Phoenix, AZ

INRC





46th meeting of the International Narcotics Research Conference in conjunction with the 77th annual College on Problems of Drug Dependence
Phoenix, Arizona, USA
June 15-19, 2015

Official Program with Poster Abstracts

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Acknowledgments
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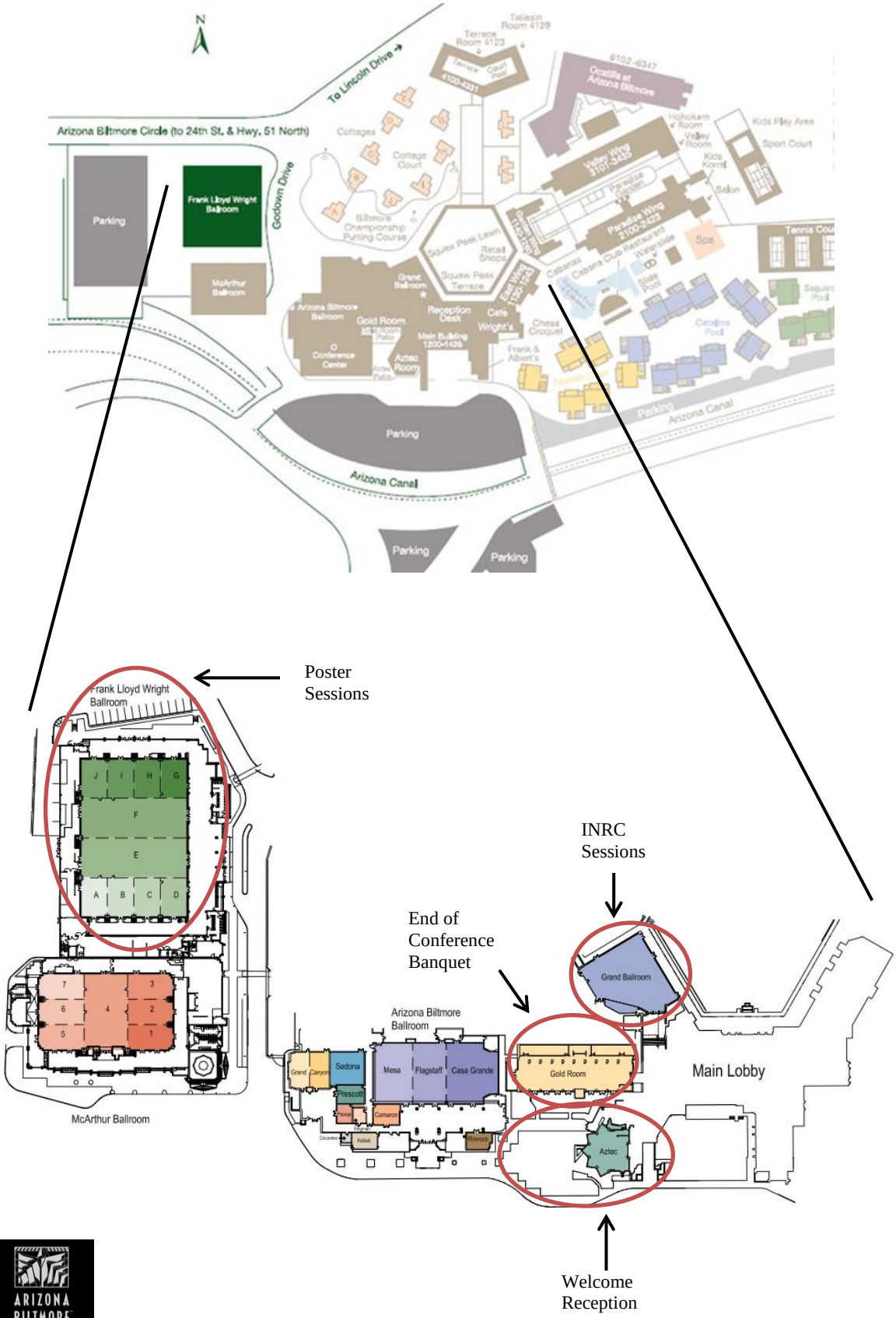
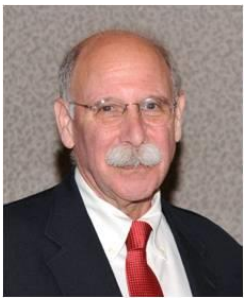
INRC- Arizona Biltmore Hotel and Meeting Floor Plans



INRC Schedule at a glance						
Time/Date	**Monday 6.15.15**	**Tuesday 6.16.15**	**Wednesday 6.17.15**	**Thursday 6.18.15**	**Friday 6.19.15**	**Saturday 6.20.15**
7:00		Registration	Registration	Registration	Registration	
8:00		8:00-9:00 CPDD/INRC Plenary 1 - Nora Volkow	8:00-9:45 INRC Plenary: John Williams & related Hot topics	8:00- 9:45 Session 5 - Vaccines to prevent Drug Abuse (Chairs: Mary Jeanne Kreek and Christopher Evans)	8:00-9:45 Session 6 - Opioid Tolerance and Hyperalgesia (Chair: Michael Morgan)	
8:30						
9:00		9:00-9:15 Break				
9:30		9:15 -11:30 Session 1 - Addiction to Prescription Opioids (Chair: Fred Nyberg, INRC President)	9:45-10:00 Break	9:45-10:00 Break	9:45-10:00 Break	
10:00			10:00- 12:00 Session 3 - Pain and Reward (Chair: Frank Porreca)	10:00-12:00 CPDD/INRC Plenary 3 - Cannabinoids (Chair: Todd Vanderah)	10:00-12:00 Session 7 - Non-Canonical Actions of Opioids (Chair: Michael Morgan)	Depart Conference
10:30						
11:00						
11:30		11:30-1:30 Lunch + Poster session (INRC, Evens)	12:00-2:00 Lunch + Poster session (INRC, Odds)	12:00-1:00 Lunch + Data Blitz (Chair: Susan Ingram)	Lunch	
12:00						
12:30						
13:00				1:00-2:00 Early Career Event- Mock Study Section (Optional)	1:00-2:00 Young Investigator Plenary (Gregory Scherrer)	
13:30		1:30-2:45 Founders Lecture - Gavril Pasternak				
14:00			2:00-4:00 CPDD/INRC Plenary 2 - Opioid Abuse (Chair: Sandra Comer)	Free afternoon	2:00-3:30 Young Investigator Session (Chair: Gregory Scherrer)	
14:30		2:45-3:00 Break				
15:00	Registration begins	3:00 - 5:00 Session 2 - Advances in Therapeutic Design (Chairs: Jay Mc Laughlin and Jane Aldrich)				
15:30						
16:00			4:00-4:15 Break			
16:30			4:15-5:15 Session 4 - Optogenetics and pain (Chair: Robert Gereau)		3:30-4:00 Break	
17:00					4:00-5:00 Business meeting	
17:30						
18:00	**Welcome reception**					
18:30					**Closing Banquet**	
19:00						
20:00						

INRC2015 Awardees



Founders' Award – Gavril Pasternak Dr. Gavril Pasternak holds the Anne Burnett Tandy Chair in Neurology at Memorial Sloan-Kettering Cancer Center and is a Laboratory Head in the Molecular Pharmacology and Chemistry Program within the Sloan-Kettering Institute. After receiving his M.D. and Ph.D. degrees from the Johns Hopkins University he completed his clinical training in Neurology at Johns Hopkins Hospital and then joined the faculty at Memorial Sloan-Kettering in 1979. He is a Fellow of the American Academy of Neurology and a Fellow of the American Neurological Association. His research has focused on opioid receptors and their mechanisms of action, with over 400 publications. He has served on the Editorial Boards of numerous scientific journals. He is a recipient of a Senior Scientist Award and a MERIT Award from the National Institute on Drug Abuse and has served on their Board of Scientific Counselors. He is a member of the Johns Hopkins Society of Scholars and has been awarded the Millenium Prize from the Norwegian University of Science and Technology, the John J. Bonica and the Raymond W. Houde Awards from the Eastern Pain Association, the Julius Axelrod Award of the American Society of Pharmacology and Experimental Therapeutics, the S. Weir Mitchell Award from the American Academy of Neurology. He has been honored with the 2014 William Potter Lecture at Thomas Jefferson University, the 6th Donald W. Benson Lectureship on Pain Medicine at the Johns Hopkins School of Medicine, the 1st Annual Machaskee Memorial Lectureship at the Cleveland Clinic and the Stitzel Lecture at West Virginia School of Medicine.

Comments about Dr. Pasternak from the Selection Committee:
Dr. Pasternak is recognized for his major contributions to the differential roles of opiate receptor subtypes in relieving pain with diminished side effects. Over the years his research has focused upon the characterization of opiate receptors. Using both ligand binding and molecular biological techniques, Dr. Pasternak has uncovered several novel receptors derived by alternative splicing of the mu opiate receptor gene. His discoveries have significantly increased our understanding of how opiates act and have led to novel, potent analgesics with reduced side effects. Also, in his recent research Dr. Pasternak has discovered some new selective opioid drugs that are much more potent than morphine with diminished adverse effects with less potential for causing physical dependence. Dr. Pasternak has been a frequent attendant at the INRC meetings throughout his career and together with his students and collaborators provided important contributions to our organization.

Young Investigator Award – Grégory Scherrer Dr. Grégory Scherrer received his PhD in Molecular and Cellular Biology from Strasbourg University in 2005 under the supervision of Dr. Brigitte Kieffer. In 2006 he joined Dr. Allan Basbaum's laboratory at UCSF for his postdoctoral training. From 2009-20012 he continued as a postdoctoral fellow with Dr. Amy MacDermott at Columbia University in NYC. In 2012 he started his own research laboratory at Stanford University as a faculty member of the Neurosciences Institute and the Departments of Anesthesiology and Molecular and Cellular Physiology. His laboratory combines a variety of experimental approaches including molecular and cellular biology, neuroanatomy, electrophysiology, opto-/pharma-cogenetics and behavior in mouse to resolve the functional organization of pain neural circuits in normal conditions and during injury- or disease-induced chronic pain, and how opioids modulate neuronal function to produce analgesia and detrimental side effects. A major goal of the Scherrer laboratory is to use novel insights into opioids' mechanisms of action develop more efficacious and safer therapeutics to treat patients suffering from pain.

Comments about Dr. Scherer from the Selection Committee: While working on his thesis, conducted under the supervision of Dr. Brigitte Kieffer, Dr. Gregory Scherrer studied the role of delta opioid receptor (DOR) in pain control, emotional response and cognitive process utilizing advanced techniques, including molecular biology and transgenic animals. Upon obtaining his PhD, he then moved to San Francisco to work with Professor Alan Basbaum at UCSF to conduct studies on the function and anatomical localization of DOR in pain processing pathways. During a post-doctoral period with Dr. Amy MacDermott, he focused on the organization of neuronal circuits that are regulated by opioids in the spinal cord. Upon finishing his work at Columbia he was recruited by Standford University as an Assistant Professor in the Department of Anesthesiology where, Greg continues his research on cellular and molecular mechanisms of pain and its control by opioids. Since the beginning of his career, Dr Scherrer's research has been original and important, resulted in several publications in high impact journals such as PNAS, PLoS One, Pain and Cell, and led to the awarding of a competitive Pathway to Independence (K99/R00) Award from NIDA to examine the analgesic actions of opioid peptides in chronic pain. Over the years Dr. Scherrer has been a regular attendee at INRC meetings and has contributed excellent science related to our organization.

Full Program

Monday, June 15

15:00-20:00 Registration

18:00-20:00 Welcome Reception
*Aztec Ballroom and patio

Tuesday, June 16

8:00-9:00 **INRC/CPDD Plenary 1 Nora Volkow Director, National Institute on Drug Abuse (NIDA)**
*****Arizona Biltmore Ballroom

9:00 – 9:15 Break

9:15-10:15 **Session 1** ***"Addiction to Prescription Opioids"****,* Chair: Fred Nyberg, Uppsala University

Thomas Clausen (University of Oslo)
Prescription opioid-abuse and dependence in Scandinavian countries

Gabriele Fischer (Medical University of Vienna)
Addiction and diversion of prescription opioids in Europe

Pauline Voon (St. Paul's Hospital, Vancouver, CAN)
Nonmedical prescription opioid use in North America

10:15 – 11:00 Hot Topics
Naoko Kuzumaki (Hoshi University, Tokyo)
Chronic pain and addiction ***poster #38*

Rachel Enga (Virginia Commonwealth University)
Precipitated oxycodone withdrawal reduces the startle reflex and bodyweight in C57BL/6J and control mice, but not in HIV-1 Tat-expressing mice ***poster #18*

Darrow Khosh-Chashm (University of Texas - Houston)
Impact of benzodiazepine use on retention and compliance, relapse, and safety in buprenorphine-maintained patients: A Literature Review ***poster #34*

11:00-11:30 Discussion

11:30-13:30 Lunch & Even numbered posters (with CPDD) *Frank Lloyd Wright Ballroom

13:30-14:45 **Founders' Lecture**
Gavril Pasternak (Memorial Sloan-Kettering Cancer Center)
40 years on drugs: It's all in the telling of the tail

14:45-15:00 Break

15:00-16:00 **Session 2** ***"Advances in Therapeutic Design"****,* Chairs: Jay McLaughlin, Torrey Pines Institute for Molecular Studies; Jane Aldrich, University of Florida

Chris McCurdy (University of Mississippi)
Sigma receptor antagonists in peripheral pain: Potential for improved diagnosis and pharmacotherapy with significantly reduced CNS liabilities

Andy Coop (University of Maryland)
UMB425: A unique opioid analgesic with lower tolerance

Jessica Anand (University of Michigan)
Development of bifunctional MOR agonist/DOR antagonist peptides: Successes and failures

Jane Aldrich (University of Florida)
Analogs of the novel macrocyclic tetrapeptide CJ-15,208 exhibit diverse opioid activity profiles

16:00 – 16:30 Hot Topics
Todd Hillhouse (University of Michigan)
Enhancement of opioid-mediated antinociception by the mu-opioid receptor positive allosteric modulator, BMS-986122 ***poster #31*

Achla Gupta (Mt. Sinai Hospital)
Collybollides are highly selective and potent agonists of kappa opioid receptors ***poster #27*

James Zadina (Tulane University)
An endomorphin analog providing prolonged antinociception with substantial reduction of multiple adverse side effects relative to morphine ***poster #103*

16:30 - 17:00 Discussion

Wednesday, June 17

8:00 - 08:45 INRC Plenary 1 - John Williams (Vollum Institute)
What is all the confusion about opioid actions on dopamine neurons?

8:45 – 9:10 Elyssa Margolis (UCSF)
Opioid actions in the VTA are diverse and depend on projection target

9:10 – 9:35 MOP in the VTA, plenary follow-up
Adrianne R. Wilson-Poe (Columbia University)
Inflammatory pain dysregulates opioid function in the mesolimbic pathway

Michael Bruchas (Washington University-St. Louis)
Spatiotemporal control of opioid signaling and behavior

9:35 – 9:45 Discussion

9:45 - 10:00 Break

10:00 - 11:45 **Session 3** ***"Pain and Reward",*** Chair: Frank Porreca, University of Arizona

Howard Fields (UCSF)

How the nucleus accumbens enables the approach or avoid decision

Frank Porreca (University of Arizona)
Endogenous opioids and reward of pain relief

Catherine Cahill (UC-Irvine)
Neuropathic pain modulates dopaminergic circuitry: A role for microglial activation

Siri Leknes (Oslo University Hospital)
Endogenous opioids in human pain and reward

11:45 - 12:00 Discussion

12:00 - 14:00 Lunch & Odd-numbered posters (with CPDD) ***Frank Lloyd Wright Ballroom**

14:00 - 16:00 **INRC/CPDD Plenary 2** ***"Novel Strategies for Reducing Opioid Abuse"*****,** Chair: Sandra Comer, Columbia University ***Arizona Biltmore Ballroom**

Kathy Cunningham (University of Texas Medical Branch, Galveston)
Prospects for serotonin therapeutics in addictive disorders

Marco Pravetoni (University of Minnesota)
Development of vaccines for opioid abuse

Jermaine Jones (Columbia University)
Assessing the ability of glial inhibitors to alter the abuse liability of opioids using laboratory models in humans

Theodore Cicero (Washington University, St. Louis)
Epidemiology on the impact of opioid abuse-deterrent medications on opioid abuse in the community

16:00 - 16:15 Break

16:15 - 17:00 **Session 4** ***"Optogenetics in Pain"***, Chair: Robert W. Gereau IV, Wash U.-St. Louis
Robert W. Gereau IV (Washington University-St. Louis)
Fully-implantable wireless optoelectronic systems for interrogation of spinal and peripheral pain circuitry

Anthony Pickering (University of Bristol)
Opto- and chemo-genetic activation of brainstem POMC neurones reveals opioid-mediated analgesic and cardiorespiratory roles

Zhizhong Z. Pan (The University of Texas MD Anderson)
Dissecting the brain's pain circuits

17:00 - 17:15 Discussion

Thursday, June 18

8:00-10:00 INRC posters on display
***Frank Lloyd Wright Ballroom**

8:00 - 9:00 **Session 5** ***"Vaccines to Prevent Drug Abuse",*** Chairs: Mary Jeanne Kreek, The Rockefeller University; Christopher Evans, UCLA

Brian Reed (The Rockefeller University)
Application of a novel small molecule vaccine platform to oxycodone immunization

Gary Matyas (Walter Reed Army Institute of Research)
Development and optimization of a heroin vaccine

Paul Bremer (Scripps Research Institute)
Design of haptens and conjugate vaccines for heroin addiction

Joel Schlosburg (Scripps Research Institute)
Selectivity, capacity, durability: putting a heroin vaccine through its paces and clearing the major hurdles towards clinical viability

9:00 – 9:30 Hot Topics
Rashmi Jalah (Walter Reed Army Institute of Research)
*Development of a combination Heroin-HIV vaccine **poster #46*

Oscar Torres (Walter Reed Army Institute of Research)
*Comparison of equilibrium dialysis (ED) combined with ultra-high performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) and competitive ELISA for the determination of dissociation constants of 6-acetylmorphine (6AM) and morphine to polyclonal antibodies **poster #88*

Lee W. Hutson (University of North Carolina-Chapel Hill)
*Interleukin-1 signaling in the basolateral amygdala mediates heroin-conditioned immunosuppression **poster #35*

9:30 – 9:45 Discussion

9:45 – 10:00 Break

10:00-12:00 **INRC/CPDD Plenary 3** ***"Cannabinoid Mechanisms and Interventions"*****,** Chair: Todd W. Vanderah, University of Arizona
***Arizona Biltmore Ballroom**

Alexandros Makriyannis (Northeastern University)
Therapeutic interventions through the modulation of CB1/CB2 cannabinoid receptor function

Aron Lichtman (Virginia Commonwealth University)
Inhibition of the 2-AG biosynthetic enzyme diacylglycerol lipase beta: Antinociception through a cannabinoid receptor independent pathway

Todd W. Vanderah (University of Arizona)
Synergistic actions of opioid/cannabinoid agents for discrete pain etiologies

Andrea Hohmann (Indiana University)
The therapeutic promise of nonpsychoactive cannabinoid analgesics

12:00 - 13:00 **Data Blitz + Lunch** Chair: Susan L. Ingram, Oregon Health and Science University

13:00 - 14:00 **Optional Early Career Event - Mock Study Section** Chair: José Moron-Concepción, Columbia University

Friday, June 19

8:00 - 9:15 **Session 6 *"Opioid Tolerance and Hyperalgesia- Real Consequences, Treatment, Mechanisms"***, Chair: Michael M. Morgan, Washington State University-Vancouver

Michael M. Morgan (Washington State University-Vancouver)
Why are there so many mechanisms of opioid tolerance?

Bradley K. Taylor (University of Kentucky)
Endogenous opioid dependence

Lori Eidson (Georgia State University)
Role of glia in opioid tolerance

Jürgen Sandkühler (Medical University of Vienna)
CNS mechanisms leading to opioid-induced Hyperalgesia

Dorit Pud (University of Haifa)
Opioids and abnormal pain perception in human

9:15 – 9:45 Discussion

9:45 – 10:00 Break

10:00 - 11:00 **Session 7 *"Non-canonical Actions of Opioids",*** Chair: Michael M. Morgan, Washington State University-Vancouver

Eamonn Kelly (University of Bristol)
GRKs, arrestins and bias at the mu opioid receptor

Lakshmi Devi (Mt. Sinai Hospital)
Regulation of opioid receptor function by post-endocytic peptide processing

Michael Lacagnina (Duke University)
Neuroimmune signaling and opioids: impact of early-life experience on glial function and addiction-related behaviors

Wendy Walwyn (UCLA)
A role for constitutively active mu opioid receptors in the recovery from chronic pain

11:00 – 11:30 Hot Topics
John Streicher (University of New England)
Heat shock protein 90 regulates mu opioid receptor signaling in vitro and in mouse periaqueductal grey ***poster #84***

Jin Xu (Memorial Sloan-Kettering Cancer Center)
Truncated six transmembrane mu opioid receptors mediate opioid analgesia ***poster #48***

11:30 - 12:00 Discussion

12:00 -13:00 Lunch

13:00 - 14:00 **Young Investigator Plenary**
Gregory Scherrer (Stanford University)
Functional organization of the opioid system in pain neural circuits

14:00 - 14:45 **Session 8 Young Investigator** (Chair: Gregory Scherrer)

Sarah Ross (University of Pittsburgh)
Dynorphin is released from spinal B5-I interneurons and functions as an inhibitory neuromodulator of itch

Steeve Bourane (The Salk Institute for Biological Studies)
Identification of functional populations of interneurons in dorsal spinal cord

14:45 - 15:15 Hot Topics

Edita Navratilova (University of Arizona)
Distinguishing brain circuits mediating pain relieving and addictive effects of systemic morphine ***poster #73***

Gina Marrone (Memorial Sloan-Kettering Cancer Center)
U50,488H requires truncated 6 transmembrane variants of the mu opioid receptor for analgesia but not side effects ***poster #39***

Erica Levitt (Oregon Health and Science University)
Mu opioid receptors hyperpolarize respiratory-controlling Kölliker-Fuse neurons ***poster #60***

15:15 - 15:30 Discussion

15:30 – 15:45 Break

15:45-17:00 Business Meeting (INRC President, Fred Nyberg)
*Special Recognition Award, Rao Rapaka (NIDA)

17:00– 18:30 Break

18:30 - end Closing Banquet *Gold Room

Saturday, June 20: Depart Conference

Talk Abstracts (Main Sessions only)
"Hot Topics" abstracts can be found in poster abstracts

Tuesday June 16th

INRC/CPDD Plenary 1

1. INRC/CPDD Plenary 1 Nora Volkow Director, National Institute on Drug Abuse (NIDA)

INRC Session 1

2. *The involvement of prescription opioid-abuse and dependence in Scandinavian countries*
Thomas Clausen (University of Oslo)

The Scandinavian countries share many similarities cultural and political characteristics, all providing health care primarily through publicly funded universal health care systems. Scandinavian countries share a common feature of well-developed national health registries, including prescription databases. , Set against this relatively similar and comparable context, the presentation will highlight and compare prevalence of opioid use, estimates of abuse and dependence from the Scandinavian region, and specifically address the involvement of prescription opioids in overdose deaths. The access to comparative data offer the opportunity for exploring further some observed differences.

3. *Addiction and diversion of prescription opioids in Europe*
Gabriele Fischer (Medical University of Vienna)

In Europe, the overall numbers of new heroin clients in treatment are declining from a peak of N = 59,000 in 2007 to N = 31,000 in 2012. However, the misuse of opioids other than heroin is an increasing concern in the EU, with more than 10% of first-time opioid clients entering specialised treatment misusing opioids other than heroin (including methadone, buprenorphine and fentanyl). In addition, prescription opioid misuse and addiction among chronic pain patients is an emerging public health issue, which may be associated with long-term opioid pharmacotherapy for these serious medical conditions. European studies report that up to 14% of patients, who receive treatment with opioid analgesics for pain relief, develop an opioid dependence.

4. *Nonmedical prescription opioid use in North America*
Pauline Voon (St. Paul's Hospital, Vancouver, Canada)

The rates of prescription opioid use in North America are among the highest in the world, and have been paralleled by a rise in opioid-related morbidity and mortality. However, many monitoring surveys currently fail to differentiate between prescription opioid use for the purpose of euphoria versus pain or withdrawal management. This presentation will report on recent findings and avenues for intervention and research in order to mitigate the individual, social and structural problems related to undertreated pain and prescription opioid use.

5. Founders' Lecture - *40 years on drugs: It's all in the telling of the tail*
Gavril Pasternak (Memorial Sloan-Kettering Cancer Center)

Opioids are unique in that our extensive clinical experience with them predated the identification of their receptor targets. Over the past 40 years, our understanding of opioid action at the molecular level has evolved to provide insights into the extraordinary complexity of opioids and their targets implied by decades of clinical observation

INRC Session 2

6. Sigma receptor antagonists in peripheral pain: Potential for improved diagnosis and pharmacotherapy with significantly reduced CNS liabilities
Chris McCurdy (University of Mississippi)

Peripheral nerve injury, as a consequence of trauma, surgery, inflammation, or other causes, is a major medical problem. This type of injury is often associated with chronic pain. About 100 Million people suffer from chronic pain in the United States. Diagnosis and treatment are still considered as unmet medical needs. Current clinical imaging methods used to evaluate chronic pain are centered on anatomic alterations, which do not necessarily reflect the origin of chronic pain. A potential biomarker associated with nerve injury and neuroinflammation is the sigma-1 receptor (S1R). In addition, S1Rs appear to play an active role in pain modulation, both peripherally and centrally. We recently identified a highly selective S1R antagonist that was transformed into a PET probe candidate and demonstrated high specificity and

selectivity for imaging S1Rs in mice, rats, and monkeys. We have utilized this probe in a rat model of nerve injury via PET/MRI. The results have helped promote the clinical use of the agent in identifying peripheral pain generators in patients suffering from neuropathic pain. Furthermore, we have investigated the cold compound and similar derivatives as potential pharmacotherapies for neuropathic pain in mouse models of nerve injury. These compounds have equipotent or superior analgesic efficacy to the clinically utilized gabapentin. The compounds have also been examined for liabilities in locomotor, rotorod, conditioned place preference and in some cases, self-administration assays. The results indicate the analgesic effects produced by S1Rs antagonists are not associated with these potential liabilities. These results confirm the ability of S1Rs to serve as potential diagnostic and analgesic agents for neuropathic pain without CNS liabilities. Funding provided by NIDA (DA023205), NIGMS (GM104932), the Center for Biomedical Imaging at Stanford University, and the State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development.

7. UMB425: A unique opioid analgesic with lower tolerance

Andy Coop (University of Maryland)

Opioids remain the primary medication for the treatment of chronic pain, but their use is significantly hindered through the development of tolerance and dependence. Previous studies have shown that the co-administration of the mu agonist morphine together with a delta antagonist attenuates the development of tolerance and dependence in rodents. Strengthened by studies with delta knock-out animals, and peptide ligands with a dual profile of mu agonism/delta antagonism, UMB425 was designed as a novel non-peptide ligand possessing the same dual profile. In mouse antinociceptive assays, UMB425 was shown to possess similar potency to morphine, but showed significantly less tolerance development when an ED90 dose was administrated twice a day for 5 days. UMB425 thus has the potential to be developed into a new clinical analgesic with important advantages over current opioid analgesics.

8. Development of bifunctional MOR agonist/DOR antagonist peptides: Successes and failures

Jessica Anand, Emily Jutkiewitz, and Henry I Mosberg (University of Michigan)

Historically, medicinal chemists and pharmacologists have sought to develop highly selective ligands for specific therapeutic targets. The prevailing thought was that the more selective a ligand was for its desired target, the fewer adverse events occur. However recently, there has been a growing consensus that the simultaneous modulation of multiple targets often generates a more desirable drug profile. This is of particular interest in the field of opioid analgesics, where it has been reported that the co-administration of a mu opioid receptor (MOR) agonist with a delta opioid receptor (DOR) antagonist produces MOR mediated analgesia, but with reduced tolerance and dependence liabilities, features that limit the clinical use of MOR agonist opioid analgesics. Early work in our lab focused on generating selective ligands for MOR or DOR as potential therapeutics. More recently, we have reexamined past "failed" selective ligands as starting points for developing bifunctional MOR agonist/DOR antagonist compounds. Using SAR and computational modeling we have developed and reported a peripherally bioavailable, constrained peptide, VRP26 that displays MOR agonist/DOR antagonist features in vitro. More interestingly, VRP26 produces antinociception in vivo, with reduced development of tolerance and dependence. We have further explored the rewarding effects of VRP26 in rodents. In parallel, we have attempted to translate the key binding elements of VRP26 to a more easily synthesized linear peptide scaffold.

9. Analogs of the novel macrocyclic tetrapeptide CJ-15,208 exhibit diverse opioid activity profiles

Jane Aldrich (University of Florida)

The macrocyclic peptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) exhibits mixed opioid agonist/kappa opioid receptor (KOR) antagonist activity *in vivo*, while its D-Trp isomer exhibits predominantly KOR antagonism. Both compounds are active after oral administration and are promising lead compounds for potential development as treatments for pain and drug abuse. We are exploring structure-activity relationships for both opioid agonist and KOR antagonist activities. We expected that conservative amino acid substitutions in these peptides would be well tolerated by opioid receptors and that the resulting analogs would exhibit similar opioid activity profiles to the parent peptides with potentially greater potency *in vivo*. The analogs exhibited diverse opioid activity profiles. The analogs generally retained opioid receptor affinities, determined in radioligand binding assays, similar to or higher than the parent peptides. As with the parent peptide, the analogs of CJ-15,208 exhibited antinociception in the mouse 55 ^{o}C warm water tail withdrawal assay *in vivo*, but with a wide range (80-fold) of antinociceptive potencies and varying opioid receptor involvement. Of particular interest was an analog that produced antinociception mediated primarily by mu opioid receptors, while exhibiting antagonism at delta opioid receptors. The analogs of [D-Trp]CJ-15,208 generally exhibited minimal opioid agonist activity, similar to the parent peptide. KOR antagonist activity proved sensitive to structural modification, with most of the analogs exhibiting loss of KOR antagonism. Thus minor structural changes had substantial impacts on the opioid activity observed *in vivo* and resulted in analogs with promising activity profiles for potential development. Research supported by NIDA grants R01 DA023924 and DA032928.

Wednesday, June 17th

INRC Plenary session

10. What is all the confusion about opioid actions on dopamine neurons?
John Williams (Oregon Health and Science University)

This presentation will focus on the action of opioids on dopamine neurons *in vivo* and in brain slices. The heterogeneity of dopamine neurons distinguished by projection area, direct opioid sensitivity and the sensitivity of afferent inputs to opioids will be presented. Finally there will be a comment on the requirements necessary to obtain reliable and reproducible results using electrophysiological methods.

11. Opioid actions in the VTA are diverse and depend on projection target
Elyssa Margolis (UCSF)

MOP receptor activation in the VTA is required for systemic opiate reward, however recent discoveries suggest that classical local circuit concepts proposed to explain how this happens need to be revised. We recently reported a MOP receptor induced direct excitatory effect on a subset of VTA neurons, and we report here that these include dopaminergic neurons that project to the infralimbic, but not the prelimbic, cortex. As we previously reported that VTA dopamine neurons that project to prefrontal cortex are inhibited by KOP receptor activation, these data raise the possibility that the VTA projection to infralimbic cortex conveys a valence difference between the aversive KOP and appetitive MOP signals from the VTA.

12. Inflammatory pain dysregulates opioid function in the mesolimbic pathway
Adrianne R. Wilson-Poe and Jose Moron-Concepcion (Columbia University)

Chronic pain triggers a myriad of compensatory processes in the central nervous system. Much of our understanding of these adaptations comes from studies in the spinal cord and other regions that are classically involved in pain transmission and modulation. However, recent work in our laboratory indicates that inflammatory pain can also influence the function and behavioral output of the mesolimbic pathway. We utilized the complete Freund's adjuvant (CFA) model to induce persistent inflammatory pain. Microdialysis, self-administration, and *in vitro* electrophysiology were used to quantify pain-induced changes in opioid function in the ventral tegmental area (VTA) and nucleus accumbens (NAc). Inflammatory pain was associated with diminished dopamine release in the NAc, as triggered by microinjection of the mu-opioid receptor (MOR) agonist DAMGO directly into the VTA. These results were complemented by *in vitro* whole-cell patch clamp experiments, which further demonstrated pain-induced MOR desensitization in the VTA in response to DAMGO. The pain-induced decrease in MOR-dependent accumbal DA release was also associated with a higher propensity to self-administer large doses (200 μg/kg) of heroin. These data indicate that inflammatory pain induces changes in the MOR system in mesolimbic regions which are typically associated with the rewarding effects of opioids. Thus, pain-induced alterations in opioid function in this pathway may contribute to enhanced opioid abuse liability in chronic pain patients. Support from the National Institute on Drug Abuse (DA034464, DA027460). None of the authors has a conflict of interest related to this research.

13. Spatiotemporal control of opioid signaling and behavior
Michael Bruchas (Washington University-St. Louis)

Optogenetics is now a widely accepted tool for spatiotemporal manipulation of neuronal activity. However, a majority of optogenetic approaches use binary on/off control schemes. Here we extend the optogenetic toolset by developing a neuromodulatory approach using a rationale-based design to generate a Gi- coupled, optically-sensitive, mu-opioid-like receptor, we term opto-MOR. We demonstrate that opto-MOR engages canonical mu-opioid signaling through inhibition of adenylyl cyclase, activation of MAPK and G protein-gated inward rectifying potassium (GIRK) channels, and internalizes with similar kinetics as the mu-opioid receptor. To assess *in vivo* utility we expressed a Cre-dependent viral opto-MOR in RMTg/VTA GABAergic neurons, which led to a real-time place preference. In contrast, expression of opto-MOR in GABAergic neurons of the ventral pallidum hedonic cold spot, led to real-time place aversion. This tool has generalizable application for spatiotemporal control of opioid signaling and, furthermore, can be used broadly for mimicking endogenous neuronal inhibition pathways.

INRC Session 3

14. How the nucleus accumbens enables the approach or avoid decision
Howard Fields (UCSF)

Howard Fields will review the evidence that the Nucleus Accumbens is not simply part of the reward circuit but can generate both approach and avoidance behaviors. Opioids acting in the Nucleus Accumbens can shift decision making to promote approach and suppress responses to pain.

15. Endogenous opioids and reward of pain relief
Frank Porreca (University of Arizona)

Frank Porreca will present evidence that relief of ongoing pain by non-opioid treatments requires release of endogenous opioids in the rostral anterior cingulate cortex.

16. Neuropathic pain modulates dopaminergic circuitry: role for microglial activation
Catherine Cahill (UC-Irvine)

Catherine Cahill will present recent evidence that peripheral nerve injury causes microglial activation in the VTA. This activation leads to disruption in reward behavior and dopamine release in the nucleus accumbens via changes in GABAergic neuronal activity.

17. Endogenous opioids in human pain and reward
Siri Leknes (Oslo University Hospital)

Siri Leknes will discuss the role of the opioid system for pain and reward. Recent evidence from pharmacological studies suggests extensive parallels between human and rodent findings not only for pain relief, but also for reward 'liking' and 'wanting' behaviours.

INRC/CPDD Plenary 2 - *Novel Strategies for Reducing Opioid Abuse*

18. Prospects for serotonin therapeutics in addictive disorders
Kathy Cunningham (University of Texas Medical Branch, Galveston)

19. Development of vaccines for opioid abuse
Marco Pravetoni (University of Minnesota)

20. Assessing the ability of glial inhibitors to alter the abuse liability of opioids using laboratory models in humans
Jermaine Jones (Columbia University)

21. Epidemiology on the impact of opioid abuse-deterrent medications on opioid abuse in the community
Theodore Cicero (Washington University, St. Louis)

INRC Session 4

22. Fully-implantable wireless optoelectronic systems for interrogation of spinal and peripheral pain circuitry
Robert W. Gereau IV (Washington University-St. Louis)

The use of optogenetics has previously required remote light sources and fiber optic delivery schemes that impose significant physical constraints on natural behaviors, and thus limit utility in typical animal behavioral studies. In this talk, we discuss technologies we have developed that combine soft, compliant neural interfaces with fully-implantable, stretchable wireless power and control systems to achieve optogenetic modulation of nearly any region of the nervous system including the spinal cord and peripheral nerves, in freely behaving animals. The utility of these devices in the study of pain will be demonstrated.

23. Opto- and chemo-genetic activation of brainstem POMC neurones reveals opioid-mediated analgesic and cardiorespiratory roles
Anthony Pickering (University of Bristol)

Beta-endorphin plays a key role in the mediation of stress-induced analgesia and is synthesised by POMC neurones in the brain. These POMC neurones located in two discrete clusters in the arcuate nucleus of the hypothalamus and also in the nucleus of the solitary tract (NTS) in the medulla. Using opto- and chemo-genetic targeting approaches we show that the NTS population of several hundred neurones exerts potent, opioid mediated effects on somatic nociception and cardiorespiratory control in vivo. These findings identify the NTS POMC neurones as being cross-modality command-like neurones and provide a proof of concept demonstration that a chemogenetic strategy can be used to generate a synthetic analgesic by targeting a defined cell population.

24. Dissecting the brain's pain circuits
Zhizhong Z. Pan (The University of Texas MD Anderson)

Thursday June 18th
Session 5

25. Application of a novel small molecule vaccine platform to oxycodone immunization

Brian Reed (The Rockefeller University)

Vaccines against drugs of abuse could potentially yield circulating antibodies which protect against the CND-mediated effects of exposure to the relevant drug, including rewarding effects, thus preventing progression to addiction. To be effective, such vaccines need to elicit a significant level of high affinity antibody, which have selectivity for the molecule of interest, and which circulate for substantial time periods. Small molecule vaccine platforms to date largely consist of conjugating a hapten closely related to the molecule of interest on a carrier protein which is injected one or more times with an immune stimulating adjuvant. We have begun investigations of alternative small molecule platforms to determine, in comparison with conventional carrier proteins. We have synthesized *Trypanasoma brucei brucei* coated with oxycodone-conjugated surface coat proteins using selective enzymatic coupling ("Sortagging" using sortase A). The approach makes use of the ability of the natural pathogen, *T. brucei*, to stimulate a vigorous immune response.

26. Development and optimization of a heroin vaccine

Gary Matyas (Walter Reed Army Institute of Research)

Development of a heroin vaccine requires that the vaccine induce antibodies that react with heroin, 6-acetylmorphine (6AM) and morphine. The 3-hydroxyl, 6-hydroxyl or the bridgehead nitrogen of heroin/morphine can be attached to carrier proteins and used as hapten immunogens. Depending on the attachment site, different faces of the heroin/morphine molecule are presented to the immune system. The immunogenicity and efficacy of chemically stable haptens containing linkers at the 3- (6-AmHap) and 6- (MorHap) positions and the bridgehead nitrogen (DiAmHap) conjugated to tetanus toxoid and adjuvanted with liposomes containing monophosphoryl lipid A were investigated. The haptens induced high titer antibodies in mice. Following subcutaneous challenge with heroin (1 mg/kg), 6-AmHap and MorHap immunized animals had low %MPE in a tail-flick antinociception assay. DiAmHap immunized animals had a 59% MPE. Variation of the MorHap density on tetanus toxoid indicated that the highest hapten density immunogens induced the highest efficacy from heroin challenge. The K_d of the antibodies to 6AM and morphine was measured by equilibrium dialysis with quantitation by UPLC-MS/MS. DiAmHap-induced antibodies had a K_d of >0.1 μM for 6AM and did not bind morphine. MorHap and 6-AmHap-induced antibodies had a K_d of <25 nM for 6AM and morphine, suggesting that the efficacy of MorHap and 6-AmHap was due to the induction of high affinity antibodies. This work was supported through a Cooperative Agreement Award (W81XWH-07-2-067) between the Henry M. Jackson Foundation and the U.S. Army Medical Research and Materiel Command and a NIDA Avant Garde award (1DP1DA034787-01). The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Department of the Army, Department of Defense, or NIH, or the U.S. Government.

27. Design of haptens and conjugate vaccines for heroin addiction

Paul Bremer (Scripps Research Institute)

Heroin is a highly addictive opioid that incurs a huge cost to society. In addition to its negative contribution to criminal activity, productivity loss and medical care, heroin can facilitate the spread of blood-borne pathogens such as HIV and HCV via intravenous administration. Furthermore, heroin addiction leads to abuse of prescription opioid analgesics such as OxyContin (oxycodone) and Vicodin (hydrocodone). Abuse of heroin and other opioids has reached epidemic levels in the past decade, precipitating a rise in overdose deaths. In pursuing therapies to curb the rise of opioid addiction, we have developed a heroin conjugate vaccine. In order to maximize efficacy, we have rigorously optimized each component of the vaccine i.e. heroin hapten structure, conjugation chemistry, carrier protein, adjuvant and dosing. After 3 immunizations, our refined vaccine elicits unprecedented levels of 'immunoantagonistic' protection from heroin; it shifts the heroin EC_{50} curves by ~25 times in mouse antinociceptive testing. The protective effects of the vaccine last over three months without boosters and can be maintained indefinitely with boosters. Furthermore, the heroin vaccine is formulated with components already approved for human use, showing ample promise for future clinical trials. *This work is supported by the National Institute on Drug Abuse (R01DA026625, F31DA037709 and K99DA037344). The authors declare no conflict of interest related to this research.*

28. Selectivity, capacity, durability: putting a heroin vaccine through its paces and clearing the major hurdles towards clinical viability

Joel Schlosburg (Scripps Research Institute)

Despite the fact that demand for treatment of opiate dependence has more than doubling in the United States in the past decade, few effective therapeutic options result in long-term abstinence. In the 1970s, researchers demonstrated proof-of-concept that administering a drug-like structure attached to an immunogenic protein can produce antibodies which effectively bind opiates in the bloodstream. However, the limitation of being easily surmounted by additional drug administration left the immunoantagonistic promise of a heroin vaccine unfulfilled for decades to follow. After recent development of new formulations, screening for acute antagonism in basic antinociceptive tests identified a potentially

effective vaccine. This vaccine was examined in an increasingly complex series of behavioral heroin screens, including: acquisition of self-administration, conditioned place preference, drug-primed reinstatement, and suppression of dependent drug escalation. Capacity of the lead formulation was estimated at 5-6 mg/kg using heroin cumulative dosing in the hot plate test, greater than the average daily intake of our dependent heroin self-administering rats. Following extensive optimization procedures focused on comparing formulations, based on quantitative capacity to block heroin *in vivo*, the final formulation now shifts the heroin dose-response curves over 25-fold versus unvaccinated animals. The blockade of heroin activity can be maintained by reboosting at least 8 months, and is now being further tested in anticipation of a transition to clinical trials. This heroin vaccine may represent an effective therapeutic for heroin addiction, and the behavioral procedures followed may demonstrate the procedures needed to identify an effective, clinically-ready drug vaccine. *Research Supported by R01DA026625, K99DA037344, and the Pearson Center for Alcoholism and Addiction Research. The authors declare no conflicts of interest for this research.*

INRC/CPDD Plenary 3 *"Cannabinoid Mechanisms and Interventions"*

29. Therapeutic interventions through the modulation of CB1/CB2 cannabinoid receptor function

Alexandros Makriyannis (Northeastern University)

The endocannabinoid system is composed of two GPCRs CB1 and CB2. It also encompasses two families of endogenous ligands represented by anandamide and 2-arachidonoyl glycerol (2AG) as well as the enzymes involved in their metabolism. A number of these protein targets are being explored as potential drug targets. I shall be discussing some recent efforts in my laboratory to identify new drug leads as well as methods we developed to carry out the drug discovery effort. (Supported by grants from NIDA)

30. Inhibition of the 2-AG biosynthetic enzyme diacylglycerol lipase beta: Antinociception through a cannabinoid receptor independent pathway

Aron H. Lichtman, Department of Pharmacology and Toxicology, Virginia Commonwealth University

This presentation will provide an overview of the consequences of pharmacological inhibition or genetic deletion of diacylglycerol lipase (DAGL)-β in the mouse LPS model of inflammatory pain. Intraplantar LPS evoked mechanical allodynia that was associated with increased expression of DAGL-β that co-localized with increased tumor necrosis factor-α, and prostaglandins in paws. DAGL-β (-/-) mice or wild type mice treated with the DAGL-β inhibitor KT109 displayed reductions LPS-induced allodynia. The antinociceptive effects of KT109 did not undergo tolerance following repeated administration and did not elicit discernable side effects (e.g., motor deficits, anxiogenic behavior, or gastric ulcers). Further work showed that KT109 reversed LPS-induced allodynia through a local site of action, and also reversed allodynia in mouse neuropathic pain models. These findings suggest that local inhibition of DAGL-β at the site of inflammation represents a novel avenue to treat pathological pain, with no apparent untoward side effects.

31. Synergistic actions of opioid/cannabinoid agents for discrete pain etiologies

Todd W. Vanderah (University of Arizona)

Neuropathic pain is a complex pain disorder that is difficult to manage. Current therapeutic options not only lack efficacy, but are also associated with adverse events. Therapeutics such as Vicodin® and Percocet® that combine opioids with NSAIDs for a synergistic analgesic effect are limited by their association with hepatotoxicity, GI irritation, constipation and the potential for abuse. Cannabinoid 2 receptors (CB2) are not known to possess psychotropic, hepatotoxic or GI effects and have been shown to be active against inflammatory pain. My presentation will focus on whether synergistic analgesic activity can be achieved by combining CB2 agonists with mu opioid agonist in models of inflammation and neuropathic pain. In addition, I will present whether CB2 agonists are effective at significantly reducing opioid-induced unwanted side effects and their ability to modify metastatic bone cancer.

32. The Therapeutic Promise of Nonpsychoactive Cannabinoid Analgesics

Andrea G. Hohmann, Department of Psychological and Brain Sciences and Gill Center for Biomolecular Science, Indiana University

Direct activation of cannabinoid CB1 receptors produces desirable therapeutic properties but also unwanted (psychoactive) side effects that limit clinical use. CB1 receptors are densely expressed in the central nervous system whereas CB2 receptors are expressed primarily in immune cells. The discovery of an allosteric binding site on the cannabinoid CB1 receptor− a site distinct from the classical (orthosteric) binding site − has fostered drug discovery efforts aimed at developing positive allosteric modulators (PAMs) of CB1 signaling that lack cannabimimetic effects associated with direct CB1 agonists. CB2 receptors, which are upregulated in response to inflammation and injury, also represent a therapeutic target. Therapeutic efficacy of CB2 agonists, classical CB1/CB2 mixed agonists and CB1 PAMs were evaluated in a mouse model of toxic neuropathy produced by the chemotherapeutic agent paclitaxel. We evaluated anti-allodynic efficacy, possible tolerance, and cannabimimetic effects (i.e., hypothermia, catalepsy, and CB_1-dependent withdrawal signs) using CB_1 knockout (CB_1KO), CB_2 knockout (CB_2KO), and wildtype (WT) mice. Chronic treatment with

THC produced tolerance to both cardinal signs of CB1 intoxication and therapeutic efficacy. Challenge with a CB1 antagonist also produced robust physical withdrawal. By contrast, CB2 agonists and CB1 PAMs suppressed chemotherapy-induced neuropathic pain without producing tolerance, CB1-dependent withdrawal, or unwanted CB1-mediated side effects. Thus, CB2 agonists and CB1 PAMs offer therapeutic potential to produce a more circumscribed and beneficial spectrum of biological effects compared with direct activation of CB1 receptors. Supported by DA037673, DA009158, and DA035068

Friday, June 19th
Session 6

33. Why are there so many mechanisms of opioid tolerance?

Michael M. Morgan, Washington State University- Vancouver

Tolerance to the antinociceptive effect of opioids develops rapidly with repeated administration. Over 50 distinct neural mechanisms for opioid tolerance have been described. Some of this variability is caused by the fact that there are different types of tolerance (e.g., associative and non-associative). In addition, distinct mechanisms for tolerance are engaged in different parts of the nervous system. For example, NMDA receptors contribute to tolerance produced by spinal administration of opioids, but not following administration into the periaqueductal gray (PAG). Distinct mechanisms of tolerance also are engaged depending on the model of tolerance (e.g., desensitization), whether the development or expression of tolerance is examined, and the opioid administered. However, problems with experimental design also contribute to the wide range of mechanisms proposed to underlie opioid tolerance. These problems include failing to recognize the difference between correlation and causation, designing experiments to distinguish changes that occur as a result of tolerance as opposed to repeated testing, and the necessity of determining whether tolerance can be altered independent of antinociception. In order to identify specific mechanisms of tolerance, a well-designed experiment must focus on specific neurons (e.g., PAG neurons), a specific type of tolerance (e.g., development of non-associative tolerance), and a specific opioid (e.g., morphine). Control groups are needed to avoid confounds such as changes that are secondary to repeated testing or alterations in antinociception. There are no conflicts of interest associated with this work. Funded by NIH grant DA015498

34. Endogenous opioid dependence

Bradley K. Taylor (University of Kentucky)

Mu opioid receptor constitutive activity (MOR_{CA}) keeps chronic pain in remission for months after the induction of inflammation with complete Freund's adjuvant in mice. Spinal analgesic dependence on MOR_{CA} was manifested with signs of cellular withdrawal upon spinal administration of inverse agonists, including MOR-G-protein uncoupling, cAMP overshoot, and neuronal ERK phosphorylation and $[Ca^{2+}]_i$ mobilization. Supraspinal MOR_{CA} dependence was revealed upon systemic or central administration of naltrexone with altered gene expression (Fos), somatomotor behaviors (paw flutters, teeth chatters, wet-dog shakes), escape behaviors (jumping, rearing, hyperlocomotion), and motivation to seek pain relief (the negative reinforcing capacity of intrathecal lidocaine in a conditional place preference paradigm).

35. Role of glia in opioid tolerance

Lori N. Eidson[1], Kiyoshi Inoue[2], Larry J. Young[2], Malu G. Tansey[2] & Anne Z. Murphy[1]

[1]Neuroscience Institute, Georgia State University, [2]Psychiatry, Emory University; Atlanta, GA

Opioid-based narcotics remain an integral part of clinical pain management with morphine being amongst the most effective drugs. However, the acute and long-term analgesic efficacy of morphine is limited by the ensuing neuroinflammatory response. The midbrain periaqueductal gray (PAG) is an essential neural substrate for opioid-mediated analgesia, and we have previously reported that chronic morphine administration activates ventrolateral PAG (vlPAG) glia via the innate immune receptor toll-like receptor 4 (TLR4), leading morphine tolerance. TLR4 activation results in a robust release of cytokines, including tumor necrosis factor (TNF), and TNF signaling increases neuronal excitability by modulating glutamate homeostasis and signaling via neuronal TNF receptor 1 (TNFR1). Our overarching hypothesis is that morphine administration increases PAG neural excitability in a TNF-dependent manner to decrease the hyperpolarizing effects of morphine and facilitate opioid tolerance. Using anti-TNF gene therapy and an anti-TNF biologic that crosses the BBB, we demonstrate that sequestration of PAG *soluble* TNF (solTNF) abolished tolerance to systemic morphine and naïve tolerance to morphine induced by intra-vlPAG injections of the TLR4 agonist LPS. Morphine tolerance was accompanied by a significant increase in vlPAG TLR4 and proinflammatory cytokine (IL-1β) mRNA and decrease in vlPAG astrocytic glutamate transporter mRNA (GLT-1 and GLAST) that was attenuated or eliminated by sequestration of solTNF. These results support our working hypothesis and indicate that morphine binds to TLR4 within the vlPAG, leading to the release of solTNF. Our results further suggest that solTNF mediates morphine tolerance in the PAG via neuronal TNFR1 signaling and augmentation of glutamate homeostasis.

36. CNS mechanisms leading to opioid-induced Hyperalgesia

Jürgen Sandkühler (Medical University of Vienna)

37. Opioids and abnormal pain perception in humans

Dorit Pud, PhD, University of Haifa, Haifa, Israel

Opioids are the cornerstone therapy for the treatment of moderate to severe acute and chronic pain. Yet, while some patients achieve proper analgesia with opioid treatment, others – with similar diagnoses – do not benefit or even experience negative effects from the same treatment. Thus, if based solely on pain diagnoses, the decision of whether or not to initiate opioid treatment is often counterproductive. Therefore, exploring the response to opioid treatment is of a significant clinical importance as well as identifying patients who are likely to benefit from opioid treatment. Clinical evidence suggests that *opioid usage per-se* can alter pain sensitivity. This has been consistently demonstrated in opioid addicts, who show paradoxical increased (opioid induced hyperalgesia=OIH) responsiveness to various painful stimuli. I my presentation I will review the alterations in pain perception in opioid addicts and in patients receiving opioids for the treatment of chronic pain and become more sensitive to pain. Better understanding of these issues can potentially optimize opioid analgesic effectiveness and mitigate opioid-related risks.

Session 7

38. GRKs, arrestins and bias at the mu opioid receptor

Eamonn Kelly[1], Eduard Sabido Aguade[2], Graeme Henderson[1] and Alexandra Cooke[1]

[1]School of Physiology and Pharmacology, University of Bristol, Bristol UK; [2]Proteomics Unit, Centre de Regulació Genòmica (CRG), Barcelona, Spain

The cellular response to a mu opioid receptor (MOPr) agonist is usually defined in terms of a single output, such as inhibition of cyclic AMP signal, but it is likely that the global cellular response will involve many intracellular signaling pathways. In terms of bias, it is increasingly important that we are able to monitor many different outputs from the receptor rather than just the basic G protein versus arrestin signals, which could miss important forms of bias. Here we have used a SILAC (stable isotope labeling with amino acids in cell culture) approach with mass spectrometry-based phosphoproteomics to begin to address this issue, monitoring the signaling induced by a 10 min treatment of HEK293 cells stably expressing MOPr with morphine or endomorphin-2 (each 30 µM). Preliminary results from the phosphoproteomics and bioinformatics analysis indicate that MOPr activation in HEK293 cells 1) leads to regulation of many signaling pathways, some of which are not normally associated with MOPr, and 2) whilst morphine and endomorphin-2 regulate the same pathways in some instances, in others they regulate different pathways, or regulate them in opposite directions. These results suggest that quantitative phosphoproteomics offers an important means to study bias. Supported by the Prime-XS European Consortium

39. Regulation of opioid receptor function by post-endocytic peptide processing

Lakshmi Devi (Mt. Sinai Hospital)

Opioid receptor function is modulated by post-activation events such as receptor endocytosis, recycling and/or degradation. While it is generally thought that the peptide ligand gets co-endocytosed with the receptor, relatively few studies have investigated the role of the endocytosed peptide and peptide processing enzymes in regulating receptor function. In this study, we focused on endocytic endothelin-converting enzyme 2 (ECE2), a member of the neprilysin family of metallopeptidases that exhibits an acidic pH optimum, localizes to an intracellular compartment and selectively processes some, but not all, opioid peptides at acidic pH. We examined the role of ECE2 in endocytic processing of opioid peptides and its effect on opioid receptor function by using selective ECE2 inhibitors. We find that the highly selective ECE2 inhibitor, S136492, and reagents that increase the pH of the acidic compartment impair receptor recycling by protecting the endocytosed peptide from degradation. This, in turn, leads to a substantial decrease in surface receptor signaling. We also find that ECE2 inhibition modulates antinociception mediated only by opioid peptides that are ECE2 substrates. Finally, we find that ECE-2 inhibition modulates stress-induced opioid analgesia. These results suggest that ECE2, by selectively processing endogenous opioid peptides in the endocytic compartment, plays a role in modulating opioid receptor activity *in vitro* and *in vivo*. This work was supported in part by NIH grants DA008863 and NS026880 (to L.A.D.)

40. Neuroimmune signaling and opioids: impact of early-life experience on glial function and addiction-related behaviors

Michael Lacagnina (Duke University)

Identifying the biological and environmental factors that influence risk or resilience to opioid abuse is an issue of significant clinical importance. While remarkable progress has been made in characterizing the neurobiological consequences of acute and repeated opioid exposure, considerably less is known about the contribution that glial cells may play in the molecular and behavioral outcomes associated with drug abuse. Here I will discuss several lines of evidence suggesting that opioids activate glia and alter expression of neuroimmune signaling pathways in the rat central

nervous system, as well as the potential mechanisms whereby early-life conditions can potently influence later-life glial activation and subsequent behavioral responding to opioids. During an acute morphine challenge there is a rapid increase in chemokine gene transcription within the nucleus accumbens (NAc) that can be blocked with the nonspecific glial attenuator ibudilast. Importantly, a neonatal handling procedure that promotes enriched maternal care also attenuates the morphine-induced chemokine response and prevents reinstatement of morphine conditioned place preference. Furthermore, neonatal handling reduces self-administration of remifentanil with no appreciable effect on operant responding for food or sucrose. The enduring effect of neonatal handling may be explained by epigenetic mechanisms altering expression of the anti-inflammatory cytokine interleukin-10 (IL-10) exclusively within microglia. Collectively, these results support the concept that neural-glial interactions in the NAc are involved in the establishment of addiction-related behaviors, and early-life environmental conditions that affect this signaling may substantially influence the reinforcing properties of opioids. Research supported by NIH/NIDA grant DA034185. No conflicts of interest are declared.

41. A role for constitutively active mu opioids receptors in the recovery from chronic pain

Wendy Walwyn,Ph.D., Dept. of Psychiatry, UCLA, Los Angeles, CA, USA

G-Protein Coupled Receptors (GPCRs) are recognized as being able to activate downstream signaling cascades in the absence of an agonist. However, such constitutive signaling is seldom observed in an endogenous, unperturbed system. We have previously shown that mu opioid receptors expressed in dorsal root ganglia neurons are no exception and do not signal constitutively in the basal state. However, deleting β-arrestin 2 or inhibiting Src increases such signaling, possibly by revealing a caveolin-dependent recycling pathway. We have recently found that chronic pain increases constitutive inhibition of calcium channels by mu opioid receptors in these primary afferent neurons. Such constitutive activity is partially responsible for the return to a normal analgesic state following chronic inflammatory pain. However, there are key differences between this adaptation to chronic pain and the β-arrestin 2 and Src-mediated constitutive activity previously observed. The pathways involved and the consequences of this adaptation to chronic pain will be discussed.

42. Young Investigator Plenary *Functional organization of the opioid system in pain neural circuits*

Gregory Scherrer (Stanford University)

Young Investigator Session

43. Dynorphin is released from spinal B5-I interneurons and functions as an inhibitory neuromodulator *of itch*

Sarah Ross (University of Pittsburgh)

Mu opioids are known to relieve pain. The effect if of kappa opioids on somatosensation, however, is less clear. Our previous work revealed that B5-I spinal interneurons are a population of inhibitory neurons that function to inhibit itch. Here we show that B5-I interneurons release the kappa opioid dynorphin. Our data indicate that spinal kappa opioids bidirectionally modulate itch sensitivity. Thus, dynorphin acts as a neuromodulator to inhibit itch in the dorsal horn of the spinal cord, raising the possibility that kappa opioids may be a broadly effective therapy for pathological itch. Finally, we show that B5-I neurons are innervated by menthol-, capsaicin-, and mustard oil-responsive sensory neurons and are required for the inhibition of itch by menthol. These findings suggest that dynorphin may be involved in the inhibition of itch by chemical counterstimuli. This research was supported by NIH grants R01 AR063772 and R21 AR064445 to S.E.R. and by grants to A.J.T. from the MRC (MR/L003430/1) and BBSRC (BB/J001082). Part of this work was supported by a grant from the Rita Allen Foundation to S.E.R, a Whitehall Foundation Research Grant to S.E.R., and the Wellcome Trust to A.J.T. None of the authors has a conflict of interest related to this research.

44. Identification of functional populations of interneurons in dorsal spinal cord

Steeve Bourane (The Salk Institute for Biological Studies)

The somatosensory system plays an essential role in protecting the body from noxious insults and the fine tuning of motor activity that ensures the flexible and coordinated limb movements animals need for locomotion, posture and volitional movements such as reaching and grasping. The dorsal spinal cord is the primary center for integrating and processing a number of these somatosensory modalities including heat, cold, pain, itch and touch. Incoming somatosensory information is processed by complex neural circuits comprised of different excitatory and inhibitory interneuron cell types (INs) that then relay this information to several brain areas via projection neurons. Changes to the dorsal horn circuitry have been implicated in the development and maintenance of somatosensory disorders like chronic pain and allodynia, which are major healthcare problems with few effective treatments. In the present study, we have functionally characterized different population of excitatory and inhibitory INs in the dorsal spinal cord and have identified discrete roles for them in processing and transmitting noxious and innocuous touch modalities. We find that the RORα INs are important for innocuous touch, while Sst excitatory neurons respond to, and relay, noxious mechanical stimuli. Supported by the Catharina Foundation, the NIH grants NS080586, NS086372. There is no author conflict of interest related to this research.

Poster Abstracts

"Evens"- Tuesday presentation; "Odds"- Wednesday presentation

1. A peripherally available mixed efficacy MOR/DOR ligand that displays reduced tolerance, dependence, and abuse liability

Jessica P. Anand1, Henry I. Mosberg2, and Emily M. Jutkiewicz1

1Department of Pharmacology and 2Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI, 48109, USA

It has been demonstrated that the co-administration of a mu opioid receptor (MOR) agonist with a delta opioid receptor (DOR) antagonist produces analgesia with limited development of undesirable neurobiological adaptations and reduced abuse liability. We have previously reported a peptide, VRP26, which displays MOR agonism and DOR antagonism in a single molecule. VRP26 contains a C-terminal β-glucoserine to improve blood-brain barrier penetration and therefore displays antinociception *in vivo* after peripheral administration. In this study we examined the ability of VRP26 to produce tolerance to its antinociceptive effects or physical dependence after chronic administration as well as its ability to produce conditioned place preference as a measure of reward. Unlike fentanyl, seven day continuous administration of VRP26 produced no rightward shift in the antinociceptive dose response curve and fewer signs of withdrawal than fentanyl. Further, antinociceptive doses of VRP26, unlike fentanyl, do not produce conditioned place preference in mice. Additionally, VRP26 does not produce increased locomotor activity, while fentanyl does, suggesting that VRP26 and fentanyl may have differential effects on the dopaminergic system. VRP26 demonstrates proof of concept that mixed efficacy opioid ligands may be better alternatives to traditional opioid analgesics for chronic pain management, producing pain relief with limited tolerance development and abuse liability.

2. Signal biased agonists of delta opioid receptors for alcohol withdrawal-induced hyperalgesia

Doungkamol Alongkronrusmee and Richard M. van Rijn

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, USA

Chronic pain and hyperalgesia are major health issue for which adequate treatment options are still lacking. Withdrawal from long-term alcohol consumption can induce hyperalgesia in both humans and rodents. Yet, the molecular mechanisms underlying this phenomenon are not well understood, which stifles development of new therapeutics. We have previously shown that delta opioid receptors (DORs) are upregulated during prolonged alcohol exposure. Moreover, alcohol withdrawal-induced hyperalgesia is exacerbated in DOR knockout mice, suggesting that DORs are promising targets for treatment of chronic pain induced by alcohol withdrawal. We therefore proposed to study how DORs modulated hyperalgesia during chronic alcohol withdrawal. We also investigated the contribution of β-arrestin in DOR-mediated analgesia using signal biased DOR agonists. For alcohol withdrawal-induced hyperalgesia, mice were exposed to alcohol either voluntarily or by oral gavage for three weeks followed by a period of abstinence during which the mice exhibited hyperalgesia. Mechanical hyperalgesia and analgesia were measured using von Frey filaments. We have found that DOR agonists are able to reduce alcohol withdrawal-induced hyperalgesia and those oral gavaged mice show robust and persistent hyperalgesia. This study will contribute to our understanding of the role of DORs in hyperalgesia and further assist in rational design of DOR agonists for potential therapeutic use in chronic pain conditions. Support by the Ralph W. and Grace M. Showalter Research Trust and the NIH Pathway to Independent Award (K99/R00). None of the authors has a conflict of interest related to this research.

3. Measurement of phasic dopamine signals in the rat nucleus accumbens core and shell in response to noxious stimuli

Christopher Atcherley1,2, Jennifer Xie2, Levi Lazarus3, Michael L. Heien3, Frank Porreca1,2,4

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Pain serves a critical function in survival and impacts all aspects of well-being. The aversiveness of pain demands a behavioral response that is associated with dopamine signaling in the nucleus accumbens (NAc). Mesolimbic dopaminergic pathways have been implicated in action selection. Both pain, and pain relief, have been shown to produce dopamine release in the nucleus accumbens, however the exact mechanism is not yet understood. In this study, we used fast scan cyclic voltammetry to capture and temporally correlate phasic dopamine signals in the NAc core (NAcc) and NAc shell (NAcs) in response to a noxious thermal stimulus. Male, S.D. rats (320 - 360 g) were anesthetized with isoflurane (0.75 – 1.0 %). The level of anesthesia was regulated to elicit a tail-flick response with a latency of 3.8 – 5.2 sec. Following anesthesia, biocompatible carbon-fiber microelectrodes were implanted into the NAcc or NAcs for fast-scan cyclic voltammetry (FSCV) enabling real-time measurements of dopamine (DA). A triangle waveform (-0.4 V to 1.3 V at 400 V/sec) was applied to the electrode every 100 ms and the resultant voltammogram recorded. The dopaminergic signal was extracted from the voltammogram by principal component regression, a mathematical tool to identify and quantify endogenous DA changes based on wave-shape. Electrode placement was optimized by electrically evoking DA release

with a stimulating electrode placed in the median forebrain bundle (24 pulse, 4 ms per pulse, 300 µA). Placement of the microelectrode was verified with histology by locating the electrode tip, which was marked with an electrical lesion (600 µA). The noxious thermal stimulus produced NAc DA efflux in both the NAcc and NAcs that was time-locked to the tail-flick. The temporal relationship to pain onset and pain offset varied in these two regions. These methods provide an approach to assess how NAc dopaminergic signaling may contribute to pain-motivated behaviors.

4. Dorsal striatal mu opioid receptors inhibit cholinergic interneuron-driven dopamine release

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Dopaminergic input to the dorsal striatum from the substantia nigra is important for many forms of striatal plasticity and striatum-mediated behaviors. The regulation of dopamine release in this brain region is complex and involves multiple neurotransmitters. The role of the mu opioid receptor (MOR) in regulating dopamine release from these inputs is unclear. Some studies show that MOR activation within the dorsal striatum decreases dopamine release, which is curious considering the absence of MOR on dopaminergic inputs. However, MORs found on cholinergic interneurons (CINs) may allow for regulation of dopamine release independent of direct effects on dopaminergic terminals. CINs make up less than 5% of striatal neurons, yet have extensively arborized axons that broadly influence neurotransmission. Acetylcholine released by these interneurons drives local striatal dopamine release by activating nAChRs on dopaminergic terminals. Recent evidence demonstrates that activation of MORs on CINs inhibits the activity of these neurons. We tested the hypothesis that MOR-mediated reduction of CIN activity is responsible for the effect of MOR agonists on dopaminergic transmission. We utilized fast-scan cyclic voltammetry to determine the effects of opioid ligands on dopamine release in striatal brain slices. The MOR agonist DAMGO inhibited dopamine release elicited by both electrical stimulation and optogenetic activation of CINs. These data demonstrate that MORs are important regulators of CIN-driven dopamine release. We are currently exploring the effects of genetic manipulations of MOR expression on CIN-driven dopamine release. No conflict of interest. Work is supported by the Division of Intramural Clinical & Biological Research of NIAAA.

5. Synaptic adaptations in CA1 area of the mouse hippocampus following conditioned place preference to morphine

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Drug addiction has a strong learning and memory component, thought to contribute to relapse (Portugal GS et al (2014) J Neurosci 34:527-38). Our aim was to examine memory-related synaptic adaptations in the mouse hippocampus following contingent and non-contingent administration of morphine. Mice were trained to exhibit morphine-induced conditioned place preference ('morphine CPP'): 1x i.p. injection per day for 4 days (alternating between 10 mg/kg morphine and saline). Immediately following CPP testing, mice were killed and ventral hippocampal slices prepared for electrophysiological recording (Nicholls RE et al (2006) Proc Natl Acad Sci USA 103:6380-5. Separate groups of animals ('non-contingent morphine') received identical injections but were confined to home cage. There were 2 control groups: 'non-contingent saline' and 'saline CPP'. Using extracellular field recordings, stimulus-induced LTP was reduced in both morphine groups ('morphine CPP' and 'non-contingent morphine') vs saline controls. But, exposure to the CPP environment ('morphine CPP' or 'saline CPP') increased both the magnitude and variance of LTP compared with non-contingent controls. Using whole-cell patch-clamp recordings, the AMPA/NMDA ratio was increased in the 'non-contingent morphine' group. The 'morphine CPP' group showed a non-significant trend to increased AMPA/NMDA ratio, but a highly significant increase in the variance. These data suggest that in vivo morphine administration induces LTP in the ventral hippocampus, but that conditioning induces more complex, possibly cell-specific, changes. Ongoing work is attempting to uncover these cell-specific effects of morphine conditioning on synaptic plasticity. Funding: BBSRC studentship; no conflict of interest

6. Mu-opioid receptor modulation of thalamo-striatal afferents.

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Opioid dependent inhibition of glutamate inputs to the striatum/Nucleus Accumbens using electrical stimulation is small and variable. This variability may result from the large number of different afferent inputs. The medial thalamus is known to send broad glutamatergic projections to both the striatum and Accumbens. Medial thalamus also expresses a high density of mu-opioid receptors, MOPr. Therefore, it was hypothesized that specific thalamo-striatal projections would be more sensitive to modulation by opioid receptor agonists than other glutamatergic afferents in the striatum— cortico-striatal, for example. The current project uses whole cell voltage clamp recordings in mouse striatum coupled with viral mediated expression of channel rhodopsin in either thalamus or cortex. Optical stimulation of thalamo-striatal and cortico-striatal afferents as well as bulk electrical stimulation of striatal inputs were used to compare the opioid sensitivity of thalamic and cortical glutamatergic inputs onto striatal medium spiny neurons. The results suggest that while cortico-striatal and bulk glutamate inputs are relatively insensitive to inhibition by MOPr activation, thalamo-striatal projections are highly sensitive

to inhibition by MOPr agonists. Thus, opioid treatment may alter the balance of glutamatergic input from thalamus and cortex to the striatum. This work was supported by the National Institutes of Health National Institute on Drug Abuse [DA08163].

7. Protein kinases C and GRK2/3 are involved in U50,488H-promoted KOPR phosphorylation

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Agonist-promoted KOPR phosphorylation plays a key role in signaling and receptor regulation. Here we characterized molecular mechanisms of U50,488H-induced KOPR phosphorylation. Neuro2a cells stably transfected with FLAG-mKOPR-6His were treated with vehicle or U50,488H and solubilized and receptor was partially purified with Ni-NTA agarose and resolved by SDS-PAGE followed by immunoblotting with specific antibodies recognizing KOPR phosphorylated at S356/T357, T363 and S369. U50,488H (0.1μM) caused phosphorylation of KOPR at S356/T357, T363 and S369 very quickly, reaching peaks at 1 min. The effect of U50,488H (2 min) was dose-dependent with the EC50 values of 0.1μM, 0.48μM and 0.24μM for phosphorylation at S356/T357, T363 and S369, respectively. The pan-PKC inhibitor GF109203X (4μM) inhibited U50,488-induced phosphorylation at S356/T357> T363>> S369. The selective PKC α and β1 inhibitor Go6976 (100nM) inhibited U50,488-induced phosphorylation at S356/T357 and S369, but not at T363. The PKC activator phorbol 12-myristate 13-acetate (PMA, 0.1μM) caused dose-dependent phosphorylation of S356/T357, S369 and T363 with the EC50 values of 6.6nM, 7.5nM and 0.2nM, respectively. PMA produced similar phosphorylation level at S369 as U50,488H, but reached the peak (at 15 min) later than U50,488H. PMA promoted lower maximal response at pT363, but higher maximal effect at pS356/T357, than U50,488H. siRNA knockdown of GRK2, GRK3 or GRK2+GRK3 reduced U50,488h-promoted phosphorylation at S356/T357, T363 and S369. These results indicate phosphorylation of KOPR at different residues is differentially regulated by GRKs and PKC. We will further examine which isoforms of PKC are involved and if GRK5/6 participate in U50,488H-induced KOPR phosphorylation. (This work was supported by NIH grants DA017302 and DA036802. The authors have no conflicts of interest.)

8. Differential phosphorylation of the KOPR by U50,488H and etorphine: quantitation with SILAC

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U50,488H promoted robust KOPR internalization, whereas etorphine caused low or no internalization. Here we used SILAC (stable isotope labeling by amino acids in cell culture) to quantitatively compare U50,488H- and etorphine-induced KOPR phosphorylation. Neuro2A mouse neuroblastoma cells stably transfected with the mouse KOPR epitope tagged with FLAG at the N-terminus and 6 x His at the C-terminus (FmK6H) were grown in the presence of Arg0, Arg6 or Arg10 for at least 10 passages to label proteins to 99%. Cells were treated with vehicle (C, Arg0), etorphine (E, Arg6) or U50,488H (U, Arg10) for 30 min and the FmK6H was purified with Nickel-NTA column followed by anti-FLAG affinity chromatography. The FmK6H was resolved as a broad band of Mr 53kDa by SDS-PAGE, in-gel digested with endoproteinase Glu-C and analyzed with LC-MS/MS. A group of mixed single-, double- and triple-phosphopeptides were identified and quantified, revealing distinct imprints of the two different ligands on KOPR phosphorylation. The two ligands promoted single-site phosphorylation primarily at T363 and S369 (U/E=2) whereas S356/T357 had basal phosphorylation and U50,488H induced only slight increases. Both agonists also induced two- and three-site phosphorylation at S356-T363, T357-S369, T363-S369, S356-T357-S369 with ratios of U/E=2, 3.4, 3.3, 5.6, respectively. Therefore, U50,488H produced higher levels of single-, double- or triple-phosphorylated form of the KOPR and U50,488H differs more markedly from etorphine in higher-order KOPR phosphorylation. In addition, because all phosphorylation occurs within the fragment RQSTNRVRNTVQDPASMRD, the unphosphorylated fragment was used to calculate the % of total KOPR being phosphorylated. About 60% and 30% of the receptors are phosphorylated following U50 and etorphine treatment, respectively. (This work was supported by NIH grants DA017302 and DA036802. The authors have no conflicts of interest).

9. Optogenetic control of the spinal cord in behaving mice.

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Spinal cord circuits perform extensive computation, process sensory and motor information, and play a critical role in the development of pathology. However, progress in understanding the function and role of these neurons using optogenetic techniques has been stymied by a lack of experimental tools for light delivery to the spinal cord. Here, we developed methods to enable optogenetic interrogation of spinal cord circuits using off-the-shelf components,

demonstrating modulation of dorsal horn neurons in freely moving animals. We use these techniques to show that activation of somatostatin interneurons is sufficient to induce pain, and investigate the pathway through which this signal is relayed to the brain. This work eliminates roadblocks in spinal cord neuroscience, facilitating a wide range of studies from other groups. This study was supported by the U.S. National Institutes of Health (NINDS R01-NS080954), and the Stanford Bio-X NeuroVentures program. AJC was supported by a Texas Instruments Stanford Graduate fellowship. None of the authors has a conflict of interest related to this work.

10. Effects of sex and genotype on corticosterone levels in a mouse model of *OPRM1* (A118G) polymorphism

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The A118G single nucleotide polymorphism (SNP) of the human mu-opioid receptor gene (*OPRM1*) is widely studied due to its association with multiple pain and addiction phenotypes in humans. To elucidate the functional consequences of this SNP, mice bearing an equivalent nucleotide substitution (A112G) in the mouse opioid receptor gene were generated (Julie Blendy, UPenn). 112G mice show pain-, stress-, and addiction-related phenotypes similar to humans bearing the 118G allele. Previous work from our group shows that the 118G allele affects physiological, agonist-, and antagonist-mediated changes in cortisol levels. To our knowledge, there are no studies of the effects of the 112G allele on corticosterone levels in mice. Thus, we determined baseline serum levels of corticosterone in stress-minimized, adult male and female mice homozygous for either the 112A (AA) or 112G (GG) allele using a commercially available radioimmunoassay. Corticosterone was higher in female mice compared to male mice (females 137.75 ± 19.06 ng/mL, males 54.45 ± 4.45 ng/mL; $p < 0.05$). Neither males (AA 50.80 ± 7.07 ng/mL, GG 57.05 ± 5.98 ng/mL; $p > 0.05$) nor females (AA 104.46 ± 18.44 ng/mL, GG 165.50 ± 16.84 ng/mL; $p > 0.05$) showed a significant effect of genotype. There was a trend toward higher corticosterone in GG mice (overall AA 77.63 ± 13.04 ng/mL, overall GG 111.28 ± 11.47 ng/mL; $p = 0.068$). This work was supported by NIH 1R01DA029147 (YZ), the Adelson Medical Research Foundation (MJK), and the David Rockefeller Graduate Program (DC). The authors declare no conflict of interest.

11. Receptor dissociation kinetics determines opioid receptor signalling bias

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Recently there has been much interest in the possibility that biased signalling at the MOPr may be effective in skewing the analgesic versus side-effect profiles of MOPr agonists. We have used a single cell type to examine the signalling efficacy of a series of opioids with varying efficacy for G-protein signalling versus endocytosis, including a novel peptidic agonist with extreme G-protein bias. We determined activation of G-protein signalling (GIRK activation), Ser375 phosphorylation (immunohistochemistry), β-arrestin-2 binding (BRET) and endocytosis (immunohistochemistry) under conditions, where possible, that association and dissociation kinetics could be determined. We and others have previously established that all regulatory steps of MOPr are reversible at the cell surface. Our major findings are that the dissociation kinetics of GIRK signalling, Ser375 phosphorylation, and β-arrestin-2 binding are highly correlated for agonists at each step of the regulatory process, with correlation coefficients of approximately 0.9. This demonstrates that reversal of each regulatory step is ligand-dissociation rate dependent rather than pathway (e.g. phosphatase kinetics) limited. Importantly, when we account for initial GIRK signalling efficacy, dissociation rate strongly predicts efficacy for endocytosis, with rapidly dissociating agonists displaying the greatest GIRK versus arresting bias. This suggests that development of high G-protein efficacy agonists with rapid dissociation rates can be systematically developed to limit adverse effects of opioids.

12. Lipophilicity Efficiency is a new paradigm for balancing receptor affinity and in vivo antinociceptive efficacy of opioid peptides

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The lipophilicity efficiency indices LLE and LELP have been recently proposed to support balanced optimization of potency and ADMET profile. Ligand lipophilicity efficiency LLE represents the activity of a ligand without the contribution of its lipophilicity. LLE consents high potency while maintaining moderate lipophilicity. Ligand efficiency-dependent lipophilicity LELP has been recently proposed to combine lipophilicity, molecular size, and potency into one composite descriptor. In this respect, we compare for the first time the lipophilic efficiency indices to affinity and *in vivo* efficacy in the field of opioid compounds. We analyzed of a mini-library of lipophilic opioid peptides, carrying a Trp equipped with methyl, nitro, and halo-substituents at various positions of the indole ring. Substitution had a strong impact on experimental MOR affinities. We also observed that the different groups at Trp influenced stability and lipophilicity. Peptides with 5-nitro and 7-Br-2-Me Trp showed moderately faster analgesia as compared to the unsubstituted compounds in mouse tail flick assay. The antinociceptive efficacy profiles nicely correlated to the calculated LLE and LELP, suggesting that lipophilicity efficiency indices might represent a new and useful predictive tool for the design of centrally or peripherally acting analgesic

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13. The evolution of amygdala neuronal ensembles encoding neuropathic pain states

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Pain is a multidimensional experience comprising sensory and emotional modules. The basolateral amygdala (BLA), classically involved in fear and anxiety, assigns an emotional valence, such as unpleasantness, to highly salient environmental stimuli. To understand how the BLA encodes the experience of aversive stimuli and how this activity changes during the development of chronic neuropathic pain we followed BLA ensemble dynamics for multiple weeks after the induction of a peripheral nerve injury. To visualize the BLA network activity in awake, freely behaving mice we implanted a small microendoscope that allowed us to image large populations of BLA projection neurons expressing the calcium indicator GCaMP6m. We monitored the evolution of neural activity patterns in the BLA in response to innocuous and noxious stimuli, before and for weeks after a peripheral nerve injury that induced neuropathic pain. Prior to nerve injury, noxious, but not innocuous, stimuli evoked time-locked Ca2+ spikes in a subpopulation of BLA neurons. Strikingly, after the establishment of neuropathic pain the neuronal ensemble responses evoked by prior innocuous stimuli transformed such that the network representation was indistinguishable from activity patterns evoked by frankly noxious stimuli. Our results point to a possible neural substrate for allodynia and show for the first time how neural ensemble coding may shape the perception of pain unpleasantness. We are presently examining the functional importance of this BLA network representation to several behavioral features of chronic pain. Studies supported by NIDA DA031777 (GS), T32-DA035165 (GC), NSF DGE-114747 (BA) and DARPA (MS). No conflicts of interest.

14. Nalfurafine shows lower propensity to cause aversion in mice and produces lower KOPR phosphorylation than U50,488H and MOM-Sal B

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KOPR agonists are potentially useful as antipruiritic agents, analgesics and water diuretics; however, their development has been limited by dysphoria. Nalfurafine, U50,488H and methoxymethyl salvinorin B (MOM-SalB) are structurally distinct selective KOPR agonists. Nalfurafine is used in Japan for treatment of uremic pruiritus in hemodialysis patients; however, surprisingly, at the therapeutic doses, nalfurafine does not produce dysphoria. Here we examined the three compounds for inhibition of compound 48/80-induced scratching, antinociceptive effect in the formalin test and aversive effect in conditioned place aversion (CPA) in male CD-1 mice to determine if there were dose differences among the three tests for each compound. The three compounds produced antinociception and anti-scratching effects in dose-dependent fashion with antinociception A50 values of 5.8 μg/kg (nalfurafine), 17 μg/kg (MOM-SalB) and 0.58 mg/kg (U50,488H) and the corresponding A50 values for antiscratching effect of 8.0 μg/kg, 70.2 μg/kg and 2.07 mg/kg, respectively. Significant aversion was seen at all doses of MOM-Sal B (10-300 μg/kg) and U50,488 (0.25-10 mg/kg), whereas nalfurafine only produced significant aversion at the 20 μg/kg. Thus, U50,488H and MOM-SalB produced CPA at lower doses than those required for antinociceptive and anti-scratching effects, whereas the reverse was true for nalfurafine. In neuro2A mouse neuroblastoma cells expressing the mouse KOPR (mKOPR), the three compounds were full agonists in promoting [35S]GTPγS binding. All three compounds induced mKOPR phosphorylation at S356/T357, T363 and S369, but nalfurafine caused T363 phosphorylation to a much lower extent. These results indicate that nalfurafine acts on the KOPR in a different manner from U50,488H and MOM-SalB, which may account for the unique in vivo pharmacological profile. In addition, our mouse models may be useful for screening for KOPR agonists with lower propensity to cause dysphoria. Supported by NIH grants (DA036802 and DA013429). The authors have no conflicts of interest to disclose.

15. Effect of a novel fentanyl derivate on mu-opioid receptor-induced G-protein activation

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The use of mu-opioid receptor (MOR) agonists in the treatment of pain is greatly limited by their side effects. Therefore, new drugs with a disease-specific action precluding sedation, addiction, respiratory depression and other adverse effects are needed. Opioids activate the G-protein coupled receptor (GPCR) MOR, leading to conformational rearrangements of inhibitory Gα and Gβγ subunits, their activation and targeting of intracellular mediators. Despite the progresses made in understanding GPCR activation, the diversity of signaling by different MOR ligands and in different tissue milieus requires further characterization. Fluorescence Resonance Energy Transfer (FRET) provides novel methodological impulses to the study of dynamic protein-protein interactions in live cells. Here, we used FRET to investigate MOR-induced G-protein

activation upon treatment with Fentanyl and Fluor-Fentanyl. The latter was derived by computer-aided drug design to selectively activate peripheral MOR during extracellular acidosis, a hallmark of inflammation following tissue injury. G-protein subunits and MOR were expressed in HEK293 cells and FRET between fluorescent Gαi1-mTq and Venus-Gγ2 was measured over time. Fentanyl induced a marked decrease in FRET, likely due to rearrangement/dissociation of G-protein complexes, which was blocked by co-treatment with naloxone. Fluor-fentanyl selectively activated G-proteins at low extracellular pH, while under physiological conditions (pH 7.4), its effects were indistinguishable from vehicle-treated cells. These data suggest that our novel fentanyl derivate activates MOR and G-proteins preferably at low pH. Supported by BMBF-VIP0272/03V0364. Conflicts of interest: Patent applications WO 2013026787 A1; WO 2013102681 A1.

16. Differential regulation of delta opioid receptor-mediated behaviors by RGS proteins in mice

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The delta opioid receptor (DOPr) is a member of the opioid receptor family of G protein-coupled receptors (GPCRs). Activation of DOPr induces antinociception and antidepressant-like effects in animal models without the constipation, respiratory depression, and abuse liability associated with mu opioid receptor agonists such as morphine. In addition, some DOPr agonists cause convulsions, hindering their development as therapeutics in humans. However, the intracellular signaling pathways that generate these behaviors have yet to be determined. Regulator of G protein signaling (RGS) proteins act as negative modulators of GPCR signaling and may differentially regulate DOPr-mediated behaviors. To investigate this, we compared behaviors induced by the DOPr agonist SNC80 in Gαo RGS-insensitive (RGSi) knock-in and RGS4 knockout mice. SNC80-induced peripherally-mediated antinociception in the acetic acid stretch assay was enhanced in both the Gαo RGSi knock-in and RGS4 knockout animals. Loss of RGS4 also potentiated SNC80-induced centrally-mediated antihyperalgesia in a nitroglycerin-induced thermal hyperalgesia assay. In the tail suspension test, RGS4 knockout mice showed no significant difference in SNC80-induced antidepressant-like effects. Furthermore, there was no change in the dose of SNC80 needed to induce a convulsion in RGS4 knockout mice. Taken together, these data demonstrate that RGS4 negatively modulates some, but not all DOPr-mediated behaviors. Because RGS proteins modulate DOPr-mediated G protein signaling, these data are consistent with G protein independent signaling mechanisms generating DOPr-mediated antidepressant-like effects and convulsions. Future studies will focus on verifying which DOPr-mediated behaviors signal through G protein independent mechanisms.

17. Enhanced enkephalin tone in the striatum facilitates locomotion following deletion of D2 receptors in striatal medium spiny neurons

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The endogenous opioid peptide enkephalin is expressed in medium spiny neurons (MSNs) of the striatum and nucleus accumbens (NAc) where, through activation of opioid receptors, enkephalin is thought to mediate morphine reward. The level of enkephalin mRNA in the striatum is regulated by the activity of dopamine D2 receptors (D2R) but it is unclear which population of D2R-expressing neurons exerts this control and whether changes in expression level have any functional significance on striatal behaviors, such as opioid-induced locomotor activity and reward. We found that a transgenic mouse with selective deletion of D2Rs from D2R-containing MSNs (MSN-Drd2-KO) show an 85% increase in enkephalin mRNA levels in the striatum compared to littermate controls. The effect of met-enkephalin on GABAA-mediated inhibitory post-synaptic currents (IPSCs) was measured in D1R-containing MSNs triggered by optogenetic stimulation of D2-MSN axon collaterals in slices containing the NAc. Met-enkephalin attenuated optogenetic-evoked IPSC amplitude equally in MSN-Drd2-KOs and controls, suggesting that opioid receptor levels are functionally equivalent. However, when enkephalin degradation was inhibited optogenetic-evoked IPSC amplitude and spontaneous IPSC frequency was decreased in MSN-Drd2-KOs, but not controls, strongly suggesting that these mice have enhanced enkephalin tone. Furthermore, systemic administration of the opioid antagonist naloxone induced a locomotor response and a state-dependent place aversion only in MSN-Drd2-KOs; however, both genotypes showed a locomotor response to oxycodone and acquired morphine conditioned place preference. These data indicate that selective deletion of D2Rs from D2R-containing MSNs enhances striatal enkephalin tone to facilitate locomotion without affecting opioid conditioned reward. The authors have no conflict of interest to declare.

18. Precipitated oxycodone withdrawal reduces the startle reflex and bodyweight in C57BL/6J and control mice, but not in HIV-1 Tat-expressing mice

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Abuse of prescription opiates, such as oxycodone, has increased in recent decades. HIV-infected individuals have an enhanced risk of opiate abuse. There is evidence that opiates worsen the cellular and behavioral toxicity symptomatic of

HIV-associated neurocognitive disorders (HAND) presumably through their interaction with the neurotoxic HIV-1 Tat protein. Previously, in agreement with clinical findings in HAND-diagnosed patients, we found significant deficits in prepulse inhibition of the startle reflex (PPI), a measure of sensorimotor gating, in transgenic mice that centrally express HIV-1 Tat. We subsequently hypothesized that even greater PPI deficits would occur in Tat-expressing vs Tat-null mice during oxycodone dependence. Physical dependence upon oxycodone was induced by administering a 10-day escalating regimen (9-33 mg/kg, b.i.d., s.c.) in Tat(+), Tat(-), and C57BL/6J mice. Startle/PPI was tested in a 25-min session under chronic and precipitated withdrawal conditions. Chronic oxycodone administration did not significantly affect startle or PPI in any group. Precipitating withdrawal with naloxone (1 mg/kg, s.c.) significantly ($p<0.05$) reduced the startle reflex and body weights in C57BL/6J and Tat(-) mice, but surprisingly not in Tat(+) mice. Also surprisingly, PPI was unaffected in all groups. Contrary to our hypothesis, these results suggest that Tat-expressing mice may be more resistant to opiate withdrawal effects. This research was supported by NIDA training grant T32DA007027 and NIH grant R01 DA018633. None of the authors have a conflict of interest.

19. Characterization of the diimidazo-diazepine TPI-2065-14, a mixed KOR and DOR agonist with peripherally-restricted activity and minimal liabilities of use.

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Agonists with mixed selectivity for kappa and delta opioid receptors could increase analgesic potency while reducing adverse effects. Moreover, we hypothesized that peptidomimetic ligands might display reduced blood brain barrier (BBB) permeability, resulting in safer peripherally-selective opioid analgesics. Accordingly, the 2065-series of fused diazaheterocyclics was synthesized from reduced tripeptides. *In vitro* screening with radioligand competition binding assays demonstrated variable opioid receptor affinity across the series, with the diimidazo-diazepine TPI-2065-14 displaying nanomolar affinity for KOR and DOR. Central (i.c.v.) or intraperitoneal (i.p.) administration of 2065-14 produced dose-dependent, opioid-receptor mediated antinociception in the mouse 55oC warm-water tail-withdrawal and acetic-acid writhing assays. The potency ratio of 2065-14 in the two assays was 32.6 times higher than morphine, and antinociception was blocked by pretreatment with naloxone methiodide only when administered on the same side of the BBB, suggesting possible peripherally-restricted activity. Confirming this, although readily detected in blood, only trace amounts of 2065-14 were detected in brain by LC-MS/MS following peripheral administration, which supports our earlier hypothesis. Administration of 2065-14 did not alter gastrointestinal transit or produce seizures at any antinociceptive therapeutic dose tested, and did not demonstrate acute antinociceptive tolerance. Even when centrally administered (i.c.v.), maximal antinociceptive doses of 2065-14 did not produce locomotor effects in the rotorod assay or cause conditioned-place preference or aversion. Together, these data support that mixed-selectivity opioid agonists may increase analgesic potency with minimal liabilities and that diimidazo-diazepine ligands may possess innate peripheral selectivity, with 2065-14 offering early therapeutic potential as a novel analgesic with fewer clinical liabilities. This work was funded by the Florida Drug Discovery Acceleration Program by the State of Florida and by R01-DA31370 (to RAH).

20. Morphine conditioned place preference induces structural plasticity of dendritic spines in the hippocampus.

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Opiate abuse is emerging problem in the US with 2.1 million people abusing opiate prescription drugs. Relapse occurs in 80% of abstinent abusers indicating a need to develop methods to prevent cravings that drive relapse. Our previous studies demonstrate that the NMDA-NR2B subunit in the hippocampus (HPC) is necessary for opiate induced behaviors. Dendritic spine morphology, controlled by actin polymerization, is regulated by NR2B-mediated Ca2+ influx and activation of the Rho signaling cascade. We have found that morphine conditioned place preference (CPP) induces a decrease in spines on CA1 pyramidal cells in the hippocampus mediated by a decrease in thin type spines. Additionally, we have found that morphine CPP increases RhoA expression in synaptosomal fractions while overall expression in unaltered following morphine CPP. Suggesting that morphine CPP alters RhoA trafficking in the hippocampus. Future studies will investigate whether the morphine CPP induced effects on thin spines and RhoA trafficking are mediated by increases in NR2B function. Studies thus far demonstrate that morphine CPP induces plasticity in the hippocampus, a brain area that sends projections to the medial prefrontal cortex (mPFC). The mPFC is also involved in the acquisition and retrieval of drug associated memories. Ongoing studies are investigating the role of vHPC-mPFC projections in reward, and in particular opiate induced behaviors. Our preliminary studies demonstrate that optogenetic activation of the vHPC can induce a CPP and future studies will determine the role of hippocampal projections to the mPFC in the development of CPP and their role in opiate craving. This work was supported by NIH grant R01 DA027460 to JMC. Authors have no conflict of interest.

21. The kappa opioid receptor agonist 16-bromosalvinorin A reduces cocaine seeking in rats

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Kappa opioid receptor (KOPr) agonists have known anti-cocaine effects but produce various side effects that limit therapeutic use. The aim of the current study was to determine if the novel salvinorin A analog 16-bromosalvinorin A (brSalA), could attenuate reinstatement of cocaine seeking preclinically with reduced side effects compared to traditional KOPr agonists. Male Sprague-Dawley rats trained to self-administer cocaine in daily 2 hour sessions were subjected to extinction followed by administration of vehicle or brSalA (0.3 mg/kg, 1 mg/kg; n=6) and drug-prime reinstatement testing (20 mg/kg cocaine, i.p). Sedative effects were evaluated by determining locomotor activity in rats for 60 min following brSalA (1 mg/kg, i.p, n=6). To evaluate the effects of brSalA on natural reward pathways, drug naïve rats were trained to self-administer sucrose pellets and effects on responding after brSalA administration (1 mg/kg, i.p, n=7) determined. Depressive effects were assessed by subjecting rats to forced swimming conditions for 5 min following an injection of brSal A (1 mg/kg, i.p, n=8-9). BrSalA significantly decreased cocaine seeking but did not reduce locomotor activity and sucrose intake or increase immobility times in rats. This highlights a specific effect of brSal A on drug seeking not due to sedation or modulation of natural reward, and the minimal side effects of brSalA at the effective dose. These findings provide information for ongoing work in developing effective anti-addiction pharmacotherapies. Support by Neurological Foundation of New Zealand. AWE was supported by a Victoria University Doctoral Scholarship. The authors have no conflicts of interest to declare.

22. Impact of linker/address region in the discovery of potent multifunctional ligands with μ/δ opioid agonist/neurokinin 1 (NK1) Antagonist Activities

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Multifunctional ligands with *μ*/*δ* opioid agonist/NK1 antagonist activities were designed and synthesized with the concept of overlapping and adjacent pharmacophores. Conformationally flexible, as well as rigid, amino acids were used as linker/address region to examine the role played by it in binding selectivity between *μ* and *δ* opioid receptors. Influence of structural changes in opioid pharmacophores in the biological profile at MOR, DOR, and NK1R were studied. The structure-activity relationships (SARs) study revealed that the *N*-terminus modification, which was expected to have influence on the biological profiles at *μ*/*δ* opioid receptors, has appreciable impact on NK1 receptor binding affinities and activities. Use of rigid amino acid at the address region enhanced the MOR binding selectivity over DOR. Functional assays showed that the ligand AKG113 (H-Tyr-D-Ala-Gly-*NMe*Phe-Gly-Pro-Leu-Trp-NH-Bn(3',5'-(CF3)2)) containing a flexible linker (i.e. Gly) has two times more agonist activity at DOR compared to that at MOR. This study led to the discovery of a potent ligand AKG177 (H-Tyr-D-Ala-Gly-*NMe*Phe-4-Apac-Pro-Leu-Trp-NH-Bn(3',5'-(CF3)2)) having a relatively rigid linker (i.e. 4-Apac) with balanced agonist activity at MOR and DOR while maintaining high antagonist activity at NK1 receptor.

23. Temporally coordinated presynaptic inhibition by enkephalin and GABA gates primary afferent input in the spinal cord dorsal horn

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Enkephalins are endogenous peptidergic agonists that can bind to both delta and mu opioid receptors (DOR and MOR) to regulate neurotransmission. Here we combined mouse genetics, neuroanatomy, optogenetics and slice electrophysiology to resolve the mechanism of action of enkephalins in the spinal cord. We generated knockin mice in which the Cre recombinase is expressed by enkephalinergic neurons (Penk-Cre mice) to identify and manipulate these neurons. In situ hybridization experiments confirmed Cre activity in neurons expressing enkephalins. We first resolved the identity of spinal enkephalinergic neurons in laminae I-III and found that they represent a heterogeneous population of GABAergic and glutamatergic interneurons. Furthermore, enkephalins were concentrated in axons and presynaptic buttons of these Penk-Cre+ interneurons, which formed axo-axonic synapses with primary afferents. We then expressed channel rhodopsin-2 in Penk-Cre+ neurons to elucidate how release of enkephalins at these synapses modulates neurotransmission between somatosensory and spinal neurons. Strikingly, we found that light-evoked depolarization of enkephalinergic neurons caused a long-lasting depression in glutamate release that followed GABA-mediated presynaptic inhibition. This enkephalinergic presynaptic inhibition is highly selective and topographically organized as it requires the presence of MOR on terminals of afferents projecting to lamina I-IIo and of DOR on afferents projecting to laminae IIi-III. Altogether our results uncover a mechanism by which the coordinate action of GABA and enkephalins provide immediate but sustained presynaptic inhibition to gate primary afferent input in the spinal cord dorsal horn. Support by NIH/NIDA

(DA031777, G.S.) and Stanford Dean's Fellowship (A.F.) None of the authors have conflicts of interest.

24. GPR171, the receptor for hypothalamic neuropeptide BigLEN, is involved in the regulation of stress and reward-related behaviors

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Neuropeptidomic studies show that proSAAS derived peptides are abundantly present in the hypothalamus and enriched in agouti related peptide (AgRP) neurons of the arcuate nucleus. We recently identified BigLEN, a proSAAS derived peptide, as an endogenous agonist of GPR171. Lentiviral shRNA-mediated knockdown of GPR171 in the hypothalamus indicates receptor involvement in modulation of food intake and metabolism. In order to explore the role of GPR171 *in vivo* non-peptidic ligands, both agonists and antagonists, are needed. Therefore, we generated a homology model of GPR171 based on the crystal structure of a phylogenetically related receptor, and virtually screened an in-house small-molecule library. This led to the identification of MS0015203 as an agonist and MS0021570 as an antagonist. Biochemical characterization demonstrated that these compounds bind to and exert their effects selectively via GPR171. Administration of MS0015203 increases receptor activation in the paraventricular nucleus and food intake; this is not seen in mice with knockdown of GPR171 in the ventral hypothalamus. Interestingly the antagonist, MS0021570, blocks the increases in food intake seen following selective activation of AgRP neurons that would lead to release of BigLEN. Immunohistochemical studies show that, in addition to hypothalamus, GPR171 is expressed in the basolateral amygdala. We find that GPR171 knockdown in this brain region not only prevents the acquisition of fear memories but also of morphine conditioned place preference. Together, these results show an involvement of GPR171 in stress and reward-related behaviors. This work was supported in part by NIH grants DA008863 and NS026880 (to L.A.D.) and E.N.B. is supported by NIDA training grant (DA007135).

25. Casein kinase- 1 epsilon deletion enhances opioid reward and is associated with increased striatal Oprm1 and Npas4 expression

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Genetic and pharmacological studies indicate that Casein kinase-1 epsilon (*Csnk1e*) contributes to psychostimulant, opioid, and ethanol behaviors. Pharmacological inhibition of CSNK1E enhanced psychomotor sensitivity to the selective mu-opioid receptor agonist fentanyl, indicating a negative regulatory role in drug behavior. Here, we tested the hypothesis that Csnk1e negatively regulates fentanyl reward using the conditioned place preference assay (CPP) in *Csnk1e* knockout (KO) and wild-type (WT) mice. KOs showed a leftward shift in the inverted u-shaped curve for opioid reward versus WTs, exhibiting enhanced reward at lower doses (0.05 mg/kg) and decreased reward at higher doses (0.2 mg/kg). No significant differences were observed in fentanyl analgesia in the 52.5°C hot plate assay (0-0.4 mg/kg) or naloxone conditioned place aversion (4 mg/kg), suggesting a neural mechanism selective for dopaminergic reward circuitry. To generate novel hypotheses regarding the molecular mechanisms that mediate enhanced opioid reward in *Csnk1e* KOs, we used transcriptome analysis via mRNA sequencing of striatum from naïve KO and WT mice to identify the transcription factor *Npas4* as the top hit (2.2-fold increase in expression; p=4.96 x10-136), supporting *Npas4* transcript covariance with morphine reward (Piechota et al., 2010). Importantly, expression of *Oprm1* (mu-opioid receptor) was higher in KOs compared to WTs (1.7-fold increase; p=2.14x10-4). The activity of one of the top upstream regulators identified by Ingenuity Pathway Analysis, STAT3, is negatively regulated by CSNK1E and *Stat3* has previously been shown to regulate *Oprm1* expression. We conclude that *Csnk1e* deletion enhances opioid reward, possibly via a STAT3-mediated increase in mu-opioid receptor expression. FUNDING SOURCES: R00DA029635 (NIDA; C.D.B.), T32GM008541, HG005692 (W.E.J.), Transformative Training Program in Addiction Science (Burroughs Wellcome 9550300872). None of the authors has a conflict of interest related to this research.

26. Development of stable dynorphinA analogues at the bradykinin 2 receptor for the treatment of neuropathic pain

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Current treatments for neuropathic pain involve opioids, non-steroidal anti-inflammatory drugs (NSAIDS), and anticonvulsants. Many of these treatments are highly efficacious for acute pain but are not very effective in neuropathic pain and have serious side effects caused by long-term administration. Treatment for this disease is difficult with conventional methods, partly because the mechanism of this disease is not well known. One target for neuropathic pain

treatment may be the blockade of Dynorphin A (Dyn A). Experimental evidence has shown that *des*-tyrosine fragments of dynorphin A can interact with bradykinin 2 receptors (B2R) to activate calcium influx through a non-opioid mechanism. This neuronal excitatory effect is proposed to underlie the pronociceptive actions of spinal Dyn A. Therefore, the development of B2R antagonists can be used to block Dyn A which can lead to novel therapeutics for pain management. We previously discovered a minimum pharmacophore for the B2R that was found to reverse thermal hyperalgesia and mechanical hypersensitivity when administered intrathecally in nerve-injured rats (spinal nerve ligation). Based on its structure, this pharmacophore is susceptible to degradation by peptidases. In an effort to improve the peptide stability, modified Dyn A analogs were designed. These modifications showed improved stability and gave us further insight into the interaction of Dyn A analogs with the B2R. This research was supported by grants from the National Institute on Drug Abuse of the NIH (P01DA006284 and R01DA013449). SMH was supported by a Marshall Dissertation Fellowship. None of the authors have a conflict of interest.

27. Collybollides are highly selective and potent agonists of kappa opioid receptors

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Ligands selective for the κ opioid receptor (κOR), both agonists and antagonists, have a potential to be developed as therapeutics in the treatment of psychiatric and neurological disorders as well as obesity. Salvinorin A, a non-nitrogenous diterpene derived from the hallucinogenic mint *Salvia divinorum*, is a highly selective and potent κOR agonist. The structure of Salvinorin A has a furyl-δ-lactone motif; this motif is also present in collybolides, sesquiterpenes extracted from the fungus *Collybia maculata*. We therefore examined whether collybolide and its sesquiterpene diastereoisomer, 9-*epi*-collybolide function as opioid receptor ligands. Competition binding assays as well as functional and trafficking assays carried out with human embryonic kidney cells expressing either μ, δ or κ opioid receptors show that collybolide, and to a lesser extent 9-*epi*-collybolide, is a highly selective κ opioid receptor agonist. Interestingly, a hemisynthetic epimer of collybolide at the level of its furan group, 7-*epi*-collybolide, exhibits inverse agonist activity. Taken together our results demonstrate that collybolides represent novel κOR agonists that could be developed as therapeutics in the treatment of drug addiction, mood disorders or pain. This work was supported in part by NIH grants DA008863 and NS026880 (to LAD.) and E.N.B. is supported by NIDA training grant (DA007135).

28. AMPA receptor positive allosteric modulators (PAMs) attenuate opioid tolerance, dependence, and opioid induced-hyperalgesia

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Development of opioid tolerance, dependence, and paradoxical hyperalgesia hinders the use of opioids for the treatment of chronic pain. It is reported that antagonists of a-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) receptors attenuate morphine tolerance and dependence. In this study, we found that positive allosteric modulators (PAMs) of AMPA receptors can also attenuate acute morphine antinociceptive tolerance and physical dependence. In mice treated with morphine (100mg/kg s.c.), acute morphine tolerance and dependence developed in 4-6 h. Treatment with aniracetam, an AMPA PAM, was able to prevent the development of morphine antinociceptive tolerance and physical dependence (86.1 ± 4.9% v.s. 16.7 ± 5.1% MPE, $p<0.001$ compared with morphine tolerance group). Aniracetam also partially reversed the established acute morphine tolerance and dependence in mice (57.1 ± 12.6% MPE, $p<0.01$). In addition, Aniracetam was effective in blocking established opioid-induced hyperalgesia (0.62 ± 0.08g vs. 0.03 ± 0.02g; $p<0.001$ for mechanical allodynia and 10.36 ± 0.82s v.s. 4.95 ± 0.34s; $p<0.001$ for thermal hyperalgesia, compared with chronic morphine group). These data suggest that positive allosteric modulators of AMPA receptors can attenuate morphine tolerance, dependence, and opioid-induced hyperalgesia.

29. Presence of spontaneous sensory hypersensitivity in mice lacking *OPRM1*

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The μ opioid receptor (MOR) is necessary to mediate the analgesic and addictive effects of opioids. Taking advantage of the MOR knockout mouse model, this study aimed to examine the functional involvement of MOR in the endogenous pain modulation circuits, by characterizing spontaneous and evoked pain behaviors in mice lacking the functional μ opioid receptor gene (*OPRM1*). We found MOR-null mice did not produce significant changes to the locomotor coordination in the rotarod test. Both the wild-type and MOR-null mice exhibited normal activities and movements in open field exploration test. MOR-null mice displayed nociceptive responses to innocuous mechanical and noxious thermal stimuli, indistinguishable from those in the wild-type littermate mice, indicting the absence of evoked sensory hypersensitivity in

MOR-null mice. We previously validated a method of employing negative reinforcement to study spontaneous pain in mice and applied the conditioned place preference (CPP) paradigm in the present study to investigate spontaneous pain in MOR-null mice. After a single trial conditioning, (clonidine: 1 µg in 5 µL of saline, *i.t.*), MOR-null mice spent significantly more time in clonidine-paired chambers than in saline-paired chambers. In contrast, clonidine did not elicit CPP in the wild-type control mice. These data suggest the presence of stimulus-independent spontaneous pain in the MOR-null mice, supporting a physiological role of the endogenous opioid system in tonic pain suppression. None of the authors has a conflict of interest related to this research.

30. The involvement of free fatty acid receptor GPR40/FFA1 signaling on the development of chronic pain

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We have demonstrated that the activation of the G protein-coupled receptor 40/free fatty acid receptor 1 (GPR40/FFA1) signaling pathway may play an important role in the regulation of the descending pain control system. Here, we examined the involvement of supraspinal GPR40/FFA1 signaling in the development of chronic pain. We used a complete Freund's adjuvant (CFA)-induced inflammatory chronic pain and postoperative pain mouse model. Mechanical allodynia and thermal hyperalgesia were evaluated using von Frey filaments and plantar test, respectively. Long-lasting hyperplasia of paw, persistent mechanical allodynia and thermal hyperalgesia were elicited in CFA-treated mice. The intracerebroventricular (i.c.v.) injection of docosahexaenoic acid (DHA) (50 µg) and GW9508 (1.0 µg), a GPR40/FFA1 agonist, significantly reduced mechanical allodynia and thermal hyperalgesia at day 7, but not at day 1, after CFA injection. These effects were inhibited by the i.c.v. pretreatment with GW1100 (10 µg), a GPR40/FFA1 antagonist. Furthermore, The repeated GW1100 treated mice significantly increased phosphorylated ERK (p-ERK) in the spinal cord after low threshold touch stimulation, and exacerbated postoperative pain-induced mechanical allodynia compared to vehicle treated mice. Our findings suggest that the activation of the supraspinal GPR40/FFA1 signaling might provide valuable information regarding a novel therapeutic approach for pain control.

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31. Enhancement of opioid-mediated antinociception by the mu-opioid receptor positive allosteric modulator, BMS-986122.

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We recently described positive allosteric modulators (PAMs) of the mu-opioid receptor. Such compounds bind to a site on the mu-receptor that is distinct from the orthosteric site for endogenous opioid peptides and opioid drugs. Mu-receptor PAMs could be analgesics themselves and/or enhance the action of traditional opioid agonists and thereby reduce the level of opioid drug required to afford pain relief. In cells expressing the mu-receptor, BMS-986122 alone did not activate GTPgamma(35)S binding or recruit beta-arrestin. However, BMS-986122 enhanced the binding affinity and/or potency of mu-opioid receptor orthosteric agonists in a probe-dependent manner with the most robust increase in affinity being seen with R(-)-methadone; BMS-986122 also showed a robust increase in the potency of R(-)-methadone to stimulate GTPgamma(35)S binding in mouse brain homogenates. Consequently, we sought to examine the effect of BMS-986122 in vivo. Using the hot-plate assay we observed that BMS-986122 given intracerebroventricularly (i.c.v.) caused a substantial increase in the antinociceptive activity of R(-)-methadone given i.c.v. or intraperitoneally. At higher doses BMS-986122 alone (i.c.v.) afforded a short-lived antinociception that was completely prevented by pretreatment of the mice with naloxone or the selective mu-antagonist beta-FNA. In addition, BMS-986122 promoted swim-stress induced antinociception. These studies are the first to demonstrate that mu-opioid receptor PAMs enhance the antinociceptive activity of opioid drugs and suggest the PAMs may also increase endogenous opioid peptide activity. Supported by the National Institute on Drug Abuse of the National Institutes of Health (T32 DA07268). The authors report no conflict of interest.

32. Exploring the contribution of the anterior cingulate cortex in opioid-sensitive descending pain modulatory circuitry

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Neuropathic pain is a complex, multidimensional phenomenon consisting of sensory and affective components. Opioids such as morphine are effective for moderate to severe pain, but have many harmful side effects. There is a

growing, urgent medical and societal need to elucidate the mechanisms and opioid-sensitive circuits by which pain and pain relief is modulated. Cortical regions may contribute to descending pathways modulating pain through projections to the peri-aqueductal gray (PAG) and the rostral ventromedial medulla (RVM). Here, we explore the effect of morphine injection at different regions of the ACC and its effect on tactile hypersensitivity in a rodent model of neuropathic pain. Adult, male Sprague-Dawley rats received nerve injury by ligation of the L5/L6 spinal nerves (SNL injury). Evoked tactile hypersensitivity was evaluated following ACC and RVM morphine injections. Rats with SNL developed tactile hypersensitivity that was not reversed by morphine injection into various region of the ACC. In contrast, morphine injection in the RVM showed increased evoked thresholds in SNL animals, as demonstrated previously. We have shown that sites in the rACC can modulate the affective components of neuropathic pain without altering the sensory component. The overarching goal of this research is to understand the nature of the neuroanatomical contributions of the ACC and its role in descending pain modulatory pathways. Further studies exploring other sites within the ACC may ultimately lead to a better understanding of opioid-sensitive circuitry. Supported by DA034975. The authors have no conflict of interest.

33. Accelerating basic research and drug discovery (from hundreds of compounds to trillions!)

Richard Houghten, Torrey Pines Institute for Molecular Studies

Over the past 25 years, Torrey Pines Institute has generated very large mixture-based peptide combinatorial libraries (10s of thousands to millions, billions and even trillions of compounds) that are systematically arranged allowing for the screening of these libraries in virtually any assay that is capable of low to medium throughput. Essentially, these libraries allow for high throughput screening of millions of compounds using an exponentially lower number of samples required to be screened. The Torrey Pines platform technology of highly diverse libraries and deconvolution strategies, have been utilized in dozens of *in vitro* assays to produce highly potent and specific individual compounds. More recently, these libraries have been screened directly in disease-relevant animal models with the hypothesis that this approach will yield more “advanced” therapeutic candidates, decreasing both the time and costs inherent in the drug discovery process. Our libraries are now being used in a robust program that blankets the State of Florida in a program known as the “Florida Drug Discovery Acceleration Program”. Examples of the successes we have had in pain modulation will be described in detail.

34. Impact of benzodiazepine use on retention and compliance, relapse, and safety in buprenorphine-maintained patients: A Literature Review.

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Benzodiazepine use is prevalent among buprenorphine-maintained patients. Our objective was to assess the impact of benzodiazepine use on compliance, retention, resolution, and safety in patients being treated with buprenorphine for opioid use disorders. We searched Ovid MEDLINE PubMed, OneSearch, and Science Direct. We did not use any date or language restrictions in the electronic searches for trials. We included all studies pertaining to buprenorphine with concurrent benzodiazepine use published between January 2002 and March 2015. The primary outcomes were compliance with treatment and relapse to illicit opioid use. Our secondary outcome was safety. We included 7 trials in this review; 4 trials were conducted in the USA, 1 in Norway, 1 in Finland, and 1 in England. Patients actively using benzodiazepines relapsed more often although one study contradicted these findings. There was a statistically significant increase in patients who tested positive for benzodiazepines and non-compliance with treatment. For issues of safety, we found that lethal overdose would be unexpected to occur in the context of controlled oral co-administration of therapeutic dosages of benzodiazepines and buprenorphine. Our findings for buprenorphine and concurrent IV benzodiazepine use was consistent with previous literature with a more than three-fold increased odds of accidental injury compared to those without a benzodiazepine prescription, with the greatest odds of injury among females. Benzodiazepine use was also associated with slowed sensory processing and psychomotor impairment. Our review confirms that benzodiazepine use among buprenorphine-maintained patients is associated with a greater risk of relapse, poor compliance, and safety concerns. There was no conflict of interest related to this research.

35. Interleukin-1 signaling in the basolateral amygdala mediates heroin-conditioned immunosuppression

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Studies in our laboratory have demonstrated that the immunosuppressive properties of heroin can be conditioned to a specific environmental context (CS), resulting in a previously neutral stimulus eliciting immunosuppressive effects. Moreover, we have shown that the nucleus accumbens shell (NAcS) and basolateral amygdala (BLA) are important neural substrates that mediate this effect. Recently, we began investigating the mechanisms that mediate heroin-conditioned immunosuppression within these regions. Cytokines have been traditionally thought of as peripheral immune molecules, but, in fact, they also serve as neuromodulators in the brain. For example, studies suggest that central interleukin-1 (IL-1) has an important modulatory role in associative learning. The present study investigated the role of IL-1 signaling within the BLA and NAcS in the expression of heroin-conditioned immunosuppression. Rats received five, 60-minute conditioning sessions, during which heroin was administered prior to placement into the CS. On test day, rats received microinfusions of interleukin-1 receptor antagonist (IL-1Ra) or saline into the BLA or NAcS 30 minutes prior to CS re-exposure. Following

re-exposure, rats received systemic lipopolysaccharide treatment to induce an immune response. Our findings demonstrate that bilateral administration of IL-1Ra into the BLA, but not the NAcS, attenuated nitrate/nitrite levels in blood plasma and expression of inducible nitric oxide synthase in spleen tissue following re-exposure to the CS, indicating IL-1 signaling in the BLA, but not the NAcS, plays an important role in heroin-conditioned immunosuppression. Supported by the National Institute on Drug Abuse (DA034721). None of the authors have a conflict of interest related to this research.

36. Blockade of dopamine D3 receptor by YQA14 attenuates methamphetamine-induced behavioral sensitization and conditioned place preference in mice

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Drug addiction is characterized by high rate of relapses to drug use after abstinence. The neural mechanisms underlying high vulnerability to relapse are incompletely understood. We have recently reported that blockade of dopamine (DA) D3 receptors by YQA14 attenuates cocaine reward and relapse to drug-seeking. In the present study, we investigated whether YQA14 similarly inhibits methamphetamine (METH)-induced locomotor sensitization and conditioned place preference (CPP) in mice. Here we report that chronic administration or a single injection of YQA14 (6.25, 12.5 and 25 mg/kg, i.p., 20 min prior to METH) significantly inhibited the acquisition and expression of METH-induced locomotor sensitization. In contrast, chronic administration of YQA14 did not alter the acquisition, while a single injection of YQA14 significantly attenuated the expression of METH-induced CPP. In addition, chronic administration of YQA14 also facilitated extinction and decreased the reactivation of METH-induced CPP. These findings suggest that brain D3 receptors are critically involved in METH's rewarding and psychomotor-stimulating effects. Thus, YQA14 deserves further studies as a potential medication for METH addiction. Support by Natural Science Foundation of China (Grant No.81102425), Project of National Science and Technology Support Program in China (2012BAI01B07), Beijing Nova Program xx2014A014, Key project of Natural Science Foundation of Beijing (7131010) and the National Basic Research Program of China (Grant No. 2009CB522008). None of the authors has a conflict of interest related to this research.

37. An opioid agonist/NK1 antagonist inhibits nociception in an animal model of neuropathic pain while lacking positive reinforcement.

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Chronic pain affects approximately 100 million Americans. Opioids are the mainstay therapy for the treatment of chronic pain. While physicians and patients alike are apprehensive about using opioids due to their side effects including respiratory depression and addiction, 259 million opioid prescriptions were written in 2012. Although opioids are the best available analgesics, they increase both positive and negative reinforcement, ultimately leading to addiction. The pro-nociceptive neurotransmitter substance p can potentiate positive reinforcement, suggesting that neurokinin-1 receptors (NK1R) located on dopaminergic neurons originating in the ventral tegmental area (VTA) of the midbrain and projecting to the nucleus accumbens (NAc) may play a role in opioid reward. Here, we show that a multivalent pharmacophore, TY032, acting as both an opioid receptor agonist and NK1R antagonist inhibits preclinical neuropathic pain without increasing extracellular dopamine in the nucleus accumbens. As tested by an infrared (IR) thermal nociception assay and von Frey filament probing, intrathecal administration of TY032 dose dependently attenuates thermal hypersensitivity and mechanical allodynia for 3 hours post injection. TY032 microinjection into the VTA did not increase extracellular dopamine release in NAc dialysate. These data indicate dual targeting of the dopamine reward circuitry may be an effective therapy with limited abuse liability warranting further investigation into how substance p signaling affects opioid induced reward in the VTA. Research supported by University of Arizona departmental funds. No conflict of interest exists.

38. Chronic pain modulates the molecular function of the opioid-reward network

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Pain is a multidimensional experience with both sensory-discriminative and motivational-affective components. The mesolimbic dopaminergic system, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is a crucial mediator of the reinforcing effects of μ-agonists. We previously demonstrated that the release of dopamine in the NAc after morphine treatment was markedly suppressed by sciatic nerve ligation. miRNAs are small, noncoding RNA molecules that direct the post-transcriptional suppression of gene expression and play an important role in regulating synaptic plasticity. In the present study, we found that sciatic nerve ligation induced a drastic decrease in the expression of miR200b and miR429 in NAc neurons. Under the tonic activation or suppression of VTA-NAc dopamine neurons by an optogenetic technique, the expression of miR200b and miR429 in the NAc was altered. Along with these changes in miRNAs, the expression of DNA methyltransferase 3a (DNMT3a), which is one of the predicted targets of miR200b/429, was significantly increased in the NAc after sciatic nerve ligation. To identify the methylated gene that is associated with this increase in DNMT3a, MBD2-seq was performed in the NAc after sciatic nerve ligation. As a result, we found some

candidate genes that were decreased and highly methylated. They included genes for intracellular enzymes that are related to the cAMP signaling pathway. These findings suggest that chronic nociceptive stimuli induce an epigenetic/post-transcriptional modification along with an attenuation of the "opioid-associated motivation/valuation circuitry".

39. U50,488H requires truncated 6 transmembrane variants of the mu opioid receptor for analgesia but not side effects

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The mu opioid receptor (MOR) contains two independent promoters associated with exon 1 and exon 11. Most exon 11 variants are truncated 6 transmembrane (TM) receptors, lacking the first TM domain encoded by exon 1. We previously reported that certain mu analgesics and the novel opioid IBNtxA require exon 11 variants for analgesia. Here, we report that the kappa opioid receptor selective analgesic U50,488H also requires exon 11 variants for analgesia, but not other actions. In contrast to wildtype controls, U50,488H failed to elicit significant antinociceptive responses in mice lacking exon 11 variants (E11 KO) following systemic, supraspinal, and spinal administration. Restoring expression of the 6TM variant mMOR-1G via lentivirus injection either spinally (i.t.) or supraspinally (i.c.v.) rescued U50,488H analgesia, confirming a role of this 6TM variant in its analgesia. Aversion and hypolocomotion are prototypical side effects of kappa agonists. Unlike analgesia, the conditioned place aversion and suppression of locomotor activity produced by U50,488H in wildtype mice were unchanged in E11 KO mice. Together, our findings demonstrate that U50,488H analgesia is dependent upon a truncated 6TM mu receptor variant and that this analgesia is separable from prototypical KOR side effects in an exon 11-dependent manner.

40. Analysis of changes in the expression of opioid- and dopamine-related genes in Parkinson's disease specific-iPS cells derived-dopaminergic neuron

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iPS cells (iPSCs) are pluripotent cells which give rise to all cells in the organism. A potential solution is to utilize reprogramming technology to generate disease-specific iPSCs. iPSC derived from patients with Parkinson's disease can then be differentiated to neurons for disease modelling. In the present study, we generated iPSCs from two Parkinson's patients and two control subjects. All of the clones differentiated into neurons included tyrosine hydroxylase-positive neurons. Under the present condition, we performed the comprehensive gene expression analysis of dopaminergic neurons derived from control iPSCs or Parkinson's disease specific-iPSCs. We found significant differences in the expression of several dopamine-related genes between control and patients. Among those, the expression level of COMT was dramatically increased in Parkinson's disease specific iPSC-derived dopaminergic neurons. Unlike dopamine-related genes, expression levels of opioid-related genes, such as μ-opioid receptor (OPRM1), δ-opioid receptor (OPRD1), κ-opioid receptor (OPRK1), proopiomelanocortin (POMC), prodynorphin (PDYN) and proenkephalin (PENK) were not changed in Parkinson's disease specific iPSC-derived dopaminergic neurons. Subsequently, we profiled DNA methylation in Parkinson's disease specific iPSCs and control iPSC-derived dopaminergic neurons using the Illumina Infinium HumanMethylation 450 BeadChips. We found differences in pattern of DNA methylation at the transcriptional start sites with CpG island of dopamine-, but not opioid-, related genes in Parkinson's disease specific iPSC-derived dopaminergic neurons. These findings suggest that change in the expression of dopamine-related genes with epigenetic modification in Parkinson's disease specific-iPSC-derived dopaminergic neurons could lead to neuronal dysfunction in Parkinson's disease.

41. Crucial role of K-opioidergic system for 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 system in tumor angiogenesis. The tumor angiogenesis is required for tumor progression with the highly expression of several activators such as VEGF. In the previous study, we demonstrated that the K opioid receptor agonist

could act as the novel endogenous angiogenesis inhibitor in vascular development. In the present study, we investigated whether K opioid receptor system could inhibit tumor angiogenesis in the process of tumor growth. We confirmed that treatment with K opioid receptor 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 K opioid receptor 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. These findings indicate that K opioidergic system may be involved in tumor angiogenesis. This knowledge could lead to a novel strategy for cancer therapy.

42. Endogenous opioid peptides and functional selectivity at the κ-opioid receptor

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The opioidergic system – composed of the μ,δ, K and NOP receptor subtypes and over 20 neuropeptides – modulates numerous animal behaviors including pain, analgesia, dysphoria, addiction and anxiety. Over the past 30+ years, pharmacologists discovered that 1) endogenous neuropeptides display low receptor selectivity between receptor subtypes, and 2) exogenous ligands can preferentially activate certain signaling pathways over others at the same receptor – a phenomenon defined as *functional selectivity*. However, the unique function of the numerous endogenous opioid peptides at a given receptor remains unclear. These studies investigate whether endogenous opioid peptides display functional selectivity. Our data demonstrate enkephalins and dynorphins differentially regulate two ubiquitous signaling modules – β-arrestin and G-protein – at the KOR. Dynorphin A and Dynorphin B swap potency rank orders for β-arrestin2 recruitment and GTPγS signaling, indicating two distinct signaling platforms are formed. Based on previously explored regulatory roles for β-arrestin and G-protein, further investigation probed whether receptor regulation was differentially regulated by the dynorphins and enkephalins. Dynorphin A and enkephalin treatment but not Dynorphin B treatment simulated JNK –a known regulatory kinase that can be scaffolded by β-arrestin. Furthermore, internalization studies showed additional changes in potency – where Dynorphin B stimulated more robust internalization than Dynorphin A. Thus, these *in vitro* assays show dynorphins and enkephalins differentially regulate signals at the KOR receptor. Observing distinct signaling and regulatory of Dynorphin A, Dynorphin B and the enkephalins *in vivo* is the major future goal of this work.

43. Changes in VTA-MOR function associated with opioid reward by neuropathic pain and chronic treatment with ethanol

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Morphine causes psychological dependence via activation of μ-opioid receptors (MOR) in the ventral tegmental area (VTA). However, μ-opioid receptor function is able to be changed under many of pathological conditions. In the present study, we investigated a possible change of VTA-MOR function and opioid reward in neuropathic pain and ethanol-dependent models. As a result, the morphine-induced place preference was significantly suppressed by sciatic nerve ligation, whereas it was enhanced by chronic treatment with ethanol. Using [35S] GTPgS binding assay, MOR-mediated G-protein activation in the VTA was significantly suppressed by sciatic nerve injury, while it was enhanced by chronic treatment with ethanol. These results suggest that alteration of VTA-MOR function by several pathological conditions may modulate the development of morphine dependence.

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44. Nalmefene: G-protein biased partial agonist of the kappa opioid receptor

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In vitro pharmacological studies of G-protein coupled receptor (GPCR) ligands have largely focused on G-protein mediated signaling endpoints (*e.g.* cyclic AMP levels). Recent indications that GPCR signaling can also proceed via non-G-protein mediated pathways, particularly β-arrestin mediated pathways, have advanced our understanding of ligand mediated signaling. Reexamination of the molecular pharmacology of receptor ligands in light of this advance is warranted. We previously demonstrated that nalmefene exhibits partial agonism of the kappa opioid receptor (KOP-r), utilizing prolactin as a biomarker of kappa agonist activity *in vivo* and GTP-gamma-S binding stimulation *in vitro* (Bart et al., 2005,

Neuropsychopharm., 30:2254-62), while also exhibiting antagonism of the mu (MOP-r) opioid receptors. Nalmefene has recently been approved for use in the European Union for treatment of alcoholism; both the antagonism of MOP-r and the partial agonism of KOP-r may contribute to its efficacy. We have compared the effects of nalmefene on KOP-r signaling via downstream G-protein mediated signaling (GTP-gamma-S binding) and arrestin recruitment. Consistent with our prior results, nalmefene exhibited partial agonism of stimulation of GTP-gamma-S binding. In contrast, investigation of ligand-directed beta-arrestin recruitment to KOP-r demonstrated that nalmefene functions as an antagonist, with minimal beta-arrestin recruitment, and full blockade of U69,593 arrestin signaling. Understanding the functional consequences of the differential signaling properties of nalmefene via KOP-r in relation to its *in vivo* effects will require further exploration.
The authors have no conflicts of interest. These studies were supported by the Robertson Development Fund and the Miriam and Sheldon Adelson Medical Research Foundation.

45. Involvement of the mesolimbic dopaminergic pathway in morphine-induced analgesia

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Morphine is frequently used for the treatment of cancer pain and moderate to severe non-cancer pain. It has been well documented that morphine analgesia is produced by activating descending pain modulatory system in brain stem and decreasing release of pain transmitter in the spinal dorsal horn. On the other hand, morphine induces rewarding behavior through the activation of the ventral tegmental area (VTA) dopamine neurons. VTA is the origin of the dopaminergic cell bodies of the mesolimbic dopamine pathway and is widely implicated in the reward, motivation and pleasure. VTA dopamine neuron projects to limbic structures, most prominently the nucleus accumbens (N.Acc.). The recent studies have shown that the reduction of neuronal activity in the N.Acc. under the neuropathic pain contributes to pain transmission. In this study, we investigated the role of the mesolimbic dopaminergic system in morphine-induced analgesia using by optogenetics and designer receptors exclusively activated by designer drugs (DREADD) system. We generated the transgenic mice expressing enhanced natronomonas pharaonis halorhodopsin (eNpHR) or Gi-coupled human muscarinic M4 DREADD (hM4Di) protein under the control of the tyrosine hydroxylase promoter. The eNpHR is activated by yellow light illumination and leading to neuronal inhibition. The hM4Di is activated by designer drug, clozapine N-oxide (CNO) and leading to neuronal inhibition. Optical suppression of VTA dopamine neurons significantly inhibited morphine-induced analgesia. As well as the result of optogenetics, CNO-induced suppression of VTA dopamine neurons also inhibited morphine-induced analgesia. These findings suggest that mesolimbic dopaminergic pathway may, at least contribute to morphine-induced analgesia.

46. Development of a combination Heroin-HIV vaccine

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There is a high prevalence of HIV-1 infection in injection drug users. We are developing a combination heroin-HIV vaccine to address this global health problem. The heroin component of the vaccine was developed by conjugating a chemically stable heroin/morphine hapten (MorHap) to tetanus toxoid (TT). To demonstrate the feasibility of a combination heroin-HIV vaccine, the TT-MorHap conjugate was mixed with a recombinant HIV envelope antigen, CN54 gp140. Alternatively, MorHap was directly conjugated to gp140 at varying hapten densities of 20-60 MorHap/gp140 molecule. Mice were immunized with 3 doses every 3 weeks using formulations containing these vaccines mixed with liposomes containing monophosphoryl lipid A as an adjuvant. The magnitude and durability of serum antibodies and inhibition of heroin-induced antinociception after subcutaneous heroin (1 mg/kg) challenge was assayed. The combination vaccines elicited high titer and durable antibodies to MorHap (~1 mg/mL) comparable to that elicited by TT-MorHap alone. Antibodies to HIV Env for the TT-MorHap+gp140 mixed vaccine and gp140-MorHap conjugate with low MorHap density of 20 were comparable to that obtained by gp140 alone (~3 mg/mL). The Env antibodies decreased for gp140-MorHap conjugates with higher MorHap densities. All mice immunized with the dual vaccines showed inhibition of heroin-induced antinociception with a % maximal potential effect (%MPE) ranging from 15-40, while MorHap-TT immunized animals had a %MPE of 8. The most promising formulations for a combination heroin-HIV vaccine were TT-MorHap mixed with unconjugated gp140 or gp140-MorHap with low MorHap density. Both induced high antibody titers to gp140 and protected mice from heroin challenge.

47. Polymorphisms in the mu opioid receptor (*OPRM1*) and monoamine genes associate with pain in sickle cell disease

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Pain is pervasive throughout a sickle cell disease (SCD) patient's life and effective pain management is suboptimal. SCD pain is heterogeneous and identifying the mechanisms that underlie this variation may aid in new therapeutic advances and management. As opioid analgesics are the mainstay in adult SCD pain therapy, candidate polymorphisms in the OPRM1 and monoamine genes have been collected for our study. Adult and pediatric sickle cell patients were recruited during routine outpatient clinic visits where blood and buccal swab samples were collected for DNA extraction and genotyping. Association studies are performed for candidate gene polymorphisms by using phenotype data gathered from PAINReportIt®, a pain assessment tool. Composite pain index (CPI) score is a marker for baseline pain and represents the multidimensional pain experience. Utilization is defined as admissions to the emergency department and/or the acute care center resulting from a sickle cell pain crisis and is a surrogate marker for acute pain in SCD. We found associations of acute and chronic SCD pain with SNPs in *OPRM1* and monoamine genes including dopamine receptor D3 (DRD3), adrenergic receptor B2 (ADRB2) and catechol-O-methyltransferase (COMT). Rs1799971 (Asn40Asp) G allele is associated with an increase in utilization rate (p=0.003) in an additive negative binomial regression analysis including covariates (ethnicity, CPI, sickle cell genotype, age, sex). Polymorphisms in the mu opioid receptor and monoamine neurotransmitter system genes may help to elucidate the variability seen in pain and opioid therapy in SCD and aid in the development of precision medicine.

48. Truncated six transmembrane mu opioid receptors mediate opioid analgesia

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Potent opioid analgesics lacking side-effects may be possible by targeting truncated six transmembrane (TM) mu receptors generated from alternative splicing of the mu opioid receptor (*Oprm1*) gene. IBNtxA (3-iodobenzoyl-6β-naltrexamide) is a potent analgesic against thermal, inflammatory and neuropathic pain and fails to produce the typical side effects associated with traditional opiates. IBNtxA analgesia is lost in an exon 11 knockout mouse lacking a set of 6TM truncated mu receptors, suggesting it acts through the 6TM truncated mu receptors. In the current study we demonstrate that truncated 6TM splice variants, including mMOR-1G, mMOR-1M and mMOR-1N, can rescue IBNtxA analgesia in a complete mu opioid receptor knockout mouse lacking all *Oprm1* splice variants due to disruptions of both exons 1 and 11. No mu opioids were active in these mice. A lentivirus containing the 6TM variant mMOR-1G or mMOR-1M or mMOR-1N administered intrathecally restored IBNtxA, but not morphine, analgesia, confirming that the truncated 6TM variants are both necessary and sufficient for IBNtxA analgesia. RT-PCR and immunohistochemistry confirmed expression of the lentivirus in the spinal cord. The lentivirus expressing mMOR-1G also restored 125I-IBNtxA binding to the 6TM-associated targets. Interestingly, U50,488H analgesia was also lost in our complete MOR-1 KO mouse, and rescued by the 6TM variants, suggesting involvement of the 6TM variants in U50,488H actions. Furthermore, we demonstrated that buprenorphine analgesia was mediated through both truncated 6TM and full-length 7TM mu receptors by the lentivirus studies in both complete MOR-1 KO and exon 11 KO mice. (Supported by DA13997 & DA029244 (Y.-X.P) and DA02615 & DA07242 (G.W.P) from the National Institute on Drug Abuse; and a core grant CA08748 from the National Cancer Institute to MSKCC)

49. Involvement of AMPA receptors in the antidepressant-like effects of the delta opioid receptor agonist SNC80

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Delta-opioid receptor agonists produce rapid antidepressant-like effects in a number of animal models. However, the mechanisms underlying these behavioral effects are unknown. The present study investigated the role of glutamatergic AMPA receptors in the antidepressant-like effects of the delta opioid agonist SNC80. The AMPA receptor antagonist 2,3-dihycdroxy-6-nitro-7-sulfoamoylbenzo(f)quinoxaline (NBQX) (0, 10, 18 mg/kg i.p.) was administered 15 min prior to SNC80 (s.c.). SNC80-induced antidepressant-like effects were evaluated 60 min later in the forced swim test in rats. In addition, locomotor activity immediately following SNC80 administration was measured with and without NBQX pretreatment. SNC80 significantly decreased immobility in the forced swim test, indicating an antidepressant-like effect. These rapid antidepressant-like effects of SNC80 were attenuated by NBQX in a dose-dependent manner. In addition, SNC80-stimulated locomotor activity was partially blocked by NBQX. These findings suggest that downstream AMPA receptor activation may play an important role in the antidepressant-like effects of SNC80 as well as other behavioral effects. This potential mechanism of action is consistent with mechanisms thought to be important in the fast-acting antidepressant effects of ketamine. Future studies will probe other mediators involved in this pathway, such as changes in glutamate

receptor ratios and mTOR signaling. The authors have no conflicts of interest and this work was supported by start-up funds to EM Jutkiewicz.

50. Novel mixed-action mu-opioid receptor (MOR) agonist/delta-opioid receptor (DOR) antagonist that prevents stress-induced reinstatement of extinguished cocaine-seeking behavior

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We hypothesized that a mixed-action MOR agonist/DOR antagonist would produce analgesia with reduced liabilities, and prevent reinstatement of extinguished drug-seeking behavior. Accordingly, we examined two mixed opioid agonist/antagonists, (-)pentazocine and a novel cyclic peptide 1, in C57BL/6J and MOR -/- mice for opioid efficacy and selectivity in the 55oC warm-water tail-withdrawal assay, and determined each compounds' effects on the reinstatement of cocaine-seeking behavior in a conditioned place preference (CPP) assay. (-)Pentazocine and 1 demonstrated full efficacy in the tail-withdrawal assay, with ED50 (and 95% CI) values of 3.3 (2.8-3.9) and 20.2 (15.1-25.8) nmol, i.c.v., respectively. Compound 1 also produced significant antinociception after oral administration (18.0 (12.7-28.8) mg/kg, p.o.). Whereas (-)pentazocine antinociception was mediated by all three opioid receptors, the antinociception produced by 1 was primarily MOR mediated. Pretreatment (>2 h, i.c.v.) with either 1 or (-)pentazocine selectively antagonized DOR antinociception. Repeated treatment with 1 or (-)pentazocine did not result in significant acute opioid antinociceptive tolerance. Acute pretreatment with 1 (30 or 100 nmol, i.c.v.) produced conditioned place aversion, whereas higher doses of (-)pentazocine produced CPP in C57BL/6J (but not MOR -/-) mice. However, after pretreatment (>2 h), both compounds dose-dependently prevented stress-induced reinstatement in C57BL/6J mice. These data suggest the therapeutic value of DOR antagonists may expand beyond the treatment of alcoholism. Moreover, with its distinct opioid activity profile, the cyclic peptide 1 is a promising therapeutic for development as both an analgesic with reduced liabilities and as a maintenance medication to reduce relapse to cocaine-seeking behavior. Funding provided by NIDA (DA018832 and DA032928) and the State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development.

51. Assessment of morphine antinociception in male and female rats on a novel pain-suppressed wheel-running assay

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Although nociception in animals is typically assessed with an acute noxious stimulus, such pain-evoked tests do not mimic the disruptive effects of pain on daily life functions. Our objective was to use suppression of wheel-running in rats as a model of pain-suppressed behavior in humans. Although a number of other pain-suppressed tests have been developed, suppression of wheel-running is expected to be a useful model because wheel-running is a natural rodent behavior, assessment takes place in the rat's home cage (a low-stress environment), and measurements can be assessed 24 hours a day (which reflects the human pain experience). Importantly, there have been no studies examining females in pain-suppressed tests, despite the greater prevalence of chronic pain in women. Thus, we studied the effect of peripheral inflammation induced by intraplantar administration of Complete Freund's Adjuvant (CFA) on voluntary wheel-running in male and female rats. Both male and female rats reach stable levels of running within 4 days, although female rats ran significantly more than male rats. Administration of CFA into the right hindpaw suppressed wheel-running in both sexes, but running recovered faster in male compared to female rats. Administration of morphine (0.32-10 mg/kg, s.c.) had both sedative and antinociceptive effects that varied depending on the dose, time post-injection, and sex of the rat. These data indicate that suppression of wheel-running may be a clinically relevant method to assess nociception. Support provided by NIH/NIDA DA027625. Authors report no conflict of interest.

52. Development of a high-sensitivity method for the detection of human exposure to fentanyl and six fentanyl analogs in urine and plasma using liquid chromatography mass spectrometry

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Increasing reports of fentanyl abuse highlight the need for sensitive and specific test methods to detect human exposure to this class of potent analgesics. During pain management sensitive and rapid test methods are required to accurately measure fentanyls, and in cases of overdose and poisoning highly specific tests are needed to determine the exact fentanyl analog of exposure. A highly-sensitive method for the analysis of fentanyl, six analogs, and five metabolites, in both human urine and plasma was developed and validated using liquid chromatography tandem mass spectrometry and a single sample preparation approach. The high-sensitivity nature of this method is well suited to quantitate concentrations of fentanyls relating to both clinical and abuse scenarios. The limits of detection of target analytes in the validated method in urine and plasma range from 0.011 to 0.0038 ng/mL. Simulated patient and overdose samples were

analyzed to evaluate the performance of the method. This high-sensitivity method uses a single sample preparation technique for urine and plasma and is able to distinguish between multiple fentanyl compounds, which is required in poisoning cases when an initial positive test warrants confirmatory analysis to determine the exact fentanyl analog of exposure. Research was supported by the Centers for Disease Control and Prevention and the Oak Ridge Institute for Science and Education. None of the authors has a conflict of interest related to this research.

53. Structural insights into function and pharmacology of the δ-Opioid receptor ligands

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The breakthroughs in crystallography of G protein-coupled receptors have led to 3D structure determination for all four Opioid receptors (ORs). Among them, the structure of δ-opioid receptor in complex with naltrindole has been solved at highest resolution (1.8 Å), revealing not only atomic details of ligand interactions, but also description of tightly bound waters and the conserved sodium binding site at the center of 7TM (seven-transmembrane) helical bundle. Most recently, serial femtosecond crystallography technology also helped to determine the structure of human δ-OR bound to the bi-functional δ-OR antagonist and μ-OR agonist tetrapeptide DIPP-NH2. Ligands with such bi-functional μ-OR and δ-OR profiles are considered as potential therapeutic alternatives to alkaloid opiate analgesics with diminished side effects. Further computational analysis of these δ-OR complexes and other ORs helps to reveal structural basis for subtype selectivity, allosteric modulation, functional and pharmacological profiles of these receptors, providing a path to structure-based discovery of improved analgesics with reduced side effects. These studies are supported by the National Institute on Drug Abuse of the NIH grants DA038858 (V.K.), DA035764 (V.C., V.K., B.L.R. and R.C.S.) and DA004443 (P.W.S.), National Institute of General Medical Sciences grants U54 GM094618 (R.C.S., V.C., V.K.) and R01 GM108635 (V.C.), and the US National Institute of Mental Health Psychoactive Drug Screening Program (B.L.R.). None of the authors has a conflict of interest related to this research.

54. Preclinical development of Salvinorin A analogues for the treatment of drug-addiction

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There are currently no effective FDA approved treatments for psychostimulant abuse. Although drugs targeting the dopamine system have some anti-addictive properties, they also have unwanted side effects that limit their therapeutic use. Activation of the kappa opioid receptor (KOPr), which regulates the function of the dopamine system, may hold the key to designing better therapeutic compounds to treat addiction. Utilising the novel neoclerodane diterpene structure of Salvinorin A, we have identified two novel, long acting, selective KOPr agonists, 16-bromo-Salvinorin A and mesyl-Salvinorin B. Male rats trained to self-administer cocaine were subjected to extinction followed by acute administration KOPr agonist and drug-prime reinstatement testing performed. Bromo-Salvinorin A (1 mg/kg), and mesyl-Salvinorin B (0.3 mg/kg) significantly attenuated cocaine-seeking. We also show these compounds have reduced side effects compared to traditional KOPr agonists and do not cause sedation in locomotor tests, conditioned place aversion (mesyl-Salvinorin B) or depression in the forced swim test (bromo-Salvinorin A) or anxiety in the light/dark test and elevated plus maze (bromo-Salvinorin A, mesyl-Salvinorin B). Characterisation of these new ligands is expected to lead to new medications to treat drug dependence, as well as, new molecular tools for chemical biology. By linking analogue design with anti-addiction effects we will be able to generate better tolerated anti-addiction compounds. Support by the Neurological Foundation of New Zealand (BMK). AE received support from Victoria University of Wellington Postgraduate Scholarship. None of the authors has a conflict of interest related to this research.

55. Loss of morphine reward and dependence in mice lacking G protein–coupled receptor kinase 5

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The clinical benefits of opioid drugs are counteracted by the development of tolerance and addiction. We provide in vivo evidence for the involvement of G protein–coupled receptor kinases (GRKs) in opioid dependence in addition to their roles in agonist selective mu-opioid receptor (MOR) phosphorylation. In vivo MOR phosphorylation was examined by immunoprecipitation and nanoflow liquid chromatography–tandem mass spectrometry analysis. Using the hot-plate and

conditioned place preference test, we investigated opioid-related antinociception and reward effects in mice lacking GRK3 or GRK5. Etonitazene and fentanyl stimulated the in vivo phosphorylation of multiple carboxyl-terminal phosphate acceptor sites, including threonine 370, serine 375, and threonine 379, which was predominantly mediated by GRK3. By contrast, morphine promoted a selective phosphorylation of serine 375 that was predominantly mediated by GRK5. In contrast to GRK3 knockout mice, GRK5 knockout mice exhibited reduced antinociceptive responses after morphine administration and developed morphine tolerance similar to wild-type mice but fewer signs of physical dependence. Also, morphine was ineffective in inducing conditioned place preference in GRK5 knockout mice, whereas cocaine conditioned place preference was retained. However, the reward properties of morphine were evident in knock-in mice expressing a phosphorylation-deficient S375A mutation of the MOR. These findings show for the first time that MOR phosphorylation is regulated by agonist-selective recruitment of distinct GRK isoforms that influence different opioid-related behaviors. Modulation of GRK5 function could serve as a new approach for preventing addiction to opioids, while maintaining the analgesic properties of opioid drugs at an effective level. Support by Deutsche Forschungsgemeinschaft Grant Nos. SCHU924/11-2 and SCHU924/15-1 and National Institute on Drug Abuse Grant No. 1R01DA031442-01A1. None of the authors has a conflict of interest related to this research.

56. Chronic junk food consumption induces aberrant cue-induced food-seeking in rats.

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The pervasiveness of highly-palatable, energy-dense foods is considered a leading cause of obesity, which is a significant public health concern. While the idea that food is addictive is under debate, overeating shares many characteristics with drug addiction, such as compulsive pursuit in the face of dire health consequences. Cues associated with palatable foods (e.g., images, odors) can trigger food-seeking, even when sated, which could lead to decreased inhibitory control over food intake. Here, we explored if a junk food diet (i.e., “cafeteria diet;” CD) impacts the ability of animals to respond appropriately to food-paired cues in a Pavlovian-to-instrumental transfer test. Male rats were first trained to press a lever to receive a food reward, subsequently they learned to associate free delivery of the reward with an auditory cue. Rats were then exposed to either normal chow (Control rats) or chow *and* CD for either 2 hrs (Binge rats) or 24 hrs (All-Day rats) per day, for 1, 3 or 6 weeks. After this, all rats were switched to 2 hrs-per-day chow access for approximately 1 week prior to testing. At test, rats were sated for 1 hour on chow, and presented with a food-paired (CS+) and neutral (CSo) cue, and lever presses and food cup entries recorded. Control rats increased lever pressing and food cup entries at the onset of the CS+, but not the CSo, as expected. Binge rats increased lever pressing to both the CS+ and the CSo, though food cup entries increased only during the CS+. All-Day rats showed markedly reduced lever pressing and food cup entries to both cues. These results suggest that chronic junk food consumption induces atypical responding to environmental stimuli predictive of food rewards, and that different dietary access produces unique behavioral abnormalities. Specifically, Binge rats generalize the excitatory properties of reward-paired cues to other, neutral cues, while All-Day rats appear insensitive to the motivational properties of either cue. Together these studies emphasize that junk food diets induce aberrant food-seeking in response to environmental cues, and this action may contribute to maladaptive feeding behavior leading to obesity.

57. Tachykinin-opioid chimeric peptide in acute pain treatment in Wistar rats

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Morphine and its derivatives are the most widely used narcotic analgesics for relieving severe acute pain. Unfortunately, the use of opioids in treating is limited due to significant side effects e.g. analgesic tolerance, addiction, respiratory depression. Numerous endogenous components that participate in the formation, transmission, modulation and perception of pain signals offer new strategies for the development of new analgesic. Substance P (SP) is one of this, the undecapeptide Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2 is widely distributed throughout the central nervous system and is highly expressed on areas that are critical for the regulation of pain influx, affective behavior and stress. For activation of the receptor neurokinin-1 (NK-1) the C-terminal sequence of SP is essential. However, not all the effects of SP are mediated through the C-terminal fragment. N-terminal fragment, such SP(1-7) induces antinociception and desensitization of several SP-induced behaviors. SP(1-7) has been suggested to modulate the expression of opiate tolerance and withdrawal behaviors in rodents. In the present study, we have synthesized the chimeric compound containing two fragments N- and C-terminal functional domains of the endogenous opioid and the tachykinin SP, respectively. In our study we have shown that the relative balance of activity between opioid and tachykinin pharmacophores can produce different effects (pro-nociceptive, antinociceptive, or neutral). The presentation will focus on the way from an idea through synthesis to the application of the new tachykinin-opioid chimeric compound (AWL 3106) in acute pain treatment. Acknowledgments: This work was supported by the European Union Grant NormoLife

58. Biological activity of peptides with both, opioid agonist and tachykinin antagonist components

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Bi- or multifunctional drugs may have much improved potency due to synergic effect or may produce fewer side effects than compounds acting at a single target. Substance P plays an important role in pain signals generation and transmission from periphery to CNS. In contrast, opioids suppress pain signals mainly through suppression of substance P release. These mechanisms of neurophysiological actions were fundamentals for invention of hybrid compounds that act as both antagonists of substance P to reduce postsynaptic activation of NK1 receptors as well as opioid agonists to activate presynaptic opioid receptors that result in decrease substance P release. We developed a new chimeric analgesics in which opioid pharmacophore is covalently hybridized with tachykinin pharmacophore that positively modulate effects of the opioid part. Synergistic enhancement of opioid analgesia and/or decrease of unwanted side-effects should result from such hybridization. Therefore, to the list of already synthesized and characterized compounds presented in the literature we elaborated new series of compounds that combine peptide opioid pharmacophore (biphalin) with 3,5 bis-trifluoromethyl-benzyl derivative, responsible for antagonist at NK1 receptor. Opioid and tachykinin pharmacophores are connected by few kinds of linkers which are derivative of hydrazine. We will present pharmacological and analgesic properties of new bifunctional peptides, which exhibit affinity to opioid (μ and δ) and NK1 receptors. Acknowledgement: This work was supported by the Polish National Science Center (NCN) DEC-2011/03/N/ST5/04725.

59. Amphipathic non-opioid dynorphin a analogs to inhibit neuroexcitatory effects at central bradykinin receptors

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Nerve injury and inflammation cause up-regulation of dynorphin A (Dyn A), an endogenous opioid ligand, in the spinal cord resulting in hyperalgesia via the interaction with bradykinin receptors (BRs).1 This is a non-opioid neuroexcitatory effect that cannot be blocked by opioid antagonists. Our systematic structure-activity relationships study on Dyn A identified a lead ligand, LYS1044: [des-Arg7]-Dyn A-(4-11), along with the key structural feature (i.e. amphipathicity) for the BRs.2 Our lead ligand showed antihyperalgesic effect in nerve injured animals and inhibited non-opioid Dyn A-induced neuroexcitatory effects in naïve animals. The inhibitory effects may be localized to the CNS and thus there would be little cardiovascular effects in the periphery. In an effort to improve the metabolic stability of our lead ligand with retained biological activities, various modifications were performed and as the results, we were able to develop very stable Dyn A analogues that possess high therapeutic potential for pathological pain states via a novel mechanism of BRs in the CNS.

60. Mu opioid receptors hyperpolarize respiratory-controlling Kölliker-Fuse neurons

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Respiratory depression is the primary cause of death from opioid overdose. Opioid-induced respiratory effects include aspiration and difficulty swallowing, suggesting impairment of the upper airways. The pontine Kölliker-Fuse (KF) controls upper airway patency and regulates respiration, in particular the inspiratory/expiratory phase transition. Given the importance of the KF in coordinating respiratory pattern, the mechanisms of mu opioid receptor activation in this nucleus were investigated at the systems and cellular level. Injection of the mu opioid agonist DAMGO into the KF of anesthetized vagi-intact rats resulted in a decrease in respiratory drive. DAMGO applied directly into the KF of the *in situ* arterially perfused working heart-brainstem preparation of rat resulted in robust apneusis. Both of these effects were rapidly reversed by the opioid antagonist naloxone. In brain slice preparations, activation of mu opioid receptors on KF neurons hyperpolarized a distinct population (61 %) of neurons. In voltage-clamp recordings the outward current produced by the opioid agonist [Met5]enkephalin (ME) was concentration-dependent, reversed at the potassium equilibrium potential and was blocked by BaCl2, characteristics of a G protein-coupled inwardly rectifying potassium (GIRK) conductance. The clinically used drug morphine produced an outward current in KF neurons with similar potency to morphine-mediated currents in locus coeruleus brain slice preparations. Thus, the population of KF neurons that are hyperpolarized by mu opioid agonists are likely mediators of the opioid-induced loss of post-inspiration and induction of apneusis. Supported by DA08163 (JTW) and DA38069 (ESL). The authors have no conflict of interest to declare.

61. GABAergic transmission and reduced modulation by endocannabinoids in adult rat rostral ventromedial medulla (RVM) neurons following persistent inflammation

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Chronic pain is a major health issue that affects ~ 20% of the population worldwide and costs >$500 billion a year. RVM neuron activity is altered during persistent inflammation induced by complete Freund's adjuvant (CFA). Using whole-cell patch-clamp recordings from *adult* rat RVM slices, we find that GABA release in the RVM is significantly increased in rats pretreated with CFA. Endocannabinoids normally inhibit presynaptic GABA release in adult RVM so that superfusion of the cannabinoid receptor 1 (CB1) selective inhibitor rimonabant (SR141716, 3 μM) increases the frequency of GABAergic miniature inhibitory postsynaptic currents (mIPSCs) by 88 ± 31%. Interestingly, there is a reduction in endocannabinoid inhibition of GABA release following CFA treatment. SR141716 increased GABA mIPSC frequency by only 13 ± 9%. Further, we find that the inhibition of GABA release by the CB receptor agonist Win55,212-2 is reduced in CFA-treated (11 ± 6%) compared with naïve rats (40 ± 5%). In contrast to cannabinoid modulation of presynaptic GABA release, the mu-opioid receptor agonist DAMGO (1 μM)-mediated inhibition of GABAergic mIPSC frequency was not different in naïve (52 ± 6%) compared to CFA-treated (52 ± 10%) RVM neurons. Increased GABA release in the RVM reduces activation of descending pain inhibitory fibers resulting in increased nociceptive transmission from primary afferents. The loss of endocannabinoid modulation in the RVM may be a key mechanism in the hyperalgesia produced by persistent inflammation. Supported by NIH (DA027625 and DA035316 SLI) and American Heart Association (13SDG14590005, MHL). There are no conflicts of interest.

62. The neuroprotective effects of Memantine on methamphetamine-induced cognitive deficits

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Abuse of amphetamine-type stimulants, especially methamphetamine (METH), has become a global public health problem. Methamphetamine can not only lead to addiction, it can also cause severe neurotoxicity, which is closely associated with cognitive deficits. The present study was undertaken to understanding the mechanisms of METH-induced cognitive dysfunction, and determining whether memantine, a non-competitive antagonist of the NMDA receptor, can prevent the METH-induced cognitive deficits induced by METH. By using an object recognition animal model to test the non-spatial cognitive memory in mice, we found that administration of METH (4 mg/kg ×4, i.p., 2 h interval) abolished long-term memory of mice in recognition task, without affecting short-term memory at 1 week after treatment. We also found that METH administration induced neuron apoptosis in the medial prefrontal cortex (mPFC) at 72 h after injection, manifested as a reduced expression of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2). More importantly, METH-induced memory impairment was prevented by pretreatment with memantine (5 mg/kg, p.o.). The present study suggested that METH-induced cognitive deficits might be related to METH-induced apoptosis in mPFC, and memantine prevented METH-induced cognitive impairment through an anti-apoptotic mechanism. The effects of memantine on preventing neuron apoptosis in the mPFC need to be further investigated. Support by the Chinese National Nature Science Foundation (81271468, 81130020), The Ministry of Education (20120073110089), Shanghai city health and family planning committee (2014ZYJB0002), China Postdoctoral Science Foundation (2014M561482). None of the authors has a conflict of interest related to this research.

63. Role of PDGF receptor and its ligands in nociception in *Drosophila*

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Recent studies suggest that platelet-derived growth factor receptor-β (PDGFR-β)-mediated signaling plays a key role in morphine tolerance and resistance to morphine analgesia in neuropathic pain. However, the exact molecular mechanism of the PDGFR downstream signaling in regulating pain is unknown. We took advantage of *Drosophila*, a highly tractable genetic model of nociception to investigate whether the PDGFR-like receptor (Pvr) and its PDGF-like ligands (Pvf1, Pvf2, and Pvf3) regulate nociception. To determine if PDGF regulation of nociception is conserved we assayed thermal and mechanical baseline nociceptive responses in Pvr gain-of-function and Pvr and Pvf loss-of-function conditions. Tissue-specific Pvr hyperactivation and overexpression in neurons required for aversive responses to noxious thermal and mechanical stimuli showed that Pvr can induce thermal and mechanical allodynia and hyperalgesia. Larvae homozygous for hypomorphic loss of function alleles and transheterozygous for hypomorphic/null alleles of Pvr were hyposensitive to noxious thermal and mechanical stimuli. Interestingly, neuron-specific RNAi targeting of Pvr led to defects only in mechanical hyperalgesia. Finally, we observed that hypomorphic loss of function alleles of Pvf2, and Pvf3 showed a hyposensitive phenotype to both thermal and mechanical stimuli. These results indicate that PDGFR and its ligands are key mediators of different nociceptive responses in *Drosophila* and suggest that PDGFR and its ligands have evolutionarily related functions between flies and mammals. They also reveal that the ligands and receptor may be acting in different

cells types to differentially mediate mechanical and thermal nociceptive responses in *Drosophila*. NIH/NIDA grant R01DA36680, H.B.G. The authors declare no conflict of interest.

64. Role of G protein-coupled receptor kinases 2 and 3 in mu-opioid receptor desensitization and internalization.

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There is ongoing debate about the role of G protein receptor kinases (GRKs) in agonist-induced desensitization of the mu-opioid receptor (MOPr) in brain neurons (see Williams et al. 2013, Pharmacol Rev 15;223-254). In the present study we have used a novel, membrane permeable, small molecule inhibitor of GRK2 and GRK3, Takeda Compound 101 (Cmpd101), to study the involvement of GRK2/3 in acute agonist-induced MOPr desensitization. We observed that Cmpd101 inhibits the desensitization of the G protein activated potassium current (GIRK) evoked by receptor- saturating concentrations of Met Enkephalin, DAMGO, endomorphin-2 and morphine in rat and mouse locus coeruleus (LC) neurons. In LC neurons from GRK3 knockout mice Met Enkaphalin-induced desensitization was unaffected implying a role for GRK2 in MOPr desensitization. Quantitative analysis of the loss of MOPr function following acute agonist exposure revealed that Cmpd101 partially reversed MOPr desensitization. Inhibition of ERK, PKC or JNK did not inhibit the Cmpd101-insensitive component of desensitization. In HEK 293 cells Cmpd101 produced almost complete inhibition of DAMGO-induced MOPr phosphorylation at Ser375, arrestin translocation and MOPr internalization. Our data demonstrate a role for GRK2 (and potentially also GRK3) in agonist-induced MOPr desensitization in the LC, but leave open the possibility that another, as yet unidentified, mechanism of desensitization also exists. Support from the MRC [MR/J013269/1], BBSRC[BB/J003506/1], Wellcome Trust Value in People award and USPHS-NIDA grant RO1DA030074. None of the authors has a conflict of interest related to this research.

65. Agonist-induced nociception/orphanin FQ peptide receptor phosphorylation revealed by phosphosite-specific antibodies

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The nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is the most recently discovered and least characterized member in the opioid receptor family (MOR, KOR and DOR). NOP receptors are widely distributed across tissues and modulate several physiological processes by its endogenous ligand nociceptin The NOP receptor is a potential target for the development of ligands with therapeutic use in several pathophysiological states (e.g. chronic and neuropathic pain). Consequently, there is increasing interest in understanding the molecular regulation of NOP receptor. Recently, we generated phosphosite-specific antibodies directed against pS351 and pT362/pS363 and a phosphorylation-independent antibody. First results show that nociception, Ro64-6198, SCH221510 and MCOPPB induce a robust phosphorylation at both S351 and T362/S363, which can be blocked by the selective antagonist J113397. Buprenorphine and norbuprenophine failed to induce a phosphorylation at these sites. In the presence of nociceptin, S351 phosphorylation occurred at a faster rate than phosphorylation of T362/S363 indicating that S351 is the primary site of agonist-dependent phosphorylation. After activation of PKC by phorbol 12-myristate 13-acetate only S351 but not T362/S363 phosphorylation is increased indicating that S351 can also undergo heterologous PKC-mediated phosphorylation. Using NOPR-GFP knock in mice, we detected phosphorylation at S351 and T362/S363 *in vivo* after application of AT202. Together, these data provide new and quantitativ insight into the molecular regulation of NOP receptors *in vivo* and *in vitro*. None oft he authors has a conflict of interest releated to this research.

66. Heterologous regulation of agonist-independent μ-opioid receptor phosphorylation by protein kinase C.

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Homologous agonist-induced phosphorylation of μ-opioid receptor (MOR) is initiated at the carboxyl-terminal S375, followed by phosphorylation of T370, T376 and T379. In HEK293 cells, this sequential and hierarchical multi-site phosphorylation is specifically mediated by G-protein coupled receptor kinases 2 and 3. In the present study, we provide evidence for a selective and dose-dependent phosphorylation of T370 after activation of PKC by phorbol esters. We used a combination of phospho site-specific antibodies, kinase inhibitors and siRNA knockdown screening to identify kinases that mediate agonist-independent phosphorylation of MOR. In addition phospho site-specific antibodies were also used to study constitutive phosphorylation at S363 of MORs in mouse brain. Activation of PKC by phorbol esters or heterologous activation of substance P receptors co-expressed with MORs in the same cell induced a selective and dose-dependent phosphorylation of T370 that specifically requires the PKCα isoform. Inhibition of PKC activity did not compromise homologous agonist-driven T370 phosphorylation. In addition, S363 was constitutively phosphorylated in both cells and mouse brain. Constitutive S363 phosphorylation required ongoing PKC activity. When basal PKC activity was decreased,

S363 was also a substrate for homologous agonist-stimulated phosphorylation. Our results have disclosed novel mechanisms of heterologous regulation of MOR phosphorylation by PKC. These findings represent a useful starting point for definitive experiments elucidating the exact contribution of PKC-driven MOR phosphorylation to diminished MOR responsiveness in morphine tolerance and pathological pain. This work was supported by the Deutsche Forschungsgemeinschaft [SCH924/11-2 and SCH924/15-1]. None of the authors has a conflict of interest related to this research.

67. Pain-induced decrease in motivation is mediated by kappa opioid receptors in the nucleus accumbens

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During chronic inflammatory pain, the nucleus accumbens (NAc) undergoes long term changes affecting mood and rewarding properties of reinforcers. The opioid system is at least partially involved in these modifications and could explain the modification of reinforcing effects of natural rewards and drugs of abuse. Kappa opioid receptor (KOR) activation in the NAc leads to dysphoria and other aversive effects. These receptors also have a role in chronic pain and adaptations in KOR system could underlie pain-induced changes in motivation. We assessed this hypothesis by microinjecting norbinaltorphimine (NorBNI) directly into the NAc shell. Motivation to self-administer food was tested 48 hours after injection of complete Freund's adjuvant (CFA), using a progressive ratio schedule of reinforcement. We then measured KOR and prodynorphin expression together with KOR function, using GTPgammaS and neuronal activation, using cfos marker. Our data reveal an increase in both KOR expression and dynorphin A-stimulated GTPgammaS binding in the NAc Shell 48 hours after CFA administration. Furthermore we showed that blockade of these receptors reverses the pain-induced decrease in motivation for food reward. These results, together with the role of KORs in mediating the negative effects of long access and chronic exposure to alcohol and drugs of abuse, further support the hypothesis of kappa opioid system involvement in the suppression of reward/reinforcement circuitry during both addiction and pain processes. This study is supported by the NIH grants DA027460 and AA020394. None of the authors has a conflict of interest related to this research.

68. Neuropeptide FF (NPFF)-induced hyperalgesia and acute opioid antinociceptive tolerance is prevented by VBJ-192, a dual-activity mu-opioid receptor (MOR) agonist-NPFF receptor antagonist

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Tolerance limits the analgesic value of MOR agonists. NPFF mediates hyperalgesia and opioid-induced tolerance through the activation of NPFF1 and 2 receptors. We hypothesized that VBJ-192, a dual-activity MOR agonist/NPFF receptor antagonist in vitro, would produce antinociception with reduced tolerance. Repeated i.c.v. administration of two MOR agonists, morphine and DAMGO, was first characterized in a model of acute antinociceptive tolerance using the mouse 55oC warm-water tail-withdrawal assay. Whereas morphine demonstrated significant acute antinociceptive tolerance with a 9.6-fold rightward shift in potency upon a second administration 8 h later, the 2.5-fold rightward shift observed with DAMGO was not significant. NPPF-receptor mediation of opioid tolerance was confirmed in tests where initial co-administration of NPFF (10 nmol) with DAMGO (1.7 nmol) significantly increased the rightward shift in DAMGO dose-response 9.7-fold. Administration of NPFF alone was sufficient to significantly shift rightward (7.6-fold) the antinociception of DAMGO administered 8 h later. Conversely, initial co-administration of the NPFF-receptor antagonist RF9 (1 nmol) with morphine (3 nmol) prevented the rightward shift of antinociceptive potency produced by a second administration of morphine. Although VBJ-192 produced antinociception lasting up to 90 min (with an ED50 value of 6.88 (4.72-9.47) nmol, i.c.v.), it also dose-dependently prevented NPFF-induced hyperalgesia in a 48oC warm-water tail-withdrawal assay for an additional hour. Unlike morphine, VBJ-192 did not reduce respiration, and repeated administration did not demonstrate significant acute antinociceptive tolerance. Together, these results confirm the mediating effect of NPFF on opioid tolerance, and suggest the potential of dual-action opioid-NPFF ligands as analgesics with fewer liabilities.
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69. Agonist-selective multi-site phosphorylation of the µ-oipoid receptor regulates ß-arrestin recruitment

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Opioid drugs are the most potent analgesics, which are used in the clinic; however, by activating the µ-opioid receptor (MOR) they also produce several adverse side effects including constipation, antinociceptive tolerance, and physical dependence. There is substantial evidence suggesting that G protein-coupled receptor kinases (GRKs) and β-arrestins play key roles in regulating MOR signaling and responsiveness. Following phosphorylation by GRKs, β-arrestins bind to phosphorylated MORs, which prevents further interactions between the receptor and G proteins even in the continued

presence of agonist resulting in diminished G protein-mediated signaling. We have previously shown that agonist-induced phosphorylation of MOR occurs at a conserved 10-residue sequence, 370TREHPSTANT379, in the carboxyl-terminal cytoplasmic tail. Morphine induces a selective phosphorylation of serine375 (S375) in the middle of this sequence that is predominantly catalyzed by G protein-coupled receptor kinase 5 (GRK5). By contrast, high-efficacy opioids not only induce phosphorylation of S375 but also drive higher-order phosphorylation on the flanking residues threonine370 (T370), threonine376 (T376), and threonine379 (T379) in a hierarchical phosphorylation cascade that specifically requires GRK2/3 isoforms. To investigate this mechanism further, we have adapted a β-galactosidase complementation assay for β-arrestin1 and β-arrestin2. Using this assay, we were able to show that activation of MOR by high-efficacy agonists such as DAMGO results in recruitment of both β-arrestin1 and β-arrestin2, whereas activation by low-efficacy agonists such as morphine results only in detectable recruitment of β-arrestin2 but not β-arrestin1. The morphine-induced β-arrestin recruitment was strongly enhanced by overexpression of GRK2 or GRK3. Conversely, siRNA knock down of GRK2 or GRK3 strongly inhibits DAMGO-induced β-arrestin recruitment. Mutation of S375 to alanine strongly inhibited β-arrestin recruitment. However, mutation of all 11 carboxyl-terminal serine and threonine residues of MOR was required to completely abolish interaction with β-arrestin1 and β-arrestin2. None of the authors has a conflict of interest related to this research.

70. Small-molecule NOP agonists reduce alcohol reward in mouse models of alcoholism

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Studies in alcohol-preferring rats suggest that dysregulation of the nociceptin opioid receptor NOP and its ligand N/OFQ are a pathophysiological consequence of excessive alcohol intake. Further, administration of N/OFQ blocks the rewarding effects of alcohol in the conditioned place preference (CPP) paradigm in mice, as well as reinstatement of extinguished alcohol CPP, suggesting that NOP agonists may be a promising approach for treating alcohol addiction. We report the efficacy of small-molecule NOP agonists in reducing ethanol-induced CPP in mice. Mice tested for baseline place preference were subjected to three conditioning trials, during which they were treated subcutaneously with vehicle or a NOP agonist, followed by ethanol (2 g/kg) or saline and confined to the vehicle-paired or drug-paired chamber for 15 min. In the afternoon of each day, mice received the alternative treatment and confined to the opposite chamber for 15 min. On day 5, mice were tested for place preference. Among the compounds tested, selective NOP agonist AT-312 dose-dependently reduced ethanol-induced CPP. AT-312 alone had no motivational effects at the dose at which it blocked ethanol CPP. The inhibitory effect of the agonists on alcohol reward was completely abolished in mice lacking NOP. Together, these data suggest that AT-312 reduced alcohol reward via the NOP receptor and was devoid of any motivational effects. Studies are ongoing to assess effect of NOP agonists in the two-bottle-choice paradigm, which is widely used as a model of alcohol-taking behaviors and reinforcement. Authors declare no conflicts of interest. Support: Contract HHSN275201300005C.

71. NPYFa, dualsteric chimeric peptide of met-enkephalin and NPFF, prevents opioid induced tolerance

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Methionine-enkephalin-Arg-Phe (MERF) is a known endogenous amphiactive analgesic peptide. Neuropeptide FF (NPFF) is reported for long lasting analgesia, role in opioid modulation and tolerance development. Based on these reports a dualsteric chimeric peptide NPYFa (YGGFMKKKPQRFamide) was designed, having Met-enkephalin (opioid) and PQRF sequence of NPFF at C-terminal which can target both opioids and NPFF receptors. The aim of the present study was to determine opioid induced analgesia upon acute treatment and its tolerance development upon chronic exposure. NPYFa demonstrated early onset, dose dependent and prolonged anti-nociception. Antagonists (μ, κ and δ receptor) pretreatment studies alone or together and with NPFF receptors antagonist demonstrated κ-opioid receptors mediated anti-nociception. RF9, NPFF receptor antagonist exhibited additive effect to NPYFa acute analgesia, suggesting participation of NPFF receptors. In addition both Eu-GTP-γS binding assay and FACS analysis further corroborated the observed acute analgesia showing significant binding with KOR and NPFF2 receptors suggesting its multiple binding nature. Further chronic (6 days) treatment effect of NPYFa showed up-regulation of protein expression of these receptors suggesting no tolerance development to the NPYFa acute analgesia. Thus, NPYFa demonstrated potent, long lasting anti-nociception without tolerance development. Hence NPYFa may prove to be a potent analgesic probe with less tolerance development.

72. Morphine analgesic potency is higher in the affective versus the sensory component of formalin-evoked pain in male and female rats

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Sensation of pain is a multifaceted process that can be divided into sensory (somatic/discriminative) and affective (emotional) components. These components play unique and important roles in the pain experience. Therefore, we

performed a side by side examination of the analgesic effects of morphine in sensory and affective components of the formalin pain model in male and female rats. The sensory component of formalin-evoked pain was measured by assessing paw flinches across a 60 min testing session; whereas the affective component was measured using the conditioned place aversion (CPA) paradigm. Our preliminary findings suggest that male and female rats exhibited similar levels of formalin-evoked flinching behavior and CPA. Morphine treatment reduced the formalin flinching behavior and the CPA in a dose dependent manner. However, the formalin-evoked CPA was blocked with lower doses of morphine than those blocking the flinching response. These findings suggest a greater potency of morphine in blocking the affective versus the sensory component of pain in rats. Moreover, formalin-evoked c-Fos expression was measured in the superficial dorsal horn of the spinal cord. Systemic morphine treatment did not reduce the formalin-evoked c-Fos expression. These findings describe a critical difference in morphine's ability to reduce sensory and affective pain and highlight the importance of considering the affective component of pain when examining various pain types and analgesics. Supported by funds provided by the Western University of Health Sciences. The authors declare no conflict of interest.

73. Distinguishing brain circuits mediating pain relieving and addictive effects of systemic morphine

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Relief of ongoing pain is a natural reward that activates the mesolimbic dopamine (DA) reward pathway. Opiates such as morphine can relieve ongoing pain by reducing sensory nociceptive transmission, however, opiates act primarily by alleviating the affective features of pain (i.e., unpleasantness). Opiates also directly activate mesolimbic pathways, an effect associated with their addictive properties. Using a rat model of neuropathic pain we assessed motivated behaviors (conditioned place preference; CPP) and performed neurochemical analyses (*in vivo* microdialysis) to show that morphine's effects on modulation of pain affect (i.e., the anti-aversive effects) occur at doses that do not alter evoked sensory responses. Additionally, the anti-aversive doses of morphine did not activate reward circuits in uninjured animals, suggesting that the anti-aversive and addictive effects are mediated by different mechanisms. We hypothesized, that in injured animals, morphine acts by engaging opioid receptors in the anterior cingulate cortex (ACC), an area implicated in pain aversiveness, to alleviate the affective features of pain. Microinjection of morphine in the ACC elicited CPP and NAc DA release in neuropathic rats without affecting evoked hypersensitivity and without activating reward circuits in uninjured states. Moreover, blockade of opioid receptors in the ACC abolished the anti-aversive effects of intravenous morphine while the rewarding effects were not dependent on the ACC. Thus, the anti-aversive and rewarding effects of morphine can be dissociated both pharmacologically and anatomically. Supported by a grant from the National Institutes on Drug Abuse (DA034975). The authors have no conflict of interest.

74. Methadone-induced neuronal injury: a possible NMDA receptor-mediated mechanism

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Methadone is a long-lasting drug used as a therapy against opiate dependency and is a commonly used pain reliever for neuropathic pain and cancer. Despite its efficacy, little is known about the damaging effects caused by methadone in the brain. Methadone is known for its affinity to the endogenous opioid receptors, such as the μ-opioid receptor, however, it has also been suggested to act as a antagonist at the *N*-methyl-D-aspartate receptor (NMDA-R). The NMDA-R is a key receptor throughout the central nervous system, and activation is considered an underlying mechanism for several cellular phenomena, such as synaptic plasticity, long-term potentiation, and neuronal survival. In this study, we assessed the neurotoxic effects of the opioid receptor agonists methadone and morphine, and the NMDA-R antagonist MK-801 on mature primary cortical cultures using a cell viability assay. We found that both methadone and MK-801 were neurotoxic to cells in a dose-dependent manner. Morphine also induced cell death, but only at high concentrations. Notably, the opioid receptor antagonist naloxone (10 μM) was unable to inhibit the effects of methadone, suggesting that neurotoxicity may be mediated by the NMDA-R. These results led us to examine the potential neuroprotective effects of the endogenous peptide growth hormone (GH), which has been reported to improve cognitive function in patients chronically exposed to methadone. Our preliminary data suggests that GH may act as a neuroprotectant in cortical cells exposed to methadone. Support: Swedish Research Council & Kjell and Märta Beijer Foundation. The authors declare no conflict of interest.

75. A potent mu-opioid agonist that is inactive in tests of abuse liability

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For many years a large proportion of novel opioid discovery focused on developing safer analgesics by modifying the structure of opium derivatives. The failure of this approach is clear: Opioid addiction and overdoses are higher than ever, and chronic pain continues to be poorly treated. Endomorphins (EM) are endogenous ligands for the pain modulating mu-opioid receptor. EM analogs were developed with the goal of providing analgesic effects comparable to morphine with reduced side effects. In reward assays, EM analogs were not self-administered (SA), did not produce conditioned place

preference (CPP), and did not elicit locomotor sensitization (LS). Morphine promoted reward behaviors in all of these paradigms and produced dopamine neuron soma size reductions in the ventral tegmental area from these rats, while analog 4 did not. We further screened EM analogs in a drug discrimination (DD) model to test the substitution effects of EM analogs for morphine. Rats were trained to associate injections of morphine with one lever for food reward, while saline cued another lever for the food. During test sessions, rats dose-dependently responded on the drug-paired lever when pre-injected with EM analogs, indicating that rats perceive the analogs as being more similar to morphine than vehicle, even though the analogs were not self-administered. Morphine, but not analogs, impaired lever responding for food. Results indicate that EM analogs could substitute for morphine with less abuse potential. This suggests a potential therapeutic role for EM analogs in the treatment of pain and opioid addiction. Funded by VA, DOD, and ONR.

76. Characterization of the NOP receptor distribution using NOP-eGFP mice

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The NOP-N/OFQ system regulates a wide range of biological actions including pain. To better characterize the receptor localization in the brain, spinal cord and DRG, immunohistochemical experiments are conducted on a knock-in mouse that expresses NOP receptor C-terminal fused with eGFP. The current study demonstrates that NOP-eGFP is widely presented throughout the brain structures including the regions related to pain, as well as coexpressed with mu receptors in specific subpopulation of DRG and brain regions. In DRG, NOP-eGFP receptors are expressed on a number of myelinated neurons indicating that the receptors are mostly expressed in A fibers. Of which, there are a small number of medium myelinated-neurons that are mostly CGRP-, suggesting that they are not the typical peptidergic A nociceptors; implying the possibility that the receptors might be on myelinated low-threshold mechanosensory neurons. The receptors are also expressed on small unmyelinated-neurons that are likely either the typical peptidergic (CGRP+/mu+) or nonpeptidergic (IB4+) C-nociceptors, which are essential to heat or mechanical pain modalities. Additionally, strong immunoreactivity of NOP-eGFP receptors is also found in specific spinal laminae I-III, the regions important for the regulation of pain, itch and touch. Altogether our immunohistochemical characterization provides us with information regarding the cell types containing NOP receptors in DRG and the lamina location in the spinal cord as well as the brain regions related to pain. It will be useful information for understanding the mechanism by which NOP receptors alter a nociceptive response. This work was funded by NIH grant (DA023281, L.T.; DA031777, G.S.).

77. Exploration of possible effects of chronic neuropathic pain on sucrose reward in rats

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Considerable co-morbidity is observed in patients with chronic pain and major depressive disorder (MDD). Anhedonia is a hallmark of depression and one of the principle diagnostic criteria for MDD. We employed a sucrose preference assay to determine whether chronic neuropathic pain influences reward in the rat. Protocols were approved by IACUC prior to performing experiments. Spinal nerve ligation (SNL) or sham surgery was performed on groups of 8-10 male Harlan SD rats by tightly ligating L5/L6 spinal nerves. Rats were given a choice of 1% sucrose or water solutions. The preference for sucrose and the total volume of fluid consumed were recorded and assessed by two-way ANOVA ($p<0.05$). Preference for sucrose was evaluated on days 2, 7, 14 and 21. No differences in sucrose preference or total volume consumed were observed between sham-operated and nerve-injured animals. When evaluated up to day 21 after SNL, there was no evidence of changes in rewarding qualities of sucrose. Improved mechanistic understanding of the possible effects of pain on reward may facilitate novel treatments for comorbid chronic pain and depression. Acknowledgments/Disclosures: Eli Lilly & Company funded these studies and currently employs AO and, DM. Conflicts of interest: The authors have no conflicts of interest to declare.

78. A bivalent ligand (MCC22) potently inhibits inflammatory and neuropathic pain via
putative MOR-CCR5 heteromers in mouse spinal cord

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Conventional opioid analgesics are not highly effective for treatment of pain associated with various chronic diseases due to tolerance and other adverse effects. Given that crosstalk between opioid and chemokine receptors reduces the potency of opioids, and reports on the existence of MOR-CCR5 heteromers in cultured cells, has led us to consider a novel therapeutic target for a more effective treatment of inflammatory and neuropathic pain. In this regard, we have designed a series of bivalent ligands (MCC series) whose members are comprised of MOR agonist and CCR5 antagonist pharmacophores tethered by variable length spacers. In LPS and CFA pretreated mice, the bivalent ligand with a 22-atom

spacer (MCC22) was exceptionally potent in the tail-flick and von Frey assays. MCC22 (i.t.) has an ED50=0.015 pmol/mouse and exhibits >700-fold enhancement of antinociception over the 20-atom spacer homolog, MCC20, and is 3500x more potent than a mixture of monovalent mu agonist and CCR5 antagonist. Molecular simulation studies of MCC22 bound to MOR-CCR5 heteromer with a TM5,6 interface is consistent with the bridging of its protomers. The 7500-fold greater i.t. vs i.c.v. potency of MCC22 in inflamed mice, suggests MOR-CCR5 heteromers reside in the spinal cord. Evaluation of MCC22 in a cisplatin-induced neuropathy mouse model revealed it to be effective at a 0.1 fmol i.t. dose. This research was supported by National Institute on Drug Abuse grant R01DA030316. None of the authors have conflict of interest related to this research.

79. Enkephalin and endorphin-based glycosides as analgesics

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Glycopeptides related to β-endorphin penetrate the blood-brain barrier (BBB) of mice to produce antinociception. Short glycopeptides (5–7 residues) produce mu agonism, delta agonism, or synergistic mu + delta agonism. By linking helical amphipathic *"addresses"* to these opioid *"messages"* it is possible to enhance their effects *in vivo.*† The molecular weights (MW) of the glycopeptides do not appear to affect BBB penetration rates, at least in the range of MW's examined so far, 550—3,500 Daltons. Mouse tail-flick assays and behavioral studies suggest that the mixed mu-delta agonism produces a powerful synergism to enhance anti-nociceptive effects while reducing sedative effects and locomotor stimulation. Use of separation-free MSN analysis was used to study the stability of glycopeptides in rat serum. We also used a nano LC-MSN system with chip-based electrospray ionization (ESI chip) to lower the detection limits for the glycopeptides. A modified on-line preservation system minimizes degradation, enabling quantifiable limits (~100 pM) of both endogenous peptides and exogenous glycopeptide drugs from the brain of a conscious unsedated mouse. We thank the National Institute of Neurological Disease and Stroke (NINDS) and the Michael J. Fox Foundation for the support of these studies. There are no conflicts of interest.

80. Chronic neuropathic pain and the resulting loss of intrinsic inhibition in the prefrontal cortex causes enduring structural, electrophysiological and behavioral adaptations

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The negative affect and emotion associated with chronic neuropathic pain suggests a rewiring of higher cognitive functions and a dysfunctional cortex. To investigate the cellular mechanisms underlying this behavioral state, we used the spared nerve injury (SNI) model of neuropathic pain in wild-type and transgenic rats to assess structural and neuronal plasticity in the prelimbic (PL) cortex. SNI treatment resulted in neuronal adaptations indicative of an enduring hyperexcitability including: 1) slowly evolving but persistent (>40d) regional C-Fos activation, 2) increased density of long/thin dendritic spine filaments as shown with ballistically loaded fluorescent DiI, 3) overactive release of calcium from the endoplasmic reticulum (ER) and 4) elevated basal firing rates (>300%). These adaptations occurred in CaMKII-positive, deep cortical (5/6) layer PL neurons contralateral to the lesion. The hyperexcitability was unaltered by antagonists for NMDA/AMPA receptors, but involved a failure of intrinsic-inhibition, mediated by the slow after-hyperpolarization (AHP). This resulted in loss of inhibitory accommodation (spike-frequency adaptation) — which could be restored by blockade of PKA, manipulation of calcium stores or stabilization of KCNQ channels with retigabine. In SNI animals, microinfusion of retigabine or baclofen/muscimol into the PL attenuated the mechanical allodynia to sham-levels. These findings demonstrate that the enduring cortical hyperexcitability induced by neuropathic pain (often attributed to NMDA-dependent LTP) may actually result from an ER calcium-dependent loss of intrinsic inhibition that measurably impacts pain-related behaviors. This work was supported by NIDA/NIH (DA033342 & DA028811). None of the authors has a conflict of interest related to this research.

81. Cell-autonomous regulation of mu-opioid receptor recycling and resensitization by substance p.

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Opioid analgesics are known to modulate the pain pathway, but whether and how pain can modulate neuronal sensitivity to opioids is less clear despite early reports of paradoxical antinociceptive effects of inflammatory neuropeptides. Here, we show that substance P (SP), a neuropeptide associated with inflammatory pain, reprograms opioid receptor

recycling and signaling and sensitizes peripheral neurons to opioid analgesics. SP, through activation of the neurokinin 1 (NK1R) receptor, increases the post-endocytic recycling of the mu-opioid receptor (MOR) in trigeminal ganglion (TG) neurons in response to endogenous opioids and fentanyl, but nor morphine. SP-mediated protein kinase C (PKC) activation is both required and sufficient for increasing recycling of exogenous and endogenous MOR in TG neurons. The target of this cross-regulation is MOR itself, as mutation of either of two PKC phosphorylation sites on MOR abolishes the SP-induced increase in recycling and resensitization. Further, SP abolishes acute tolerance to fentanyl-induced, but not morphine-induced, antinociception in mice. Our results define a physiological pathway that cross-regulates opioid receptor recycling via direct modification of MOR and suggest a mode of homeostatic interaction between the pain and analgesic systems. S.L.B. was supported by an NIH T32 grant NS007433, A.A.P. was supported by NIH DA031243, and M.A.P. was supported by NIH DA024698 and DA036086. None of the authors has a conflict of interest related to this research.

82. Collybolide, a novel scaffold to study kappa-opioid receptor pharmacology

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Collybolide, recently discovered to be a potent and selective kappa opioid receptor (KOR) agonist, has become a prospective pharmacological target due to a furyl-δ-lactone core structure similar to salvinorin A, the first non-nitrogenous ligand with a unique mode of binding to KOR. Understanding how collybolide achieves its remarkable selectivity for human KOR (hKOR) is an essential step to study kappa opioid pharmacology by establishing the SAR of collybolide to KOR. Through our multidisciplinary collaboration, we are developing a diverse library of collybolide analogues using two complementary approaches, i.e. semi-synthesis and diversity-oriented total synthesis. In the semi-synthetic approach, we are pursuing a rational structural diversification of the natural collybolides. Diversity-oriented total synthesis of collybolide is currently underway starting from sugars, an inherently rich source of chirality provided by Mother Nature. The absolute configuration of two natural collybolides (from the fungus *Collybia maculata*) and their conformational plasticity were determined by X-ray crystallography and dynamic NMR, respectively. Additionally, hKOR has been obtained as a recombinant protein stabilized in amphiphilic media and will be used for planned SAR studies *in vitro*. A theoretical simulation of human KOR (hKOR) in complex with collybolide suggests that the sesquiterpene interacts with hKOR-selective Tyr residues. Altogether, these studies will help in establishing the SAR of collybolide at KOR for discovering novel therapeutic agents to treat pain and substance abuse. Support by OU Start-up (to I.S.) and NIH grants DA008863 and NS026880 (to L.A.D.). None of the authors has a conflict of interest related to this research.

83. Interaction between methylphenidate, methadone and different antidepressant drugs in mice, and possible clinical implications

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Methylphenidate (MPH), a psychostimulant used for the treatment of attention deficit hyperactivity disorder (ADHD), is widely used by patients treated with antidepressants and / or methadone maintenance treatment. Preclinical studies in rats showed that MPH has an analgesic effect when given alone or in combination with morphine. In the present experiments we studied the interaction of acute doses of MPH with sub threshold doses of methadone and different antidepressant medications. Next, we studied the interaction of increasing doses of MPH with chronic methadone using implanted mini pumps. For these goals we performed the hot plate assay on mice. The addition of a sub threshold dose of venlafaxine, desipramine and clomipramine to MPH produced significant augmentation of MPH antinociceptive effect with each medication ($p<0.05$). No interactions were found when sub threshold doses of escitalopram and methadone were added to acute doses of MPH. On the other hand, addition of increasing doses of MPH to chronic methadone given for 2 weeks using osmotic mini pumps induced augmentation of the antinociceptive effect of chronic methadone exclusively at high dose of MPH. These findings may implicate the need of an excessive attention to the administration of MPH given to MMT patients. The findings of the no interaction between MPH and escitalopram may hint to the possibly safe co-administration of methylphenidate and SSRIs to depressed ADHD patients. Further studies are needed before these possible clinical implications can be validated. Supported by (i) The Dr. Miriam and Sheldon G. Adelson Center for the Biology of Addictive Diseases. (ii) The Ari and Regine Aprijaskis Fund, at Tel-Aviv University. None of the authors has a conflict of interest related to this research.

84. Heat shock protein 90 regulates mu opioid receptor signaling in vitro and in mouse periaqueductal grey

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Recent efforts to develop opioids with fewer side effects have focused on signalling proteins responsible for different aspects of the response, and targeting these regulators with functionally selective drugs and other strategies. To find new targets for this approach, we mined proteomic databases to identify unknown candidates for mu opioid receptor (MOR) regulators. One such candidate was Heat shock protein 90 (Hsp90), a ubiquitous, highly expressed, signalling regulator, which is altered in brain by chronic opioid treatment. We treated MOR expressing CHO, HEK, U2OS, and SH-SY5Y neuroblastoma cells with 17-AAG, a selective Hsp90 inhibitor, followed by the MOR agonist DAMGO. We found changes in evoked signalling and protein expression that varied by cell type. These changes included strongly decreased βarrestin2, MOR, and Akt expression, alterations in ERK signalling baseline and evoked DAMGO response, and increased Hsp70 expression. We continued these studies in vivo by intracerebroventricular injection of 17-AAG into mouse brain, followed by vehicle or DAMGO. We found an increased ERK signalling baseline and abolished DAMGO induction in the periaqueductal grey, along with increased Hsp70 expression. Furthermore we found that 17-AAG treatment did not alter the acute anti-nociceptive response to DAMGO in the tail-flick test. Studies are in progress to test the effect of 17-AAG treatment on chronic opioid induced tolerance and dependence, which we expect to be decreased. These findings demonstrate that Hsp90 has a strong regulatory role in MOR signalling in vitro and in vivo, and suggest the possibility of Hsp90 inhibitor co-therapy to improve opioid therapeutic index.

85. Implication of COPB1 in intracellular retention of the delta opioid receptor

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As opposed to most G protein-coupled receptors, the delta opioid receptor (DOPr) is poorly addressed at the plasma membrane. Indeed, under normal conditions, only a small proportion of DOPr can escape the endoplasmic reticulum (ER) and reach the plasma membrane. Most recently, we have described an important role for T161 phosphorylation by cdk5 in mediating morphine and inflammation-induced up-regulation of DOPr. In this study, we sought to better describe the molecular mechanisms involved in the regulation of DOPr trafficking. We investigated the role of the putative COPB1 binding motifs located in the intracellular loops (ICL) of DOPr. In particular, we identified a COPB1 binding motif within the consensus cdk5 phosphorylation motif in the ICL2 of the DOPr. Using confocal microscopy, co-immunoprecipitation and GST-pulldown assays we found COPB1 to colocalize and interact with the COPB1 binding motifs in ICL2 and ICL3 of DOPr. Most interestingly, the replacement of T161 by phosphomimetic residues decreased the ability of COPB1 to bind the ICL2 of DOPr. We finally studied the impact of mutations altering the COPB1 binding motifs on the surface expression of Flag-DOPr in HEK cells using an ELISA assay and found a major role for the two di-lysine binding sites in the retention of DOPr into the ER. Together, our results suggest that COPB1 is involved in the intracellular retention of DOPr and that the phosphorylation of T161 can interfere with COPB1 binding to ICL2 promoting the escape of DOPr from the ER, increasing its expression at the plasma membrane. This work was supported by the Canadian Institute of Health Research grant #MOP-123399.

86. Peripheral mu-opioid receptor activation attenuates ongoing neuropathic and inflammatory pain in rats

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Ongoing pain, a common clinical presentation in patients suffering from tissue or nerve injury, is difficult to assess in pre-clinical studies. Using a novel assay, conditioned place preference (CPP) that relies on reward from pain relief, we investigated if DALDA, a hydrophilic mu-opioid receptor (MOR) agonist, can prevent spontaneous pain in rats with neuropathic (SNL) and inflammatory insults. SNL rats spent significantly more time in DALDA (10 mg/kg s.c.) paired chamber during post-conditioning, compared to pre-conditioning. DALDA did not induced CPP in naive rats, suggesting that DALDA's effects in SNL rats is likely due to the reward from pain relief. Intraperitoneal pretreatment with the peripherally restricted MOR-preferring antagonist, methylnaltrexone (5 mg/kg), blocked DALDA-induced CPP in SNL rats, suggesting a peripheral mechanism for DALDA's effects on ongoing pain. Systemic treatment with resiniferatoxin (0.1 mg/kg i.p) blocked DALDA-induced CPP in injured rats, suggesting an inhibition of TRPV1-expressing neurons is important to DALDA-induced CPP. DALDA pretreatment also inhibited ongoing pain behavior at 15-60 min (phase II) after

intraplantar formalin injection (50 μL, 1%) and attenuated the subsequent development of mechanical hyperalgesia. In neurophysiological studies, spontaneous activity of WDR neurons in SNL rats was significantly decreased at 20-30 min after DALDA, but not vehicle, treatment. Pre-treatment with methylnaltrexone blocked the inhibitory effect of DALDA on WDR neuronal activity. Our findings suggest that DALDA alleviates ongoing neuropathic and inflammatory pain in rats by activating peripheral MORs, especially those expressed in TRPV1-positive neurons. Further studies are required to delineate the underlying cellular and molecular mechanisms. Conflict of interest: There is no conflict of interest. Grant/Other Support: NIH Grant NS70814, NS26363.

87. Galectin-3 is upregulated in primary afferent neurons and satellite glial cells following peripheral nerve injury, but not in spinal neurons or microglia

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Galectins are a class of carbohydrate binding protein that bind beta-galactoside sugars and have been implicated in cell adhesion, communication, and immune responses. Galectin-3 was shown to be upregulated in microglia following stroke and has thus been used as a marker for microglia activation. Here we wanted to determine the contribution of microglial gal-3 to neuropathic pain. We performed unilateral sciatic nerve axotomy and, strikingly, failed to observe galectin-3 in activated spinal microglia. Rather, we observed a significant increase in galectin-3-immunoreactivity in the central terminals of IB4+ and CGRP+ nociceptors in laminae I-IIo of the dorsal horn. Corresponding galectin-3 upregulation was observed in sensory neuron cell bodies in lumbar dorsal root ganglia following injury. We observed no galectin-3 in spinal cord neurons, astrocytes, or microglia before or after sciatic nerve injury. However, following direct injury to the spinal cord galectin-3 is expressed by a subset of microglia and invading macrophages. We also found an upregulation of galectin-3 in dorsal root ganglion satellite glial cells associated with somata of IB4+ nociceptors. We are currently investigating the mechanism of action of galectin-3 in nociceptors and SCGs to test the hypothesis that galectin-3 might be a signaling molecule that regulates nociception or regeneration following nerve injury. Regardless of galectin-3 mechanism of action, our results reveal that either distinct microglial populations, or distinct gene expression programs in these cells, are engaged in spinal microglia following peripheral and central injuries. Support by NIH/NIDA (DA031777, G.S.), FAER Research Fellowship (V.L.T) and NDSEG Fellowship (E.S.).

88. Comparison of equilibrium dialysis (ED) combined with ultra-high performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) and competitive ELISA for the determination of dissociation constants of 6-acetylmorphine (6AM) and morphine to polyclonal antibodies

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Vaccines to drugs of abuse function by producing antibodies that prevent the drug from crossing the blood-brain barrier. *In vivo*, heroin rapidly is metabolized into 6AM and morphine. Thus, a heroin vaccine must induce high titer and high affinity antibodies to heroin, 6AM and morphine. ED and competitive ELISA were performed on sera from mice immunized with DiAmHap, 6-ProxyHap and MorHap haptens tetanus toxoid (TT) conjugates. The concentrations of the drugs were quantified using ED-UPLC/MS/MS and dissociation constant (Kd) was calculated by plotting [Bound Drug] vs [Free Drug]. The Kd was calculated by competitive ELISA using the opiates as inhibitors and hapten-BSA conjugates as coating agents. Immunization of mice with 6-PrOxyHap-TT and MorHap-TT conjugates induced antibodies that have high affinities for both 6AM and morphine (Kd< 25 nM), while DiAmHap-TT conjugates induced antibodies that do not bind morphine and had low affinities for 6AM (Kd> 100 nM) as measured by ED-UPLC/MS/MS. In contrast, competitive ELISA yielded apparent Kd that were in the μM range. Due to the chemical instability of heroin, the Kd for heroin cannot be determined by either of the methods. We described a method that utilized ED-UPLC MS/MS that provided a more accurate apparent dissociation constants (Kd ~ nM) than competitive ELISA (Kd ~ high μM) for the measurement of antibodies to opiates. This work was supported through a Cooperative Agreement Award (W81XWH-07-2-067) between the HJF and the U.S. Army Medical Research and Materiel Command and an Avant Garde award from NIDA (NIH grant no. 1DP1DA034787-01).

89. The NOP Receptor Antagonist SB612111, but not the Agonist AT-202 Blocks Ethanol Drinking in Mice

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The NOP receptors is found throughout the brain, spinal cord, and dorsal root ganglia and is involved in many CNS pathways including those for pain and reward. It has been demonstrated repeatedly that both N/OFQ, the endogenous ligand for NOP, and small molecule agonists can block conditioned place preference (CPP) of many abused drugs, and can reduce extracellular dopamine levels in the nucleus accumbens. Results with self-administration studies have been less conclusive. N/OFQ does not block heroin self-administration in rats, and small molecule agonists have had equivocal results in blocking self-administration of alcohol in rats and opiates in monkeys. When exploring alcohol consumption in mice using the one-bottle and the two-bottle choice drinking in the dark (DID) paradigms we found the NOP agonist AT-202 to have no effect on alcohol consumption at non-sedative doses. Conversely, the NOP receptor antagonist SB612111 was able to attenuate alcohol consumption in both types of experiments. At 30 mg/kg SB612111 reduced alcohol consumption by 30% when 20% alcohol was present for 4 h in the one-bottle DID. SB6121211 was more effective, reducing alcohol consumption by 50%, when mice were exposed to both 15% ethanol and water for 4h. This surprising result suggests that there is a difference between the reward inducing actions of the CPP and self-administration tests, and that the NOP system influences these two different drug abuse paradigms differently. The mechanism by which NOP antagonists attenuate alcohol drinking behavior is current unclear. Supported by NIDA grant 1R01DA023281-01S1.

90. Structure-based approaches to designing bifunctional NOP-mu opioid receptor ligands from NOP-selective scaffolds

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Nociceptin/OrphaninFQ (N/OFQ), the endogenous peptide ligand for the NOP receptor, has been shown to decrease opioid-induced reward and tolerance development. The antinociceptive activity of selective NOP agonists, however, is dependent on the route of administration and the pain modality. We have previously hypothesized that NOP-mu opioid bifunctional agonists, possessing mu opioid-based antinociceptive efficacies and reduced tolerance/rewarding properties due to NOP agonist efficacy, may have desirable profiles as 'non-addicting analgesics'. We reported the design of such NOP-mu bifunctional agonists from the NOP-selective dihydroindolin-2-one class of NOP agonists. Ro 64-6198, the well-studied selective NOP agonist from the 1,3,8-triazaspirodecanone class of NOP agonists, only shows antinociceptive activity in models of acute thermal pain and inflammatory pain when administered systemically. Here, we report the design of bifunctional NOP-mu agonists from this NOP-selective 1,3,8-triazaspirodecanone scaffold using NOP and mu opioid receptor structure-based approaches. We designed rational structural modifications of Ro 64-6198 that increased its binding affinity at the mu opioid receptor without adversely affecting its NOP affinity. The resulting NOP/mu ligands maintained NOP full agonist efficacy and had varying levels of mu agonist efficacy to afford several new bifunctional NOP-mu agonists. These drug design approaches for obtaining the desired bifunctional profiles, guided by receptor structural information as well as the detailed pharmacological characterization of binding affinities and functional efficacies, will be presented. Authors declare no conflicts of interest. This work was supported by NIDA grant R01DA027811 (NZ).

91. Synaptic plasticity in the medial prefrontal cortex: role of α7 nicotinic acetylcholine receptors

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The medial prefrontal cortex (mPFC) is a key brain region implicated in drug-related associative learning (1). α7 nicotinic acetylcholine receptors (nAChR) have a modulatory role in the mPFC (2) and α7 nAChR antagonism can inhibit reinstatement to opioid drug seeking (3). We have explored the mechanisms by which α7 nAChRs modulate excitation in the mPFC as a pre-requisite to investigating their influence on drug-associated learning.

We measured spontaneous EPSCs and IPSCs in brain slices from drug naïve mice, in response to α7 nAChR positive allosteric modulator (PNU-120596), selective agonist (PNU-282987) and antagonists (MLA). Functional somatic α7 nAChRs on inhibitory interneurons and presynaptic α7 nAChRs on glutamatergic terminals were discriminated. This enables α7nAChRs to play a complex dynamic role in both excitation and inhibition in the mPFC, with endogenous tonic acetylcholine preferentially enhancing excitatory transmission via α7 nAChRs. Using field recordings to study stimulus-induced plasticity in mPFC slices, both inhibition and activation of α7 nAChRs reduced stimulus-induced LTP, whilst MLA enhanced LTD. Optogenetic strategies are currently being applied to define the glutamatergic input pathways bearing α7 nAChRs. These studies will pave the way for an analysis of the roles of α7 nAChRs in the mPFC in drug-related learning. Funded by an MRC studentship and bursaries from Bath Alumni, British Pharmacological Society and Guarantors of Brain to MU. We declare no conflict of interest.

92. Mitragyna alkaloids revisited: pharmacology of mitragynine and related natural products

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Mitragynine, an indole alkaloid isolated from the Southeast Asian rubiaceous plant *Mitragyna speciosa*, is one of the few examples of opioid natural product small molecules that are structurally unrelated to morphine. *Mitragyna*-based herbal products (often marketed as *Kratom*) are legally available in the US. Anecdotal reports indicate they are used recreationally, as pain relievers, and to prevent opioid withdrawal. Mitragynine, as well as its naturally occurring congeners such as 7-hydroxymitragynine and mitragynine pseudoindoxyl, have received attention in the past decades because they produce antinociceptive effects in rodents. . However, the published pharmacological data on this alkaloid family is very diverse, making the comparison of results difficult. Our goal was to pharmacologically characterize mitragynine, related natural products, and several semi-synthetic analogs. Mitragynine, 7-hydroxymitragynine, mitragynine pseudoindoxyl, and their analogs were evaluated in cloned opioid receptor transfected cell lines in *in vitro* radioligand binding assays, [35S]GTPγS functional assays, and β-arrestin biased agonism assays. All compounds were also evaluated *in vivo* in tail flick analgesia assays in the absence and presence of antagonists selective for several opioid receptor subtypes. Our presentation will discuss the implications of the results in opioid drug discovery and possibilities to diversify this template to develop novel analgesics. *Supported by DA034106 to SM from NIDA.*

93. Synthesis and pharmacology of a dual kappa-delta opioid receptor agonist which blocks cocaine conditioned place preference

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Opioids can be used to treat opioid, alcohol, and potentially cocaine abuse disorders. Analgesics with selectivity for a particular opioid receptor family have prototypical side effects including abuse liability (MOR), dysphoria (KOR), and seizures (DOR). An important goal of opioid drug development is to eliminate these unwanted effects. We have recently discovered a new class of opioid analgesics targeting 6-transmembrane splice variants of MOR (6TM/E11) with high affinity. The lead compound in this class, 3-iodobenzoyl-β-naltrexamine (IBNtxA) displays potent antinociception without side-effects associated with activation of traditional opioid receptors. During our SAR studies on IBNtxA, we discovered an opioid ligand (MP1104) with picomolar binding affinity for all opioid receptors. In functional assays *in vitro*, it was 574 and 107-fold more potent than prototypic KOR and DOR agonists, respectively. *In vivo,* MP1104 was 18-fold more potent than morphine. We used both selective opioid antagonists and knockout mice to determine the relative contributions of opioid receptor families to MP1104 analgesia. MP1104 appears to produce analgesia exclusively through KOR and DOR receptors. MP1104 did not precipitate seizures at doses 45-fold higher than the analgesic ED50. When evaluated in conditioned place preference/aversion assays, MP1104 showed no rewarding or aversive effects at a dose three times higher than its analgesic ED50, but attenuated cocaine-conditioned place preference. There are presently no FDA approved drugs to treat cocaine addiction. MP1104 may represent an attractive strategy to treat pain as well as cocaine dependence without the characteristic side effects seen with opioid analgesics. *Supported by DA034106 to SM from NIDA*

94. Beta-arrestin1 preferentially mediates analgesic tolerance to SNC80 in a ligand-biased manner

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Ligand directed signaling via the delta opioid receptor (DOPR) has important implications given the potential therapeutic uses of delta agonists in the treatment of chronic pain and emotional disorders. We had previously shown that repeated injection of the high-internalizing delta agonist (+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide or SNC80, produced acute behavioral desensitization while the low-internalizing delta agonist N,N-diethyl-4-(phenyl-piperidin-4-ylidenemethyl)-benzamide or ARM390 did not. Since β-arrestins are well known to regulate G protein-coupled receptors signaling and trafficking, we therefore investigated the behavioral significance of ligand-specific interactions between β-arrestin 1 and the DOPR. In the Complete Freund's Adjuvant (CFA) tail model of inflammatory pain, mice lacking β-arrestin 1 showed enhanced and longer lasting pain-relieving effects of SNC80, and decreased behavioral tolerance following repeat exposure to the agonist. In contrast, ARM390 produced similar analgesic effects and no tolerance in both WT and KO animals. Following chronic treatment, the absence of β-arrestin 1 attenuated the extent of tolerance to SNC80, but not to ARM390. Furthermore, chronic treatment with SNC80 abolished delta-agonist induced GTPγS binding in WT brain membranes, whereas DOPR-G protein coupling remained intact in KO mice. Overall, these results indicate that DOPR agonists interact with β-arrestins in a ligand-biased manner, and that the high-internalizing agonist SNC80 preferentially recruits β-arrestin 1. This work was supported by the NIH Grant DA031243, the Shirley and Stefan Hatos Research Foundation, and the Dept. of Psychiatry at the University of Illinois at Chicago. None of the authors has a conflict of interest related to this research.

95. Cocaine self-administration induces changes in synaptic transmission and plasticity in ventral hippocampus

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Allowing rats extended access to cocaine self-administration is thought to recapitulate key aspects of cocaine addiction. Understanding the mechanisms that underlie drug-induced neuroadaptations that persist in the brain after protracted abstinence is crucial for developing therapeutic interventions. Whole-cell voltage-clamp and extracellular recording technique were used to assess changes in neurotransmission and long-term potentiation (LTP) in the CA1 region using a ventral hippocampal slice preparation. Rats allowed to self-administer cocaine daily, including “long access” (6hr) sessions, exhibited an increase in the AMPA/NMDA current ratio and enhanced excitatory transmission following 3-5 weeks of abstinence. Inhibitory transmission was also significantly decreased in long access animals and the AMPA/NMDA ratio measured in the absence of GABAergic blockers was greatly enhanced. We also observed a significant reduction of LTP magnitude. Conclusions: These findings suggest the presence of synergistic effects of enhanced AMPA and diminished GABA neurotransmission under physiological conditions in the CA1 region of cocaine-taking animals, supporting the conclusion that persisting enhancement of AMPA-mediated transmission and concomitant inhibition of GABA-mediated transmission promoted a chronic state of potentiation that partially occluded further LTP. As a result, this increased output from the ventral hippocampus to other limbic areas such as the nucleus accumbens would be expected to contribute to sensitization-like responses mediating the escalation of drug intake and promote the reinstatement of drug-seeking behaviors. Supported by the National Institute on Drug Abuse, NIH (DA016302 to J.J.W.). None of the authors has a conflict of interest related to this research.

96. Biphasic effects of K opioid receptor agonist U50488H in anxiety-related behaviors

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Accumulative evidences indicated that K opioid receptor (KOR)/dynorphin system is involved in regulating states of emotion and motivation. There is a general consensus that activation of KORs produced negative affective states, such as dysphoria in human and depressive-like or anxiety-like effects in rodents. However, a number of studies demonstrated that administration of KOR agonists produced anxiolytic effects in rodents. The molecular mechanism underlying the biphasic effects of KORs in anxiety-related behavior was unknown. In the present study, we tested the effects of different doses of K agonist U50488H on anxiety-related behavior in mice. We found that systemically administration of low dose of U50488H (0.125 mg/kg, s.c.) produced anxiogenic effects in the elevated plus maze test, whereas higher dose of U50488H (2.5 mg/kg, s.c.) produced anxiolytic effects. The low dose-dependent effect of U50488H was completely blocked by pretreatment with Kantagonist norBNI, whilst higher dose-dependent effect was partially attenuated by norBNI. pERK was used as neuronal activity marker to study the neurobiological substrates responsible for the biphasic effects of U50488H. We found that U50488H anxiogenic effect was paralleled with robust increased pERK activity in the nucleus accumbens, whereas U50488H anxiolytic effect was paralleled with robust increased pERK activity in the hypothalamus and septum. Thus, the present study firstly provided the neurobiological substrates to explain the biphasic effect of KORs in anxiety-related behaviors. Support by grant 81401107 from National Natural Science Foundation of China. None of the authors has a conflict of interest related to this research.

97. Identification and connectivity of spinal neurons that express delta opioid receptors

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Here we used DOR-GFP reporter mice as well as in situ hybridization and electrophysiological recordings in wildtype mice to identify spinal neurons that express the delta opioid receptor (DOR). In the dorsal horn, DOR+ neurons are concentrated in lamina II inner. Coimmunolabeling revealed that these DOR+ neurons co-express calbindin, somatostatin and PKCgamma, identifying them as excitatory interneurons known to process mechanical stimuli. Consistent with this idea, mechanical stimulation of the mouse hindpaw induced c-Fos expression in DOR+ neurons. Whole-cell patch clamp recordings in slices showed that DOR+ neurons in lamina II inner displayed either single, delayed or gap action potential firing patterns, consistent with their excitatory nature. Dorsal root stimulation experiments indicated that these DOR+ neurons receive Adelta or Abeta afferent inputs. Furthermore, DOR agonists activate GIRK channels, reducing excitability to myelinated low-threshold mechanoreceptor inputs. We also report striking dorso-ventral segregation in the identity of DOR+ neurons, and DOR presence in motor control circuits. Thus the majority of DOR+ neurons located in lamina III and more ventral coexpress markers of inhibitory neurons, including NOS+ interneurons in lamina III, parvalbumin+ neurons in lamina IV-V, and engrailed 1+ V1 interneurons in the ventral horn. Our results suggest that beside their action on primary

afferent mechanoreceptors, DOR agonists could also decrease injury-induced mechanical hypersensitivity by a postsynaptic action on lamina II excitatory interneurons. Support by NIH grants (DA031777, G.S; T32GM089626, D.W.), a FAER fellowship (V.L.T.) and a HHMI fellowship (S.A.L.). None of the authors has a conflict of interest related to this research.

98. The relationship between reward perception and analgesic effects of heroin in rats

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Individual differences in sensitivity to pain and reward likely reflect a combination of genetic, developmental, and environmental factors. In this study, we investigated potential correlates of heroin reward perception in conditioned place preference (CPP) in Sprague-Dawley rats. In the same animals, we also conducted a hot-plate experiment to investigate potential correlations of the analgesic and reward effects of heroin. For CPP, animals were exposed to heroin (1 mg/kg) or saline during conditioning. On test day, total time spent in the unconditioned chamber was subtracted from total time spent in the conditioned chamber. For hot-plate, response latency was measured after heroin (1mg/kg) or saline. In CPP, a t-test revealed a significant difference between the saline (n=10) and heroin (n=12) groups [t(18.65)=2.285, p=0.0342], with the heroin group spending greater time in the heroin-paired chamber. Substantial individual variability was observed within the heroin group: the upper quartile (n=3) showed high CPP (mean=411.4$\pm$29.20 s) and the lower quartile (n=3) exhibited no CPP (mean=-62.53$\pm$52.58 s). Heroin resulted in significantly increased latency in the hot-plate assay compared with saline, indicating an analgesic effect. Additionally, a positive correlation was observed in the heroin group between % maximum response in hot-plate (at 15 minutes) and increased time spent in the heroin-paired chamber [r(9)=0.66, p=0.026, n=11]. Our findings suggest a relationship between heroin-induced reward and pain perception. Authors have no conflicts of interest. This work was supported by a David Novick Postdoctoral Fellowship (CW) and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (MJK).

99. The role of the hippocampal and medial prefrontal cortex α7 nicotinic acetylcholine receptors in morphine primed reinstatement to conditioned place preference

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Nicotinic acetylcholine receptors (nAChRs) play a key role in addiction-related behaviours (Rahman et al (2015) Front Neurosci 8:426). We have shown that the α7 nAChR antagonist methyllycaconitine (MLA) can selectively inhibit reinstatement to morphine-CPP (Wright et al., companion abstract). The aim of this study was to investigate the mechanisms of this effect. Stimulus-induced LTP in the medial prefrontal cortex (mPFC) was attenuated by the presence of α-bungarotoxin, a neurotoxin known to antagonise the α7 nAChR. Using autoradiography we examined potential changes in AMPA and NMDA receptor binding in mice that had undergone MLA treatment at morphine reinstatement. There were no significant changes in NMDA receptor binding (using [3H]MK801) but significant changes were observed in [3H]AMPA binding in the hippocampus, but not in other brain regions examined (including mPFC, nucleus accumbens, amygdala, VTA). These findings suggest potential roles for the hippocampus and the mPFC in the α7 effects. Behavioural experiments using intracranial infusions of MLA into hippocampus or mPFC are ongoing. Supported by funding from BBSRC and a CASE studentship with RenaSci, Nottingham, UK. None of the authors has a conflict of interest related to this research.

100. α7 nicotinic receptor antagonism selectively reduces reinstatement to morphine-conditioned place preference

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Maintaining long-term abstinence and preventing relapse after re-exposure to drug-associated cues is the main challenge for treating drug addiction. Nicotinic acetylcholine receptors (nAChR) have been implicated in responses to drugs of abuse other than nicotine (Rahman et al (2015) Front Neurosci 8:426). The aim of this work was to characterise the role of α7 nAChRs in morphine reward learning using conditioned place preference (CPP). The α7 nAChR antagonist methyllycaconitine (MLA) was used to determine if these receptors contribute to specific stages of drug-paired learning, namely acquisition, reconsolidation or reinstatement of morphine-CPP. In 7-8week old C57BL/6J mice MLA (4mg/kg, s.c), given 20min prior to a conditioning dose of morphine (10mg/kg, i.p) or post-test trial, had no effect on the acquisition, reconsolidation or expression of morphine-CPP. However, when given 20min prior to a priming dose of morphine (5mg/kg, i.p), MLA (4mg/kg, s.c) significantly inhibited drug-induced reinstatement. These results suggest that α7 nAChR may selectively control reinstatement to morphine-CPP. Supported by funding from BBSRC CASE studentship with RenaSci, Nottingham, UK. None of the authors has a conflict of interest related to this research.

101. Evidence for the involvement of hippocampal α7 nicotinic acetylcholine receptors in morphine primed

reinstatement to conditioned place preference

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Nicotinic acetylcholine receptors (nAChRs) play a key role in addiction-related behaviours (Rahman et al (2015) Front Neurosci 8:426). We have shown that the α7 nAChR antagonist methyllycaconitine (MLA) can selectively inhibit reinstatement to morphine-CPP (Wonnacott et al., companion abstract). The aim of this study was to investigate the mechanisms of this effect. Using autoradiography we examined potential changes in AMPA and NMDA receptor binding in mice treated with either saline of MLA at morphine reinstatement. There were no significant changes in NMDA receptor binding (using [3H]MK801) but morphine reinstatement significantly increased [3H]AMPA binding in the hippocampus, but not in other brain regions examined (including mPFC, nucleus accumbens, amygdala, VTA). The selective increase in the hippocampus was partially antagonized by MLA, linking α7 nAChR activation to glutamatergic synaptic plasticity in the hippocampus. Morphine reinstatement following intracranial infusions of saline or MLA directly into the hippocampus and mPFC are ongoing. Supported by funding from BBSRC CASE studentship with RenaSci, Nottingham, UK. None of the authors has a conflict of interest related to this research.

102. Identification and expression of a truncated OPRK1 splice variant with the novel termination codon in human post-mortem brain

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A novel splice variant of the human kappa-opioid receptor (*OPRK1*) was identified by RT-PCR amplification and sequence analysis of a human whole brain cDNA library (Clontech). The new OPRK1 splice variant (h*OPRK*1tm1) is characterized by a deletion of 88 bp at 5´-end of exon 3. The deletion in this region produces a premature translation termination codon. Translation of this variant would be predicted to result in the protein of 90 amino acids in length, retaining the extracellular N-terminal region, the first transmembrane domain (TM1) and five amino acids of the first intracellular domain. To compare the level of expression of the *hOPRK1*tm1with that of full-length *OPRK1* in human post-mortem brain tissues we have performed a quantitative RT- PCR assay in two brain regions, the nucleus accumbens and anterior cingulate in ten HIV-negative subjects, obtained from the Manhattan HIV Brain Bank. The *hOPRK1*tm1specific PCR primer was designed to span the junction site of the canonical exon 2 and the new truncated exon 3. We have found a substantial *hOPRK1*tm1expression levels in the nucleus accumbens and anterior cingulate, although lower than the full-length *OPRK1* ($p<0.001$, two-tailed t-test). Further studies are needed to elucidate potential functions of the truncated splice variant of *OPRK1* in humans. Support: Dr. Miriam and G. Sheldon Adelson Medical Research Foundation (MJK), NIMH-U24-MH100931 (SM). The authors declare no conflict of interest.

103. An endomorphin analog providing prolonged antinociception with substantial reduction of multiple adverse side effects relative to morphine

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Opioids acting at the mu opioid receptor (MOR) are the most effective analgesics, but multiple adverse side effects of available MOR agonists limit their use. Endomorphins (EMs) are potent and selective agonists at MOR. We synthesized several metabolically stable EM analogs and selected four for in-depth analysis of antinociceptive vs. side-effect profiles in the rat. The analogs showed high affinity, selectivity, and efficacy at MOR relative to delta and kappa opioid receptors. Morphine induced significant conditioned place preference and self-administration, while the analogs did not, consistent with reduced abuse potential. At equi-antinociceptive doses, respiratory depression was significantly reduced for all analogs; analogs 1 and 4 did not induce respiratory depression at doses producing significantly longer antinociception than morphine. Motor impairment on a rotorod was less, and antinociception longer, after analogs 2 and 4 than after morphine. Morphine induced cognitive impairment in the Morris water maze while several analogs did not. Tolerance was determined with cumulative dosing before and after 7-day intrathecal administration by osmotic minipump. Analogs were initially 30-fold more potent than morphine and only produced a 13-fold shift in the dose-response curve vs a 38-fold shift by morphine, reflecting significantly less tolerance. Morphine produced significant glial activation (increased GFAP, Iba1 and pp38 staining), while the analogs did not. In summary, our novel EM analogs, especially analog 4, show a highly favorable profile relative to morphine as potent analgesics with reduced 1) reward/abuse liability, 2) respiratory depression, 3) motor impairment, 4) cognitive impairment 5) tolerance and 6) glial activation. Funded by the VA, DOD, and ONR.

104. Small-molecule NOP agonists reduce alcohol reward in mouse models of alcoholism

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Studies in alcohol-preferring rats suggest that dysregulation of the nociceptin opioid receptor NOP and its ligand N/OFQ are a pathophysiological consequence of excessive alcohol intake. Further, administration of N/OFQ blocks the rewarding effects of alcohol in the conditioned place preference (CPP) paradigm in mice, as well as reinstatement of extinguished alcohol CPP, suggesting that NOP agonists may be a promising approach for treating alcohol addiction. We report the efficacy of small-molecule NOP agonists in reducing ethanol-induced CPP in mice. Mice tested for baseline place preference were subjected to three conditioning trials, during which they were treated subcutaneously with vehicle or a NOP agonist, followed by ethanol (2 g/kg) or saline and confined to the vehicle-paired or drug-paired chamber for 15 min. In the afternoon of each day, mice received the alternative treatment and confined to the opposite chamber for 15 min. On day 5, mice were tested for place preference. Among the compounds tested, selective NOP agonist AT-312 dose-dependently reduced ethanol-induced CPP. AT-312 alone had no motivational effects at the dose at which it blocked ethanol CPP. The inhibitory effect of the agonists on alcohol reward was completely abolished in mice lacking NOP. Together, these data suggest that AT-312 reduced alcohol reward via the NOP receptor and was devoid of any motivational effects. Studies are ongoing to assess effect of NOP agonists in the two-bottle-choice paradigm, which is widely used as a model of alcohol-taking behaviors and reinforcement. Authors declare no conflicts of interest. Support: Contract HHSN275201300005C.

105. The role of mu opioid receptors in D1 and D2 neurons in opioid reward-related behaviors

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The abuse of prescriptions opioids and fatalities from opioid overdose has increased exponentially over the last decade. Yet our understanding of the molecules involved and underlying neural circuitry mediating opioid reward is limited. We have begun to examine the role of specific mu opioid receptor populations in neurons that also express dopamine receptor 1 (D1) or dopamine receptor 2 (D2), important striatal intermediates of the mesolimbic reward pathway. Using conditional mu opioid (mu) receptor mice generated by Kieffer and colleagues (PMC3771900) bred with D1 or D2 Cre recombinase mice, mu receptors were deleted from D1 or D2 neurons. Opioid reward-related behaviors; opioid-induced hyperlocomotion or opioid self-administration, were then assessed. We found that mice lacking mu receptors in D1 neurons showed a reduction in both the locomotor effect and sensitization profile of morphine (10mg/kg), and oxycodone (10mg/kg), yet the locomotor profile of cocaine (20mg/kg) remained unchanged. Conversely, mice lacking mu receptors in D2 neurons showed enhanced sensitization to oxycodone (10mg/kg). Intravenous self-administration of oxycodone (0.25mg/kg/inf 2h access) revealed several deficits of the self-administration profile; a reduction in the number of infusions earned and of goal-directed opioid seeking in mice lacking mu receptors in D1 neurons. Collectively, these initial data show that mu receptors expressed in D1 or D2 neurons play specific roles in opioid-reward related behaviors. These data also suggest that opioid reward results from an interplay of mu receptor signaling in D1 and D2 neurons. Supported by NIDA (DA005010: JDJ, BK, CJE, WW and 5T32DA024635-07: DP) and the Hatos Foundation.

106. Coupled activation of primary sensory neurons contributes to chronic pain

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Primary sensory neurons in the dorsal root ganglia (DRG) play an essential role in initiating pain by detecting painful stimuli in the periphery and sending signals to the spinal cord via their axons. Pathological conditions such as inflammation and nerve injury can sensitize DRG neurons, causing heightened pain sensitivity and often leading to chronic pain. Although the mechanisms of hypersensitivity of individual neuron have been extensively studied, how DRG neurons function at a population level under physiological and pathological conditions is unclear due to the lack of proper tools. By specifically expressing a genetically-encoded Ca^{2+} indicator in almost all dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons in Pirt-GCaMP mice. Using this technique, we developed an *in vivo* live imaging technique which allowed us to simultaneously monitor the activation of >1,700 neurons/DRG in response to mechanical stimulation applied to the receptive field in live mice. Using this powerful technique, we revealed a striking neuronal coupling phenomenon that is adjacent neurons tend to activate together in mice with inflammation or nerve injury, which rarely happens in naïve animals. The coupled activation occurs among various sizes of neurons including small-diameter nociceptors and large-diameter low-threshold mechanoreceptors. The transferring of non-membrane permeable dye between coupled-activating neurons suggests that the coupled activation is likely due to non-diffusible cell-to-cell communication. Combining pharmacological and genetic approaches with the imaging technique, we found the coupling

phenomenon is mediated by the upregulation of gap junction in satellite glial cells surrounding DRG neurons after injury. Blocking gap junction significantly attenuates neuronal coupling in the DRG and mechanical hypersensitivity. Therefore, neuronal coupling represents a new form of neuronal plasticity in the DRG and by "hijacking" neighboring neurons through gap junction it contributes to pain hypersensitivity. Finally, this study creates a new way of characterizing the physiological properties and functions for developing new pain- or other modality-specific drug target for a treatment with few side effects.

107. Comparative effects of a semi-synthetic salvinorin A analog, 16-Br-salvinorin A in a translational neuroendocrine biomarker assay, and on sedation scores

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The neoclerodane salvinorin A, from the plant *Salvia divinorum,* is a potent high efficacy KOP-r agonist, and has several "on-target" undesirable effects, including sedation, aversion and hallucinogenesis. KOP-r ligands may have useful pharmacotherapeutic properties in modulating mood diseases and addictive diseases. Synthetic efforts are examining whether novel salvinorin A analogs can show differential potency in causing KOP-r agonist effects *in vivo*, as compared to the aforementioned undesirable effects. Recent studies with the novel analog 16-Br-salvinorin A (Riley et al., 2014; J. Med. Chem. **57:**10464-10475) showed that this compound retained high potency KOP-r agonist effects *in vitro*; it was also able to decrease reinstatement of cocaine self-administration in rats, in the absence of locomotor sedative-like effects. We examined the effects of 16-Br-salvinorin A in a translational neuroendocrine biomarker assay of KOP-r agonist effects (prolactin release), in male non-human primates (n=3), while also monitoring its ability to produce sedative-like effects common in reference KOP-r agonists. 16-Br-salvinorin A (0.0032-0.1 mg/kg, i.v.) was equieffective to salvinorin A (0.001-0.032 mg/kg), but was approximately 3-fold less potent in the biomarker assay. However, 16-Br-salvinorin A caused little or no sedation, as scored with an observational rating scale over this dose range (non-blinded observer rating). These studies indicate that novel salvinorin A analogs may retain KOP-r agonist effects, but with a potentially lower burden of undesirable "on-target" effects. We gratefully acknowledge funding primarily by NIH-NIDA grant DA 018151. None of the authors has a conflict of interest related to this research.

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We look forward to seeing you next year in Bath, England for INRC 2016, June 10-14

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