. In this study, we assessed the effects of tramadol, an analgesic drug mediated by opioid receptors, using a mouse model of global cerebral ischemia. Male ddY mice were subjected to 30 min of bilateral carotid artery occlusion (BCAO). The development of hind paw mechanical hyperalgesia was measured using the von Frey test. Mechanical allodynia was significantly and dose-dependently suppressed by intraperitoneal (i.p.) tramadol (10 or 20 mg/kg). These effects which peaked at 10 min and continued for at least 60 min were inhibited by naloxone (a nonselective opioid receptor antagonist, 1 mg/kg, i.p.). Tramadol antinociception was significantly negated by beta-funaltrexamine (a selective μ-opioid receptor antagonist, 20 mg/kg, i.p.) but not naltrindole (a selective δ-opioid receptor antagonist, 5 mg/kg, i.p.) or nor-binaltorphimine (a selective κ-opioid receptor antagonist, 10 mg/kg, i.p.) after 5 min, by b-funaltrexamine and nor-binaltorphimine but not naltrindole after 10 min, and by all selective opioid receptor antagonists at 15 and 30 min after tramadol treatment. These results suggested that antinociception induced by tramadol through various opioid receptors was time-dependent. Furthermore, it is possible that the opioid receptors involved in tramadol-induced antinociception change over time with metabolism of this drug. Support by Grants-in-Aid and by special coordination funds from Grants-in-Aid for Scientific Research (C) (16K10988)

112 - In Vitro And In Vivo Profile Of PPL-101 And PPL-103: Mixed Opioid Partial Agonist Analgesics With Low Abuse Potential

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Opiates are the most effective and widely used treatments for acute and chronic pain. However, problems associated with morphine and other standard opioid analgesics severely limit their effectiveness in the clinic. PPL-101 and PPL-103, derived from morphine and morphinan ring systems contain a chiral N-substituent, which confers a combination of high binding affinities and partial agonist activities at mu, delta, and kappa opioid receptors, leading to unique in vivo pharmacology compared to other conventional opioids. Acute antinociceptive and reward acquisition of PPL-101 and PPL-103 were assessed in mice using the tail flick assay and CPP paradigm, respectively. The reinforcing effects of these compounds were assessed in rats using the self-administration paradigm. In mice, PPL-101 and PPL-103 produced antinociception reaching maximal effects equivalent to morphine at approximately one third and one-tenth of morphine's dose, respectively. PPL-101-induced antinociception was attenuated following pretreatment with the kappa antagonist JDTic, but not the mu opioid antagonist beta-FNA. In mice, PPL-101 and PPL-103 produced dose-dependent decreases in activity, similar to other kappa agonists, however, they did not produce conditioned place aversion, and in fact elicited a trend towards CPP. In rats, neither PPL-101 nor PPL-103 were self-administered when substituted for morphine. Collectively, these results indicate that mixed opioid receptor partial agonists can produce potent antinociceptive activity with a lack of dysphoria in mice and without being self-administered in rats. Compounds with this profile could be superior analgesics with greatly reduced addiction liability and fewer side-effects compared to traditional opiates.

113 - Effects of Heroin on Prosocial Behavior in Rats

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Previous research indicates that rats are capable of prosocial or empathetic behavior. Under normal conditions, when a “free” rat is placed in the vicinity of rat trapped in a restraint, the rat will release or “save” the other rat from confinement. Human empathy research shows a correlation between heroin use and lower levels of empathy. This study was aimed to determine the effects of heroin on prosocial behavior in rats. For two weeks, rescuer rats were given the opportunity to save their cage-mate, and the occurrence and latency to free the confined rat was recorded. After the saving behavior was established rats were randomly selected into either the sucrose (control) or heroin (treatment) group in which they were allowed to self-administer heroin (0.06 mg/kg/inf, i.v.) or sucrose pellets (orally) for 10-14 days. Next, rats were retested for saving behavior once daily for 3 days, during which they had a choice between freeing the trapped rat or continuing to self-administer their respective reinforcer. Results indicated that sucrose rats continued to save their cage-mate instead of self-administering sucrose, whereas heroin rats chose to self-administer heroin and not save their cage-mate. Thus, rats with a history of heroin self-administration show deficits in prosocial behavior, consistent with specific diagnostic criteria for opiate use disorder. Since activity in the insula is known to be correlated with both drug craving and important abilities needed for social interaction, future studies are planned to attempt to restore prosocial behavior following heroin intake using chemogenetic approaches.

114 - Characterization of the endogenous small-sized peptides released in the nucleus accumbens related to pain modulation

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A growing body of evidences indicates the alteration of excitability in the nucleus accumbens (N.Acc) under the chronic pain. They suggest that the neuronal activity *via* chemical signaling in the N.Acc plays a role for pain modulation. However, there is little knowledge about what kind of small molecules are involved in this event. In this study, we investigated the role of the small molecules released from the N.Acc induced by pain or administration of analgesics. We generated mice expressing channerhodopsin-2 in the nociceptive neurons with the injection of AAV6-hSyn-ChR2(ET/TC)-EGFP. Two weeks after AAV injection, we collected the dialysate in the N.Acc after the activation of the nociceptive neurons by optical stimulation. We also collected the dialysate in the N.Acc. after the systemic administration of morphine. Then we performed comparative metabolome analysis by fourier transform mass spectrometry (FT-MS). In the present study, 89 metabolites were identified in the N.Acc. Among those, N-acetylaspartylglutamate (NAAG) and acetoacetate were decreased after the activation of the nociceptive neurons, whereas both were increased after the systemic administration of morphine in the N.Acc. As theoretically considered, direct injection of the NAAG into the N.Acc significantly attenuated the nociceptive stimuli induced by the activation of the nociceptive neurons by optical stimulation. These results suggest that NAAG released in the N.Acc. could be, at least in part, associated with pain modulation.

115 - Automated detection of Mu Opiod Receptor recycling reveals distinct subpopulations of exocytic events

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GPCR internalization and recycling-dependent resensitization is an integral part of how cells regulate their responsiveness to intercellular signaling. Receptor recycling has previously been assessed either through gross measures of cell surface receptor density using antibody labeling or through monitoring of individual exocytic events using TIRF microscopy. We have used quantitative image analysis of single exocytic events of the Mu Opioid Receptor, coupled with a machine learning algorithm, to identify distinct subpopulations of these exocytic events that can provide insight into the multiple regulated and constitutive recycling pathways present in cells. This insight into recycling can help elucidate the regulation of receptor recycling in basal conditions, as well as provide opportunities for modifying specific recycling pathways to change cellular responsiveness.

116 - Development of a Virtual Reality Paradigm for in vivo Hippocampal Imaging During Morphine Conditioned Place Preference

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Long term associations between a specific environment and drugs of abuse, such as opioids, can trigger craving and relapse in people with previous history of drug misuse. These maladaptive memories are partly formed by structural and functional changes in the dorsal hippocampus. Using *ex vivo* imaging approaches, our lab has shown that morphine conditioned place preference (mor CPP) development decreases the number of dendritic spines on hippocampal CA1 neurons through NR2B-containing NMDA receptors. Yet the timing and dynamics of these events and their potential relationship to the association between morphine reward and context are unknown. To observe neural networks in real time as mor CPP and reinstatement take place, we have designed a virtual reality conditioned place preference (VR-CPP) paradigm paired with two-photon imaging. The three chamber VR-CPP apparatus contains a neutral middle chamber and two conditioning chambers containing distinct visual cues. Mice are head fixed in the VR environment and allowed to freely run on a Styrofoam ball suspended by air pressure. Movement of the ball is tracked by a computer mouse, converted to forward and yaw velocities by custom written software in LabView, and then fed to a virtual reality engine written in Matlab, which updates the visual scene permitting the animal to navigate through the VR environment. The mor CPP protocol (Portugal et al., 2014) is then executed in the VR environment. When combined with two-photon *in vivo* imaging, this novel behavioral paradigm allows unprecedented spatio-temporal resolution in following structural and activity changes that accompany mor CPP.

117 - Activation, Desensitization and Trafficking of total phosphodeficient MORs in the MOR-knockout rat

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Desensitization and trafficking of receptors is thought to be dependent on the phosphorylation of multiple sites on the C-terminus of MORs. Both processes were examined in locus coeruleus (LC) neurons in brain slice preparations. Wild type and mutant MORs were virally expressed in a MOR knockout rat. Both receptors had an N-terminal GFP tag such that trafficking of receptors induced by the application of agonists could be examined. All phosphorylation sites on the C-terminus were mutated to alanine in a construct termed total phosphodeficient (TPD) MOR. Whole cell recordings and imaging experiments were carried out in preparations expressing either wild type or the TPD MOR. Activation of both receptors induced outward currents. Application of a saturating concentration of [Met]^{5}enkephalin (30 μM) resulted in a peak and decline in current over a period of 10 min in experiments from wild type receptors. In experiments with the TPD MOR the decline in the current was significantly reduced, indicating a dramatic reduction in desensitization. Imaging of membrane-associated receptors was carried out using an anti-GFP nanobody conjugated with Alexa594. The wild type receptors internalized within 10 min following the application of ME, however there was no internalization of the TPD MORs. The results show that acutely TPD MORs function similar to wild type but that desensitization and trafficking is dramatically disrupted.

118 - Inflammatory Pain Changes The Expression and Function Of CB1 Receptors in The Periaqueductal Gray

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The periaqueductal gray (PAG) mediates the antinociceptive properties of analgesics, including opioids and cannabinoids. Administration of either opioids or cannabinoids into the PAG induces antinociception, and the presumed mechanism of analgesia is the disinhibition of GABAergic neurons. However, most studies characterizing the antinociceptive properties of cannabinoids in the PAG have been conducted in naive animals. Few studies have reported on the role of CB1 receptors in the PAG during conditions which would prompt the administration of analgesics; namely, in chronic pain. In this study, we used the CFA model to characterize CB1 receptor expression and function during persistent inflammatory pain. Using cellular fractionation and western blot analysis, we demonstrate that 48 hours after CFA injection, there is a significant upregulation in the expression of synaptic CB1 receptors. To assess whether this protein upregulation induces a functional change, we measured the anti-hyperalgesic action of WIN 55,212-2, and used whole cell patch clamp electrophysiology to identify pain-induced alterations in the PAG. By studying the potency of WIN during conditions in which analgesics would normally be administered, we have unveiled a novel compensatory change in the descending pain pathway during persistent pain. Opioids are still the most commonly prescribed analgesics for chronic pain. Because there are neuroanatomical and functional interactions between PAG CB1 and mu-opioid systems, these pain-induced changes provide critical insight into the analgesic efficacy of these drugs during persistent pain, and may aid the development of novel pharmaco-therapies.

119 - Naltrexone and Nalmefene Attenuate Cocaine Place Preference but Differentially Effect Cocaine Locomotor Sensitization

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INTRODUCTION/METHODS: Previously we showed that nalmefene (NMF) exhibits partial agonism at kappa opioid receptors (KOPr), while also exhibiting antagonism at mu opioid receptors, using in vitro GTPγS binding stimulation and in vivo prolactin release as an biomarker of kappa agonist activity in humans (Bart et al., 2005, Neuropsychopharmacol, 30:2254-62). Here we examine the effect of low (1 mg/kg) and high (10 mg/kg) dose naltrexone (NTX) and NMF on cocaine place preference and locomotor sensitization. RESULTS: Both doses of NTX and high dose NMF significantly reduced cocaine place preference. Conversely, a significant place avoidance was observed for high dose NTX and both NMF doses. Interestingly, blockade of cocaine induced locomotor sensitization was observed with both NTX doses and low, but not high, dose NMF. CONCLUSIONS: Overall, NTX and NMF block the hedonic effects of cocaine and induce a negative/aversive subjective state when administered alone. However, unlikely high dose NTX, a significant locomotor sensitization to cocaine was observed with high dose NMF pretreatment (as measured by crossovers during CPP conditioning) suggesting potential pathway or receptor specific differences between NTX and NMF in vivo. This is an important step in understanding the potential mechanism(s) of action of NTX and NMF for the development of more efficacious pharmacological treatments of substance use disorders. Support: the Gary R. Helman Postdoctoral Research Fellowship (KAW), the Robertson Development Fund (EB, BR, MJK), and the Miriam and Sheldon Adelson Medical Research Foundation (MJK).

120 - A Novel assay for measuring human μ-opioid receptor's functional activity

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Traditionally GTPgS binding and adenylyl cyclase (AC) activity inhibition are often used to measure μ-opioid receptor (MOR) activation. We developed a novel assay in which intact live cells can be used to develop new ligands (agonist or antagonist) for human mu-opioid receptor. Chinese hamster ovary cells were stably transfected with human MOR, and μ-opioid receptor's expression was confirmed by immunohistochemistry and binding affinity assay. CHO cells expressing mu were seeded in 96-well plates, loaded with membrane potential-sensitive fluorescent dye for 60 minutes, and then treated with or without mu agonists. CHO-MOR cells hyperpolarized upon the treatment of its own agonist, which is probably mediated by G protein-coupled inwardly-rectifying potassium channel or other certain potassium channel. Membrane potential change induced by CHO-MOR activation can be detected only through membrane potential-sensitive fluorescent potassium dye but not through calcium dye. The resulting fluorescence changes could be recorded in real time. The μ-opioid receptor agonist DAMGO has an EC50 of ~3.28nM in mu cells, and 100nM DAMGO-induced μ-opioid receptor activation can be inhibited by μ-opioid receptor antagonist naloxone in a dose-dependent manner, with an IC50 of is ~40nM. This novel functional assay provides a simple, quick and safe method for real-time measurement of mu receptor activation or inhibition through their action on native CHO cells expressing human MOR. This method can greatly facilitate the drug discovery for opioid receptors.

121 - POTENTIAL OF CIRCULATING MU-OPIOID RECEPTOR AND TOLL-LIKE RECEPTOR-4 ACTIVATION IN MORPHINE-ADMINISTERED MICE

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In this study, we set up new cell-based assays to quantify circulating receptor activation potential and unveiled the ability of morphine and its metabolites present in biological samples to activate the μ-opioid receptor (MOR) and toll-like receptor-4 (TLR-4). Alphascreen cyclic AMP (cAMP) assay and MOR overexpressing HEK293 cells were employed to quantify the MOR activation. HEK-Blue hTLR4 were utilized to measure TLR-4 activation. Both assays were standardised using morphine, its MOR-active metabolite morphine-6 glucuronide (M6G) and its MOR-inactive, but TLR4-active metabolite morphine-3 glucuronide (M3G) in the presence/absence of plasma. Specificity was verified using opioid and TLR-4 antagonists or inhibitors. These methods were then employed to measure the receptors activation potential in the circulation of morphine-treated mice (1 or 10 mg/kg every 12 h for 3 days). Plasma spiked with morphine or M6G could activate MOR, whereas M3G, which lack MOR activation potential, led to moderate but consistent TLR-4 activation. Plasma from morphine-treated mice showed MOR activation potential, reversed by MOR antagonists. Morphine administration led to TLR4 activation at time points where M3G is measured in plasma, but also to TLR4-independent NF-κB activation via elevation of circulating cytokines such as TNFα at time points where M3G is not detected. Our study has established new methods to evaluate the effect of perioperatively administered opioids on the biology of cancer cells and other prominent tumour-associated cells such as macrophages. Furthermore, the study suggests a novel non-opioid-mediated role for morphine administered perioperatively to cancer surgery patients.

122 - Alternatively spliced mu opioid receptor C termini impact the diverse actions of morphine

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Extensive 3′ alternative splicing of the mu opioid receptor gene *OPRM1* creates multiple C-terminal splice variants. However, their behavioral relevance remains unknown. The present study generated 3 mutant mouse models with truncated C termini in 2 different mouse strains, C57BL/6J (B6) and 129/SvEv (129). One mouse truncated all C termini downstream of *Oprm1* exon 3 (mE3M mice), while the other two selectively truncated C-terminal tails encoded by either exon 4 (mE4M mice) or exon 7 (mE7M mice). Studies of these mice revealed divergent roles for the C termini in morphine-induced behaviors, highlighting the importance of C-terminal variants in complex morphine actions. In mE7M-B6 mice, the exon 7–associated truncation diminished morphine tolerance and reward without altering physical dependence, whereas the exon 4–associated truncation in mE4M-B6 mice facilitated morphine tolerance and reduced morphine dependence without affecting morphine reward. mE7M-B6 mutant mice lost morphine-induced receptor desensitization in the brain stem and hypothalamus, consistent with exon 7 involvement in morphine tolerance. In cell-based studies, exon 7–associated variants shifted the bias of several mu opioids toward **β**-arrestin 2 over G protein activation compared with the exon 4–associated variant, suggesting an interaction of exon 7–associated C-terminal tails with β-arrestin 2 in morphine-induced desensitization and tolerance. Together, the differential effects of C-terminal truncation illustrate the pharmacological importance of *OPRM1* 3′ alternative splicing.

123 - Design and synthesis a novel κ opioid receptor agonists isolating both sedation and aversion.

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Highly selective κ opioid receptor (KOR) agonists were expected to be potent analgesic drugs without morphine-like side effects derived from μ opioid receptor. Nalfurafine is the only KOR agonist without both addiction and aversion in the efficacious dose, which was launched in Japan in 2009 as an antipruritic agent for kidney dialysis and chronic liver disease patients.[1] However, nalfurafine could not be applied to use for analgesic because of the sedative effect derived from the KOR. Accordingly, we have attempted to design and synthesize nalfurafine derivatives without the sedative effect. The detailed conformational analysis and structure activity relationship studies of nalfurafine led us to the hypothesis that the orientation of the 6-amide side chain would significantly influence the affinity for the KOR and the side effects derived from KOR, like aversive and sedative effects.[2-3] On the basis of this hypothesis, we designed and synthesized the 4,5-epoxy morphinan derivatives with a bicyclo[2.2.2]octene skeleton whose 6-amide side chain oriented to the upper side of the C-ring. The final obtained derivative YNT-1612 showed the higher agonist activity for the KOR than that of nalfurafine. Furthermore, YNT-1612 showed no sedative and aversive effects even at much higher dose than that of nalfurafine. We will report the design, synthesis, and the pharmacological effects of YNT-1612 derivatives.

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124 - The effects of chronic stress on alcohol consumption in μ-opioid receptor knockout mice

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Adverse life experiences are associated with an increased risk of developing alcohol use disorders (AUD). Female drinking levels and drinking problems are increasing and more closely approximating those seen among men. One factor that has not been adequately examined in previous studies of the genetic contributions to AUD is sex, which is a substantial shortcoming in the field given that there are significant influences of sex on alcohol consumption patterns and alcoholism in humans and in animal models. Genetic factors, such as allelic variation in opioid receptor system genes, have a substantial influence on alcohol consumption, but only a limited set of such genetic influences on behavioral activity associated with forced drinking have been examined. In our previous study, disturbances of mu-opioid receptors influenced the effects of isolation-rearing on ethanol consumption in a sex-dependent manner. The preset study was based on the hypothesis that the effects of restrain stress on ethanol consumption, would be also influenced by both sex and the functioning of mu-opioid receptor systems. The effects of restrain stress on ethanol intake were assessed using a two-bottle home-cage consumption (8% v/v ethanol vs. water) paradigm in male and female wild-type and mu-opioid gene knockout mice. Restraint stress modestly increased ethanol consumption in female mu-opioid knockout mice but not in female and male wild-type mice. The study shows that disturbances of mu-opioid receptors influences the behavioral consequences of ethanol consumption following stress in a sex-dependent manner.

125 - Commonly used opioids induce neurotoxic effects in neuronal cell cultures

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Non-medical use of opioids is a global problem affecting health, social and economic welfare. The frequency of prescription drug abuse is increasing and the consequences of this abuse are devastating. In addition to unintentional overdose death there are several opioid-related disorders. Chronic non-medical use of opioids has been proposed to cause cognitive dysfunction by inhibiting neurogenesis and inducing neuronal apoptosis. Furthermore, as many of the opioid abusers are young adults with still undergoing development of the central nervous system it is of importance that these neurotoxic events are further evaluated.

The study is part of a larger project were the toxic effects of the opioids methadone, morphine, ketobemidone, fentanyl, oxycodone, buprenorphine, as well as the opioid antagonist naloxone are evaluated. The purpose of the present study is to examine the neurotoxic effects of the commonly used opioids in neuronal cell cultures.

The toxic effects of commonly used opioids were studied on neuronal cell cultures. The cells were given the opioid as a single dose for 4h and 24h, or as repeated treatment during 72h. Thereafter, the opioid induced toxicity was analysed by measuring mitochondrial activity and cell cytotoxicity. The results indicate that common prescription opioids induce neurotoxic effects to different extent. Primarily, differences in neurotoxicity depend on treatment time and dose.

The present study suggests that opioids induce neurotoxic effects on neuronal cell cultures. The result further highlights the problems associated with opioids and can provide further insight on how to counteract these problems.

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