Photo credits: Dr. Tamara Markovic - Washington University in St Louis
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Welcome to International Narcotics Research Conference – INRC – conference in 2021!

We are pleased to welcome you to our second online conference and share with you another year of opioid science advancements.

This year, we will host the meeting using three platforms: Slack, Zoom and Gather Town.

The latter will hopefully allow further interactions for our attendees. Posters boards will be accessible 24h a day for the duration of the meeting to allow for ALL time zones to visit at convenient times.

Feel free to contact speakers/poster presenters and plan a meet up on Gather Town!

Let’s enjoy some science. See you online!

⚠️ Rules for the Conference:

• On all of the meeting’s platforms, we encourage you to display your full first and last name and include your preferred pronouns.

• Please be respectful in all comments and interactions. The INRC Committee will not allow any form of disrespect or discrimination at our conferences. Any issues can be reported to internationalnarcoticsresearch@gmail.com. Please see our Code of Conduct for further details.

• NO RECORDING. All talks will be recorded and available on our website for a limited amount of time after the conference. It is very important to abide by these rules to encourage open communication and trust within our community.

• Keep yourself muted during all talks to limit background noise.

• To ask questions during webinars, please use the “Q&A” function. These questions will be considered by the moderators to ask the speaker. Please use the “Chat” function for any other comments pertaining to the talk.

• Please do not distribute the links to the conference. We are trying our best to avoid being ‘zoom-bombed’ by not distributing them widely. Anyone who wants to attend can register here for $10 for members and $20 for non-members. If it’s an issue of money, please email us at internationalnarcoticsresearch@gmail.com and we are happy to provide a code to waive the fee.

• Have fun and enjoy the science.
Links

International Narcotics Research Conference

Stay in Touch!

Gather  Join our town!
https://gather.town/invite?token=obrx91Ha
Passcode: @INRC2021

Check out our Website!
inrconference.org

Join our Slack Community!

Follow us on Twitter!
@INRCmeeting

https://us02web.zoom.us/j/86309190905
Meeting ID: 863 0919 0905
Passcode: 061221
Gather Town

Join our town!
https://gather.town/invite?token=obrx91Ha
Passcode: @INRC2021
INRC upholds a strong code of conduct to protect and encourage scientists to share their work. The Code of Conduct aims to outline this and increase the conversations and collaborations around scientific topics. This Code applies to all platforms hosted by the INRC.

Recording Restrictions

Presentations will be recorded by the I.N.R.C. for rebroadcast with permission from presenters and posted on the INRC website with restricted (password protected access) for attendees who cannot participate during the live session. Unauthorized recordings by attendees will not be allowed. Prior to quoting or publishing any information presented at a conference in any publication, written or electronic, written approval of the contributing member must first be obtained.

Without previous written consent of the contributing member, the audio or video recording of oral presentations and discussions, the photography/screen-shooting of slides, and printed or electronic quotes from papers, during the conference is strictly prohibited.

These restrictions apply to each attendee and are intended to cover social networks, blogs, tweets or any other publication, distribution, communication or sharing of information presented or discussed at the conference. Each attendee acknowledges and agrees to these restrictions when registration is accepted and as a condition of being permitted to attend. Each attendee assumes sole responsibility for the protection and preservation of any intellectual property rights in such member’s contributions to a conference. If you become aware of someone making unauthorized recordings, please immediately email this information directly to: internationalresearchconference@gmail.com.
Unauthorized Sharing of Conference Links

Our attendance is limited to license constraints. To ensure that all attendees can access the meeting, access to the conference must be limited to paid registration. **Sharing the access links with anyone is thus prohibited.** Individuals must register individually and can ask for a fee waiver if necessary.

Virtual Conference Best Practices

To avoid unwanted disruptions (i.e. “zoom-bombing”) attendees should **not share any links to the virtual conference rooms.** Attendees, unless presenting, will turn their microphones off. Any questions addressed to the speaker should be typed in the chat window. Moderators will select questions within this chat window and share them with the speaker or ask the attendee to unmute to ask their question according to the time remaining within the time allotted. Cameras may be on or off depending on personal choice, although keeping your camera on may foster a better experience for the speaker as they deliver their talk to the audience. A virtual background will be shared with the speakers if they decide to use it.

Inappropriate Behavior Policy

The INRC has always been encouraging open and honest intellectual debate as part of a welcoming and inclusive atmosphere. The INRC will foster rigorous analysis of all science presented or discussed in a manner respectful to all attendees. To help maintain an open and respectful community of scientists, the **INRC does not tolerate illegal, disrespectful or inappropriate behavior, including harassment of any kind.** The INRC condemns inappropriate or suggestive acts or comments that demean another person by reason of her/his/their gender, gender identity or expression, race, religion, ethnicity, age or disability or that are unwelcome or offensive to other members of the community or their guests. Any allegations of any such behavior will be considered and analyzed by the INRC committee on a case by case basis, and violations will result in immediate removal from the conference. Please report inappropriate behavior to: internationalnarcoticsresearch@gmail.com.

If any member of the INRC board becomes aware of illegal or inappropriate behavior, the member will report this to the rest of the INRC board. Immediate reporting is important to allow the INRC the opportunity to properly assess the situation and fashion an appropriate response that addresses the problem while being sensitive to the concerns of all who are affected. Those who violate this policy will be removed from participating in the conference to the best of the INRC board’s capability.

In exchange for the privilege of participating in a conference, I assume all such risks arising out of my participation, and I also release, agree to indemnify, and hold harmless, the INRC, and its officers, directors, employees, agents, successors and assigns from all claims and lawsuits arising out of such injury, illness, or damage.

Privacy Policy

The INRC is committed to protecting the privacy of its website visitors and conference attendees. Attendee information will not be shared unless given explicit permission.
Schedule

All events on Zoom (Passcode: 061221) except for red squares (Social/Posters), which are on Gather Town (Passcode: @INRC2021).

- **Session #1** - New Drug Development (Moderator: Jay McLaughlin), **Session #2** - Opioid Use Disorder (Moderator: Kabir Lufty), **Session #3** - Respiratory Depression (Moderator: Erica Levitt), **Session #4** - Opioid Receptors - Signaling (Moderator: Meritxell Canals), **Session #5** - Opioid Circuits (Moderator: Grégory Scherrer).

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<td>INRC Welcome Reception</td>
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<td>Dr. Nora Volkow</td>
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<td>Sweta Adhikary</td>
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Introduction
11:00am – 11:10am
Welcome

Plenary Talk
11:10am – 11:40am
Dr. Nora Volkow
The Opioid Crisis: Current Status and Scientific Findings

Talks session #1
11:40am – 12:40pm
New Drug Development
Moderator:
Jay McLaughlin

Speakers:
11:40am – 11:50am
Susruta Majumdar
Structure-based design of a functionally selective bitopic ligand for the µ-opioid receptor
11:50am – 12:00pm
Vsevolod Katritch
Bioorthogonal covalent antagonists for prolonged treatment of fentanyl overdose
12:00pm – 12:10pm
Chiara Ruzza
In vitro and vivo pharmacological characterization of the clinically viable NOP receptor antagonist BTRX-246040
12:10pm – 12:20pm
Shannan McClain
A photoactivable opioid agonist and antagonist for in vivo photopharmacology
12:20pm – 12:30pm
Julie Sanchez
Signaling profile of dual-target MOR and D3R ligands as potential non-addictive analgesics

Day 1 • July 12th

12:30pm – 12:40pm
Peng Huang
NCP, a dual mu and kappa opioid receptor agonist, is a potent analgesic without reinforcing or aversive properties

Remembrance
12:40pm – 12:50pm
Dr. Alan Gintzler remembrance: Larry Toll
Dr. Mary-Jeanne Kreek remembrance: Brian Reed

Posters Session #1
12:50pm – 2:00pm
Posters
Roundtable #1
Social: Gather Town (Passcode: @INRC2021)
Dr. Nora Volkow
Director of the National Institute on Drug Abuse (NIDA) at the National Institutes of Health

The Opioid Crisis: Current Status and Scientific Findings

Nora D. Volkow, M.D., is the Director of the National Institute on Drug Abuse (NIDA), which supports most of the world’s research on the health aspects of drug abuse and addiction. Dr. Volkow’s scientific research was instrumental in demonstrating that drug addiction is a disease of the human brain and, as NIDA Director, her work has promoted research that improves the prevention and treatment of substance use disorders. As a research psychiatrist, Dr. Volkow pioneered the use of brain imaging to investigate the toxic and addictive effects of abusable drugs. Her studies documented disruption of the dopamine system in addiction with its consequential functional impairment of frontal brain regions involved with motivation, executive function and self-regulation. She has also made important contributions to the neurobiology of obesity, and ADHD and has published more than 840 peer-reviewed articles, written more than 100 book chapters and non-peer-reviewed manuscripts, co-edited a Neuroscience Encyclopedia and edited four books on neuroimaging for mental and addictive disorders.
Susruta Majumdar
Center for Clinical Pharmacology, University of Health Sciences & Pharmacy and Washington University School of Medicine, St. Louis, USA

Abdelfattah Faouzi, Haoqing Wang, Saheem A. Zaidi, Tao Che, Jeffrey F. Diberto, Qianhui Qu, Michael J Robertson, Manish Madasu, Amal El Daibani, Kevin Appourchaux, Tiffany Zhang, Samuel T. Slocum, Ying Xian Pan, Ream Al-Hasani, Bryan L. Roth, Jay P. McLaughlin, Georgios Skiniotis, Vsevolod Katritch, Brian Kobilka and Susruta Majumdar*.

**Structure-based design of a functionally selective bitopic ligand for the µ-opioid receptor**

Like for many Family A GPCRs, Na+ acts as a negative allosteric modulator at the µOR. The Na+ binding pocket has been identified in inactive-state crystal structures of several GPCRs revealing a key interaction with a highly conserved Asp residue at the cytoplasmic end of TM2. In the µOR, there is a polar channel that connects the orthosteric binding pocket with the Na+ binding pocket. Given the strong allosteric effect of Na+, we chose to explore the functional effect of engaging the Na+ pocket by structure-based design of a library of bitopic ligands based on a fentanyl scaffold. Functional studies suggest that bitopic ligand interactions with the Na+ pocket control their efficacy and functional selectivity profiles for both Gi/o/z subtypes and arrestins. We obtained cryoEM structures of µOR in complex with the two most potent bitopic agonists validating their design, and highlighting key interactions between the guanidine group of bitopics and the sodium binding pocket.

Vsevolod Katritch
Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, USA

Vsevolod Katritch*, Saheem A. Zaidi, Joice Thomas, Valery V. Fokin, Shainnel O. Eans and Jay P. McLaughlin

**Bioorthogonal covalent antagonists for prolonged treatment of fentanyl overdose**

Saving the lives of many patients who overdose from fentanyl and its derivatives requires a potent antagonist which is longer-acting than naloxone. Here we report discovery of a highly potent and long-lasting mu-Opioid antagonist which completely reverses fentanyl action in mice for more than 24 hours.
Chiara Ruzza
Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy
Chiara Ruzza*, Joaquim Azevedo Neto, Federica Ferrari, Sabrina Rizzo, and Girolamo Calo

In vitro and vivo pharmacological characterization of the clinically viable NOP receptor antagonist BTRX-246040

BTRX-246040 (also known as LY2940094) is a novel NOP antagonist that has been already studied in humans. In the present study BTRX-246040 has been tested in vitro in the following assays: calcium mobilization in cells expressing NOP and classical opioid receptors and chimeric G proteins, BRET assay measuring NOP interaction with G proteins and β-arrestins, the label free dynamic mass redistribution assay, and the electrically stimulated mouse vas deferens. In all assays BTRX-246040 behaves as a pure, potent and selective NOP antagonist. In vivo, BTRX-246040 has been tested in mice in the forced swimming test (FST) and in the learned helplessness test (LH), in a curative protocol and in a preventive protocol. We demonstrated that BTRX-246040 evokes antidepressant effects in mice in the FST (active doses 10 and 30 mg/kg) and in the LH test (30 mg/kg), and, when given before the induction sessions, it prevents the development of helplessness. This study, performed with a clinically viable NOP antagonist corroborate the hypothesis that NOP antagonists can be useful not only as antidepressant drugs, similar to classical antidepressants, but are also worthy of investigation as preemptive treatments in patients with severe risk factors for depression.

Shannan McClain
University of California, San Diego, USA
Shannan McClain*

A photoactivable opioid agonist and antagonist for in vivo photopharmacology

Light-sensitive drugs afford precise control over the time and location of drug delivery; advantages that can drive mechanistic studies and reduce side effects in a clinical setting. Here we discuss the development of photoactivatable or “caged” variants of two clinically used opioid drugs: the agonist “PhOX” (photoactivatable oxymorphone) and the antagonist “PhNX” (photoactivatable naloxone). We demonstrate that these drugs cross the blood brain barrier and thus can be used in vivo to study reward and pain processing circuits in mice.
Julie Sanchez
Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, Queen’s Medical Centre and Centre of Membrane Protein and Receptors, Universities of Birmingham and Nottingham, United Kingdom

Julie Sanchez*, Alessandro Bonifazi, Vsevolod Katritch, Amy Hauck Newman, J. Robert Lane, Meritxell Canals

Signaling profile of dual-target MOR and D$_3$R ligands as potential non-addictive analgesics

Opioids are still the mainstay treatments for severe acute pain, despite their abuse liability and severe side effects. Targeting the dopamine D$_3$ receptor (D$_3$R) with antagonists or partial agonists has the potential to reduce the abuse liability of opioids without reducing their antinociceptive effects, thus improving opioid safety. Here we characterise novel dual-target ligands that bind to both the µ-opioid receptor (MOR) and D$_3$R with the aim of progressing towards the discovery of non-addictive analgesics.

Peng Huang
Center for Substance Abuse Research, Temple University Lewis Katz School of Medicine, Philadelphia, USA

Peng Huang*, Danni Cao, Chongguang Chen, Bashi Huang, E Andrew Townsend, Matthew Banks, Conrad Kenden Ho, Yan Zhang and Lee-Yuan Liu-Chen

NCP, a dual mu and kappa opioid receptor agonist, is a potent analgesic without reinforcing or aversive properties

While both MOR agonists and KOR agonists have analgesic effects, they produce opposite hedonic states, euphoria and dysphoria, respectively. KOR agonists have been shown to reduce the rewarding effects of MOR agonists. We hypothesize that compounds with dual MOR and KOR agonist activities may be effective analgesics with low probability of producing dysphoria or addiction. We found that in in vitro [35S]GTPγS binding assay, NCP, a 4,5-epoxymorphinan compound, displayed potent KOR full agonist activity and MOR partial agonist activity (58%) with a moderate KOR/MOR selectivity (6.4x). NCP is also a low-potency full agonist at the DOR with high KOR/DOR selectivity (107x). In CD-1 mice, NCP (s.c.) reduced licking time in the late phase of the formalin test and decreased the number of writhing in the acetic acid writhing test in a dose-dependent manner with A50 values of 47.6 µg/kg and 14.4 µg/kg, respectively, indicating potent antinociceptive activity. Pretreatment with both beta-funaltrexamine (β-FNA) (32 mg/kg, s.c.) and norbinaltorphimine (norBNI) (32 mg/kg, i.p.) or norBNI (32 mg/kg, i.p.) alone, but not β-FNA (32 mg/kg, s.c.) alone, blocked NCP-induced antinociception in the acetic acid writhing test, indicating KOR-mediated effects. However, unlike the prototypic kappa agonist U50,488H, NCP did not inhibit compound 48/80-induced scratching, cause conditioned place aversion (at 40 and 80 µg/kg, s.c.), impair rotarod performance or inhibit locomotor activity (at 80 µg/kg, s.c.). In intravenous self-administration, NCP did not function as a reinforcer at 1, 10, or 100 µg/kg/infusion in rats trained to self-administer heroin (32 µg/kg/infusion). These results indicate that NCP produces potent analgesic effects without causing aversion, sedation, motor incoordination or reinforcing effects. Therefore, dual MOR/KOR agonists may be promising as an avenue for developing non-addicting analgesics.
Plenary Talk
11:00am – 11:30am
Dr. Yasmin Hurd
Translating neuroscience advances towards novel treatments for opioid abuse

Talks session #2
11:30am – 12:30pm

**Opioid Use Disorder**

Moderator:
Kabir Lufty

Speakers:

11:30am – 11:40am
Fereshteh Nugent
Potentiation of glutamatergic synaptic transmission onto lateral habenula neurons following early life stress and intravenous morphine self-administration in rats

11:40am – 11:50am
Dillon McGovern
Ventral Tegmental Area glutamate neurons contribute to cue-induced oxycodone seeking behavior

11:50am – 12:00pm
Brady Atwood
Prenatal opioid exposure reprograms the behavioral response to future alcohol reward

12:00pm – 12:10pm
Sweta Adhikary
Chronic opioid use disrupts kinase regulation of other GPCRs

12:10pm – 12:20pm
Stephanie Puig
Novel mechanisms of peripheral opioid tolerance: involvement of PDGFRB and keratinocyte signaling

Talks session #3
12:20pm – 1:20pm

**Respiratory Depression**

Moderator:
Erica Levitt

Speakers:

12:20pm – 12:30pm
Sebastian N. Maletz
Respiratory-controlling brainstem nuclei activated by opioids and hypercapnia

12:30pm – 12:40pm
Beth Weise
Brain penetrant, but not peripherally restricted, synthetic cannabinoid-1 receptor agonists promote morphine-mediated respiratory depression

12:40pm – 12:50am
Brian Ruyle
Opioid-Induced Respiratory Depression involves opioid receptors at both central and peripheral sites

12:50am – 1:00pm
Damiana Cavallo
The ability of fentanyls and other opioids to produce respiratory muscle rigidity correlates with their agonist efficacy

1:00pm – 1:10pm
Rob Hill
Mitragynine respiratory depression in mice is mediated and metabolically limited by its active metabolite 7-OH mitragynine

1:10pm – 1:20pm
Khadija Nefzi
Screening of novel heterocyclic peptidomimetics for peripherally-restricted opioid agonist activity for antinociception with fewer liabilities

Posters Session #2
1:20pm – 2:00pm
Posters
Roundtable #2
Social: Gather Town (Passcode: @INRC2021)
Dr. Yasmin Hurd
Professor of Psychiatry and Neuroscience
at the Icahn School of Medicine in New York, USA

Translating neuroscience advances towards novel treatments for opioid abuse

Dr. Yasmin Hurd is Professor of Psychiatry and Neuroscience at the Icahn School of Medicine in New York, USA. She is an internationally renowned neuroscientist whose translational research examines the neurobiology of drug abuse and related psychiatric disorders with primary focus on opioid abuse and the developmental effects of cannabis. She is highly published in the field and leads a team of investigators in molecular biology, behavioral neuropharmacology, genetics and neuroimaging to study the human brain as well as translational animal models. Dr. Hurd is also Director for the Addiction Institute within the Mount Sinai Behavioral Health System which covers one of the largest addiction populations in the US providing clinical care supported by science-based medicine and advanced state-of-the-art research. Based on her high impact accomplishments and her advocacy of drug addiction education and health, Dr. Hurd was inducted into the National Academy of Medicine that complements other honors she has received in the field.
Fereshteh Nugent
Edward Hebert School of Medicine, Department of Pharmacology, Uniformed Services University, Bethesda, USA

Ludovic D. Langlois, Rina Y. Berman, Ryan D. Shepard, Sarah C. Simmons, Mumeko C. Tsuda, Shawn Gouty, Kwang H. Choi and Fereshteh S. Nugent*

Potentiation of glutamatergic synaptic transmission onto lateral habenula neurons following early life stress and intravenous morphine self-administration in rats

Here, we explored how maternal deprivation (MD) as an early life stressor affects intravenous morphine self-administration (MSA) acquisition and sucrose preference as well as glutamatergic synaptic function in lateral habenula (LHb) neurons of adult male rats self-administering morphine. We found that MD significantly reduced morphine intake, triggered anhedonia-like behavior in the sucrose preference test, and was associated with persistent glutamatergic potentiation 24h after the last MSA session.

Dillon McGovern
Department of Psychology & Neuroscience, University of Colorado Boulder, Boulder, USA

Dillon McGovern*, Abigail Polter, Emily Prevost, Annie Ly, Declan Mulcahy

Ventral Tegmental Area glutamate neurons contribute to cue-induced oxycodone seeking behavior

Ventral tegmental area (VTA) GABA neurons regulate dopamine neuron activity via a local projection modulated by the mu-opioid receptor. A subset of neurons within the VTA, defined by the presence of the vesicular glutamate transporter (VGluT2), also regulate dopamine neuron activity via a local projection but their role in drug-seeking behavior is unknown. We first identified that select subsets of VTA VGluT2+ neurons express the mu opioid receptor. Further, optogenetic activation of local projections from VTA VGluT2 neurons results in mu-opioid receptor sensitive currents. These data led us to hypothesize that VTA VGluT2 neurons participate in opioid-seeking behavior. To test this hypothesis, we leveraged an oral oxycodone self-administration paradigm to determine the functional role of VGluT2+ VTA neurons in both oxycodone self-administration and cue-induced drug-seeking during reinstatement.
Brady Atwood
Department of Pharmacology & Toxicology, Indiana University School of Medicine, Indianapolis, USA
Brady Atwood*

Prenatal opioid exposure reprograms the behavioral response to future alcohol reward

The opioid crisis has contributed to an increasing number of infants exposed to opioids during the prenatal period, but the long-term impact of prenatal opioid exposure on offspring brain and behavior remain largely undetermined. No studies to date have examined the effect of prenatal opioid exposure on future sensitivity to alcohol reward, which is one of the most likely substances the growing population of children with prenatal opioid exposure will encounter as they mature. We will discuss a recently developed translational mouse model of prenatal methadone exposure (Grecco et al., eLife 2021) and present new data showing that prenatal methadone exposure increases distinct alcohol reward-related behaviors in males and females.

Sweta Adhikary
Oregon Health & Science University, Portland, USA
Sweta Adhikary*, Omar Koita, Joe Lebowitz, William T. Birdsong, John T. Williams

Chronic opioid use disrupts kinase regulation of other GPCRs

Chronic treatment by opioids differentially alter kinase regulation of somatostatin receptors in the Locus Coeruleus. This heterologous effect is agonist specific and mediated by sustained signaling by partial agonists.
Stephanie Puig
Department of Psychiatry, University of Pittsburgh, Pittsburgh, USA

**Novel mechanisms of peripheral opioid tolerance: involvement of PDGFRβ and keratinocyte signaling**

Cellular mechanisms underlying peripheral analgesic tolerance caused by local peripheral delivery of opioids remain unknown. Using behavioral pharmacology and optogenetics we discovered that repeated optogenetic stimulation of keratinocytes in opioid naïve mice, cause tolerance to morphine in a platelet-derived growth factor receptor beta (PDGFR-β)-dependent manner. These findings bring to light that keratinocytes and PDGFR-β signalling are major players in the mechanisms underlying tolerance to peripheral administration of morphine.
Sebastian N. Maletz
Department of Pharmacology and Therapeutics, University of Florida, Gainesville, USA

Sebastian N. Maletz*, Brandon T. Reid, Adrienn G. Varga, Erica S. Levitt

Respiratory-controlling brainstem nuclei activated by opioids and hypercapnia

Impaired chemoreflex responses are a central feature of opioid-induced respiratory depression. Paradoxically, hypercapnia and opioids are both known to induce cFos expression in respiratory-controlling brainstem nuclei, but the effects of hypercapnic challenges with concurrent opioid administration remain untested. Using a combination of genetic labeling for the mu opioid receptor and immunohistochemistry, we examined the activation of neuronal populations in three opioid-sensitive brainstem nuclei involved in respiratory control.

Beth Weise
Department of Pharmacology, University of Arizona, Tucson, USA


Brain penetrant, but not peripherally restricted, synthetic cannabinoid-1 receptor agonists promote morphine-mediated respiratory depression

Utilizing whole body plethysmography, we sought to define the roles of central versus peripheral CB1R activation on respiratory function alone and in combination with morphine. As shown previously the synthetic cannabinoid, AM356 10 mg/kg, induced respiratory depression on its own; while here we show the peripherally restricted CB1 agonist (PrNMI 0.3 and 0.6 and 1 mg/kg) did not. Of further interest, the combination of this peripherally restricted CB1 agonist, PrNMI 0.3 and 0.6 mg/kg, and morphine significantly prevented the respiratory depression induced by morphine, however, AM356 with morphine enhanced respiratory depression.
Damiana Cavallo
School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, United Kingdom

Damiana Cavallo*, Eamonn Kelly, Graeme Henderson and Ana Paula Abdala Sheikh

The ability of fentanyls and other opioids to produce respiratory muscle rigidity correlates with their agonist efficacy

In this study we have sought to characterise the ability of fentanyl and other opioid agonists to induce respiratory muscle rigidity, by recording electromyographic (EMG) signal of the diaphragm, external and internal intercostal muscles, in the in situ decerebrated and arterially perfused rat preparation. We found that the ability of opioid agonists to affect EMG amplitude of respiratory muscles is not a property of only fentanyl derivatives but correlates with their agonist efficacy at the μ opioid receptor and not with their lipid solubility solely.
Khadija Nefzi
Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, USA
Khadija Nefzi*, Shainnel O. Eans, Adel Nefzi, and Jay P. McLaughlin

Screening of Novel Heterocyclic Peptidomimetics for Peripherally-Restricted Opioid Agonist Activity for Antinociception with Fewer Liabilities

Given that most peptides poorly cross the blood–brain barrier after peripheral administration, we hypothesized that a new series of peripherally-selective peptidomimetic opioid agonists would demonstrate antinociception without CNS-mediated liabilities. Fifteen peptides were screened in mice with the 55°C warm-water tail-withdrawal assay, with three showing dose-dependent antinociception equivalent to morphine after peripheral administration, and one (AN2638-33) demonstrating peripherally-selective opioid activity. Consistent with this, AN2638-33 produced significantly less respiratory depression than morphine. In conclusion, the correlation between peripherally-restricted opioid activity and the absence of liabilities suggests that peripherally-selective peptidomimetics can serve as safer analgesics.

Rob Hill
Queen’s Medical Centre, University of Nottingham, Nottingham, United Kingdom
Rob Hill*, Andrew Kreugel, Jonathan Javitch, Rob Lane, Meritxell Canals

Mitragynine respiratory depression in mice is mediated and metabolically limited by its active metabolite 7-OH mitragynine

Low overdose rates following Kratom consumption have prompted examination of its major opioid alkaloid, mitragynine. Mitragynine respiratory depression in mice appears to have a ceiling effect mediated by the rate limited production of its major active metabolite 7-OH mitragynine.
Talks Session #4
11:00am – 12:00pm

Opioid Receptors - Signaling

Moderator: Meritxell Canals

Speakers:

11:00am – 11:10am
Eamon Kelly
Fentanyl binds to the µ-opioid receptor via the lipid bilayer and transmembrane helices

11:10am – 11:20am
Gissell Sanchez
The role of phospholipase C-β3 (PLCβ3) in opioid signaling

11:20am – 11:30am
Alexander R. French
Real-time assay for simultaneous recruitment of arrestin isoforms to delta opioid receptor

11:30am – 11:40am
Nokomis Ramos-Gonzalez
Carfentanil is an arrestin-biased agonist at MOPr

11:40am – 11:50am
Javier Cuitavi
Microglial activation alters mu-opioid receptor internalization, activity and expression

11:50am – 12:00pm
Yu-Jun Wang
Alteration of twinfilin1 expression underlies opioid withdrawal-induced remodeling of actin cytoskeleton at synapses and formation of aversive memory

Talks Session #5
12:00pm – 1:00pm

Opioid Circuits

Moderator: Grégory Scherrer

Speakers:

12:00pm – 12:10pm
Chong Chen
A cortico-ponto-cerebellar circuit for placebo analgesia

12:10pm – 12:20pm
Xinyi Jenny He
Convergent, functionally independent signaling by mu and delta opioid receptors in hippocampal parvalbumin interneurons

12:20pm – 12:30pm
Khairunisa Ibrahim
Activation of dorsal hippocampal excitatory neurons induced reinforcing behaviors and an increase in nucleus accumbens neuronal activity

12:30pm – 12:40pm
Emmanuel Darcq
Opiates and habenula: Implication of MOR habenular neurons in aversive / depressive states

12:40pm – 12:50pm
William Birdsong

12:50pm – 1:00pm
Nicole Mercer Lindsay
Mapping the connections between the motor cortex and pain circuitry

Plenary Talk
1:00pm – 1:30pm
Dr. Julie Kauer
GABAergic afferents to the VTA: synaptic plasticity, opiate sensitivity, and behavioral outputs.

Conclusion & Social
1:30pm – 2:00pm
Social: Gather Town (Passcode: @INRC2021)
Eamon Kelly
School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, United Kingdom
Sutcliffe KJ, Corey RA, Charlton SJ, Sessions, RB, Henderson G & Kelly E*

Fentanyl binds to the µ-opioid receptor via the lipid bilayer and transmembrane helices

The synthetic opioid, fentanyl, is driving opioid overdose deaths in the USA and worldwide. Contributing to fentanyl’s lethality are its high potency, rapid onset of action, and reduced sensitivity to reversal by the antagonist naloxone. Here, we use coarse-grained molecular dynamics simulations and free energy calculations to examine how fentanyl binds to its pharmacological target, the µ-opioid receptor. We find that fentanyl concentrates in the lipid membrane, before binding to the µ-opioid receptor by diffusing through the membrane and receptor helices. This novel binding pathway may explain fentanyl’s high potency and poor naloxone reversibility compared to other opioids.

Gissell Sanchez
Department of Pharmacology, University of Michigan, Ann Arbor, USA
Gissell A. Sanchez*, Alan V. Smrcka, Susan Ingram, Emily Jutkiewicz

The role of phospholipase C-β3 (PLCβ3) in opioid signaling

The µ-opioid receptor (MOR) is a Gi-protein coupled receptor (GPCR) responsible for opioid-induced analgesia and undesired effects such as constipation, respiratory depression, and addiction. Upon binding of an agonist, the Gαi and the Gβy/subunits dissociate to signal downstream effectors. One such effector is phospholipase- Cβ3 (PLCβ3) that can be activated by both Gβy subunits and Gαq. PLC enzymes hydrolyze phosphatidylinositol-4,5-bisphosphate (PIP2) to produce diacylglycerol (DAG) and inositol triphosphate (IP3). Knockout of PLCβ3 in mice, and small molecule inhibition of Gβγ-PLC interactions potentiate opioid-induced antinociception. Based on these data we hypothesized that MOR-dependent activation of PLCβ3 opposes opioid-induced antinociception. To test for MOR-dependent PLC activation we established an assay using a fluorescent DAG sensor in HEK-293 cells. Cells expressing MOR were treated with saturating concentrations of DAMGO or morphine but no detectable PLC activation was observed. Since PLCβ3 is synergistically activated by Gαq and Gβy subunits, we hypothesized that MOR-dependent PLC activation may require a coincident signal from a Gq-signaling pathway. We used HEK293 cells expressing MOR with or without co-expression of M1 muscarinic receptors—a representative Gq-protein coupled receptor—to test this model. Synergistic activation of PLC was observed upon simultaneous addition of subsaturating concentrations of the muscarinic agonist carbachol, and MOR agonists morphine and DAMGO. Strong synergy was also observed when activating endogenous muscarinic receptors in HEK293 cells (likely M3). When cells were treated with pertussis toxin (PTX), the synergy driven by the co-activation of both types of GPCRs was lost suggesting that Gβy activation from MOR is necessary for PLC synergy. To test the physiological relevance of PLC synergy, we used electrophysiological techniques to test the effects of coincidental G-protein activation of PLCβ on MOR-dependent inhibition of GABA release in presynaptic neurons in the periaqueductal grey (PAG) of mice. PAG slices pre-treated with either a Gβγ inhibitor, showed enhanced DAMGO-induced inhibition of GABA release. In conclusion, coincidental activation of MOR and Gq-coupled receptors lead to synergistic activation of PLCβ that ultimately results in inhibition of opioid signaling in presynaptic neurons of the PAG.
Alexander R. French
Department of Medicinal Chemistry and Molecular Pharmacology and Institute for Integrative Neuroscience, Purdue University, West Lafayette, USA

Alexander R. French*, Yazan J. Meqbil, and Richard M. van Rijn

Real-time assay for simultaneous recruitment of arrestin isoforms to delta opioid receptor

Arrestin isoforms β-arrestin1 and β-arrestin2 have been long known as important regulators of opioid receptors, and recent studies now suggest they also have unique physiological roles at these receptors. Ideally, biased drugs could be designed to take advantage of these isoforms’ distinct roles, but current assays for arrestin recruitment only examine a single isoform in a cell, and therefore do not replicate isoform competition. This study overcomes this limitation by developing a method for recording simultaneous luminescence readouts of β-arrestin1 and β-arrestin2 recruitment to the delta opioid receptor.

Nokomis Ramos-Gonzalez
School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, United Kingdom

Nokomis Ramos-Gonzalez*, Sam Groom, Sukhvinder Bancroft, Katy Sutcliffe, Richard B. Sessions, Chris Bailey, Graeme Henderson, Eamonn Kelly

Carfentanil is an arrestin-biased agonist at MOPr

This work focussed on using in vitro techniques to assess the interaction of different fentanyl analogues with the mu-opioid receptor. Bioluminescence resonance energy transfer (BRET) was used to measure drug-induced G-protein activation and arrestin recruitment to MOPr. The agonists studied were morphine, DAMGO, fentanyl, carfentanil, alfentanil and sufentanil. Relative to DAMGO, carfentanil displayed arrestin bias in this assay whilst the other fentanyl analogues were not biased. We are currently undertaking MOPr trafficking studies to determine the functional significance of this bias, as well as undertaking Molecular Dynamics simulations to explore the structural basis for carfentanil’s bias.
**Javier Cuitavi**
Department of Pharmacy and Pharmaceutical Technology and Parasitology, University of Valencia, Burjassot, Spain

Javier Cuitavi*, Pere Duart-Abadia, Julie Sanchez, Jesús D. Lorente, Isabel Fariñas, Meritxell Canals and Lucía Hipólito

**Microglial activation alters mu-opioid receptor internalization, activity and expression**

Neuroinflammation and neuroimmunity play a very important role in the development of addiction and pain. In fact, previous research shows how proinflammatory cytokines increase the expression of the OPRM1 gene. However, not much more information is available regarding this matter. Herein, we present how microglial activation alters MOR internalization, activity and expression.

**Yu-Jun Wang**
Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Yu-Jun Wang*, Chuan Yu, Wei-Wei Wu, Yun-Yue Ju, Yao Liu, Chi Xu, Jian-Dong Long, Gui-Ying Zan, Xiang-Yan Wei, Le-Sha Zhang, Jing-Rui Chai, Zhong Chen, Jing-Gen Liu

**Alteration of twinfilin1 expression underlies opioid withdrawal-induced remodeling of actin cytoskeleton at synapses and formation of aversive memory**

Exposure to drugs of abuse induces alterations of dendritic spine morphology and density that has been proposed to be a cellular basis of long-lasting addictive memory and heavily depend on remodeling of its underlying actin cytoskeleton by the actin cytoskeleton regulators. However, the actin cytoskeleton regulators involved and the specific mechanisms whereby drugs of abuse alter their expression or function are largely unknown. Twinfilin (Twf1) is a highly conserved actin-depolymerizing factor that regulates actin dynamics in organisms from yeast to mammals. Despite abundant expression of Twf1 in mammalian brain, little is known about its importance for brain functions such as experience-dependent synaptic and behavioral plasticity. Here we show that conditioned morphine withdrawal (CMW)-induced synaptic structure and behavior plasticity depends on downregulation of Twf1 in the amygdala of rats. Genetically manipulating Twf1 expression in the amygdala bidirectionally regulates CMW-induced changes in actin polymerization, spine density and behavior. We further demonstrate that downregulation of Twf1 is due to upregulation of miR101a expression via a previously unrecognized mechanism involving CMW-induced increases in miR101a nuclear processing via phosphorylation of MeCP2 at Ser421. Our findings establish the importance of Twf1 in regulating opioid-induced synaptic and behavioral plasticity and demonstrate its value as a potential therapeutic target for the treatment of opioid addiction.
Chong Chen
Department of Cell Biology and Physiology, UNC Neuroscience Center, The University of North Carolina at Chapel Hill, Chapel Hill, USA

Chong Chen* and Grégory Scherrer

A cortico-ponto-cerebellar circuit for placebo analgesia

The placebo effect is a learning process wherein individuals experience a benefit through verbally-elicited expectations, cues, and/or contextual conditioning. Here, we examined the mechanisms that underlie placebo analgesia by combining neural circuit tracing with recording and manipulation of neural activity in freely moving mice. We provide evidence that a bi-synaptic excitatory circuit linking the anterior cingulate cortex and the cerebellum via the pontine nucleus encodes, and is sufficient for, the analgesia associated with the expectation of pain relief.

Xinyi Jenny He
Division of Biological Sciences, Neurobiology Section, University of California San Diego, La Jolla, USA

Xinyi Jenny He*, Janki Patel, Connor E. Weiss, Xiang Ma, Brenda L. Bloodgood, Matthew R. Banghart

Convergent, functionally independent signaling by mu and delta opioid receptors in hippocampal parvalbumin interneurons

Mu and Delta opioid receptors (MORs and DORs) are both present on parvalbumin basket cells (PV-BCs) in the CA1 region of the hippocampus, but it is unclear if they functionally interact. Using photoactivatable opioid neuropeptides, we find that MORs and DORs inhibit PV-BCs through partially occlusive signaling pathways that terminate on somato-dendritic potassium channels and presynaptic calcium channels, with DORs exhibiting greater ligand-sensitivity and faster kinetics. In assays for cross-desensitization and heteromer formation, we did not find evidence for crosstalk between endogenous MORs and DORs, implying that MOR/DOR functional interactions are not a preordained outcome of co-expression in neurons.
Khairunisa Ibrahim
Washington University School of Medicine, St. Louis, USA
Ibrahim Khairunisa*, Massaly Nicolas, Frye Hannah, Yoon Hye-Jean, Sandoval Rossana, Post William, Idowu Olayinka, Williams Sidney, Thomas L Kash, Kravitz Alexxai, Morón Jose Antonio

Activation of dorsal hippocampal excitatory neurons induced reinforcing behaviors and an increase in nucleus accumbens neuronal activity

The role of dorsal hippocampus (dHPC) in driving reinforcing behavior has yet to be explored although recent publication showed a functional projection from the dHPC to the nucleus accumbens (NAc) in retrieving “place-reward” appetitive memories. Our results shows that photo-activation dHPC not only induced reinforcing behaviors but also led to an increase in neuronal activity in the NAc, suggesting that the dHPC-NAc projections may encode reinforcing values that trigger reward seeking. Currently, we are investigating the varying activation response of the dynorphin and enkephalin neuronal population in the NAc with photo-stimulation of dHPC.

Emmanuel Darcq
Douglas Hospital Research Center, Dep. of Psychiatry, School of Medicine, McGill University, Montreal, Quebec, Canada and Université de Strasbourg, INSERM, France
Emmanuel Darcq*, Julie Bailly, Florence Allain and Brigitte L. Kieffer

Opiates and habenula: Implication of MOR habenular neurons in aversive / depressive states

The mu opioid receptor (MOR) is the major target for analgesic and abused opioids. Interestingly, MOR have their highest expression in the habenula (Hb), an emerging brain center for aversion processing. We recently reported a specific role of habenular MORs in the expression of aversion to Naloxone (Boulos et al. 2019). The goal of this project is to investigate how neurons expressing MOR in the Hb contribute to approach/avoidance behaviors and negative affects. We tested the hypothesis that MOR-neurons of the Hb (Hb-MOR neurons) projecting to IPN are critical to modulate aversive states, using optogenetics and MOR-cre mice (Bailly et al. 2020). We found that Hb-MOR neurons activation contribute to aversive state by increasing avoidance and depressive like behaviors. Our interpretation is that these Hb-MOR neurons drive aversive state expression and that endogenous opioid or opiates would limit this aversion.
William Birdsong
Department of Pharmacology, University of Michigan, Ann Arbor, USA

William Birdsong*, Elizabeth Jaeckel, Alberto Perez-Medina, Erwin Arias-Hervert


The medial thalamus sends axonal projections to the striatum and prefrontal cortex and both projections are regulated by mu-opioid receptor signaling. Chronic opioid treatment induces analgesic tolerance but tolerance does not develop to all opioid-mediated effects. Here we show that, within the same cell population, tolerance to morphine differentially develops based on the site of axon projections.

Nicole Mercer Lindsay
Department of Biology, CNC Program, Stanford University, Stanford, USA and Department of Cell Biology and Physiology, UNC Neuroscience Center, The University of North Carolina at Chapel Hill, Chapel Hill, USA

Mercer Lindsay, Nicole*, Schnitzer, Mark J., and Scherrer, Grégory

Mapping the connections between the motor cortex and pain circuitry

Modulation of motor cortex activity using transcranial magnetic or electrical stimulation has been shown to relieve chronic pain; however, how the motor cortex is connected with and influences activity in pain circuits is poorly understood. Here we use intersectional strategies with cutting edge viral and genetic tools to identify motor cortex nociceptive neurons and trace their connectivity, with a focus on pain experience-related regions such as the amygdala, periaqueductal gray, and thalamus. Finally, we used calcium imaging of neuronal activity of thousands of neurons throughout the neocortex to determine how motor cortex responds to painful stimuli.
Dr. Julie Kauer
Professor of Psychiatry and Behavioral Science,
Stanford University School of Medicine

GABAergic afferents to the VTA: synaptic plasticity, opiate sensitivity, and behavioral outputs.

Dr. Julie Kauer received her PhD in Pharmacology at Yale University, and has been a faculty member at Duke University School of Medicine and Brown University prior to her recent move to Stanford. For over twenty-five years, Dr. Kauer’s work has focused on the study of neuronal excitability, synaptic transmission and plasticity in the context of drug addiction, stress and pain. She has served as Associate Editor for the Journal of Neuroscience, was a member of the APS Editorial Board of Physiology, has served on the Editorial boards of Physiological Reviews and the Journal of Neurophysiology, and currently is a Reviewing Editor for eLife. She was the elected Chair of the Gordon Research Conference on Synaptic Transmission in 2006, and was an invited Special Lecturer at the annual Society for Neuroscience meeting in 2008. She has served on the NIH study section, MNPS, and the Board of Scientific Counselors for NINDS. In 2012, she was elected Fellow of the American Association for the Advancement of Science in recognition of her work on synaptic function and plasticity.

Dr. Kauer’s laboratory uses electrophysiological, optogenetic, behavioral and mouse genetic approaches to identify brain and spinal cord circuitry underlying addiction and pain. The lab currently has two major research areas. The first project focuses on plasticity at inhibitory GABAergic synapses in the ventral tegmental area (VTA), how different GABAergic inputs regulate the local circuit, and their modulation by stress and drugs of abuse. They have found that kappa opioid receptors gate this plasticity, and become persistently active after acute stress for a period lasting at least five days, and have linked this observation to stress-induced relapse to cocaine-seeking in rodents. Blocking kappa opioid receptors even days after the initial stress insult prevents stress-induced relapse to cocaine-seeking. In a second major project, the Kauer lab is investigating synaptic plasticity and persistent alterations in excitability in pain circuitry in the dorsal horn of the spinal cord and the midbrain periaqueductal gray, two regions highly sensitive to opiate analgesia. Brief optogenetic activation of the nociceptive afferents triggers long-lasting alterations in firing properties of dorsal horn neurons, and the lab is characterizing these excitability changes as well as the synaptic output of the same neurons in the brain.
Roundtables

Moderators:
Drs. Ream Al-Hasani, Lucia Hipolito, Anne Murphy

'Let's talk about sexism in academia'
Discuss Sexism in Academia and its impact on Professional Development. We will examine and consider means to build up Equity and Inclusive Environments for Women Scientists, foster discussion on best practices to support undergraduate and graduate students, postdocs, and faculty through their personal and professional growth. All INRC members at all levels are encouraged to attend this session.

Moderators:
Drs. Matthew Banks, Jessica Higginbotham, Lucia Hipolito, Tamara Markovic, Nicolas Massaly, Steve Negus, Jose Moron-Conception, David Reiner

'Rodent Models of Pain and Opioid Consumption'
Discuss the significance and limitations of active opioid seeking in rodent models of pain. We will examine recent data and analyze the differences in the models used to assess how different paradigms, and acute vs persistent pain may impact the observed behavioral outcome. We encourage all level INRC attendees interested in pain and opioid consumption to join us and participate in this roundtable.
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<td>HDAC5 in the rat nucleus accumbens suppresses intrinsic excitability and heroin seeking in a cell type-specific manner</td>
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<td>Murine brain gene expression during neonatal opioid withdrawal versus human placental DNA methylation following in uteroopiod exposure: identifying shared gene networks to bridge preclinical and clinical neonatal opioid withdrawal syndrome (NOWS) research</td>
<td>Borrelli</td>
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<td>RGS-insensitive mice define roles of presynaptic mu opioid receptor (MOR)-Gα and Gαi subunit coupling in inhibition of GABA release</td>
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<td>Inhibition of spinal cord Hsp90 enhances SrcKinase and Protein Kinase C signaling to increase opioid anti-nociception</td>
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<td>The ability of fentanyl and other opioids to produce respiratory muscle rigidity correlates with their agonist efficacy</td>
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<td>Semi synthetic diversification of mitragynine template leading to partial agonists with safer analgesic profiles</td>
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<td>Agonist-promoted phosphorylation and internalization of the kappa opioid receptor (KOR) in mouse brains: Lack of correlation with conditioned place aversion</td>
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<td>Sturaro</td>
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<td>Jaeckel</td>
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