KappaCon 2021

The 6th Conference on the

"Therapeutic Potential of Kappa Opioids in Pain and Addiction"

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https://wustl-hipaa.zoom.us/meeting/register/tJEofumuqjgrHdHhRL5fHnaKnChP6zx-MZ6T

If you are still are not able to connect, please email Nicolas Massaly (nmassaly@wustl.edu) for assistance.

Interacting during Zoom sessions

For Zoom sessions, please keep your microphone muted unless you are presenting or asking a question. In the spirit of interactivity, we ask that you keep your video camera on when you are comfortable.

We ask that attendees **reserve questions to the end of each talk** or to the discussion periods, unless the inquiry is a point of clarification. Participants can ask questions by

- using the “raise hand” feature
- writing in the chat that you have a question
- writing your question in the chat

Moderators will call on those who indicate they have questions during discussion periods.

Breakout rooms will be available. You should be able to move yourself into a breakout room, for instance if you would like to step away from the session and chat with another attendee. There will be an **IT breakout room** where anyone with Zoom, gather.town, or Slack issues or questions can go for assistance.

To avoid unwanted disruptions (e.g. "zoom-bombing") **attendees should not share the links to the virtual conference sites**.

Instructions for connecting to gather.town sessions*

Access the gather.town space here:

https://gather.town/app/zHQyjMa3KQHzBmy3/KappaTherapeutics

You must use the email address that you registered for the conference with as your gather.town log in email.

*Guidelines adapted from: https://events.library.cmu.edu/aidr2020/wp-content/uploads/sites/6/2020/10/Gather.town-instruction.pdf

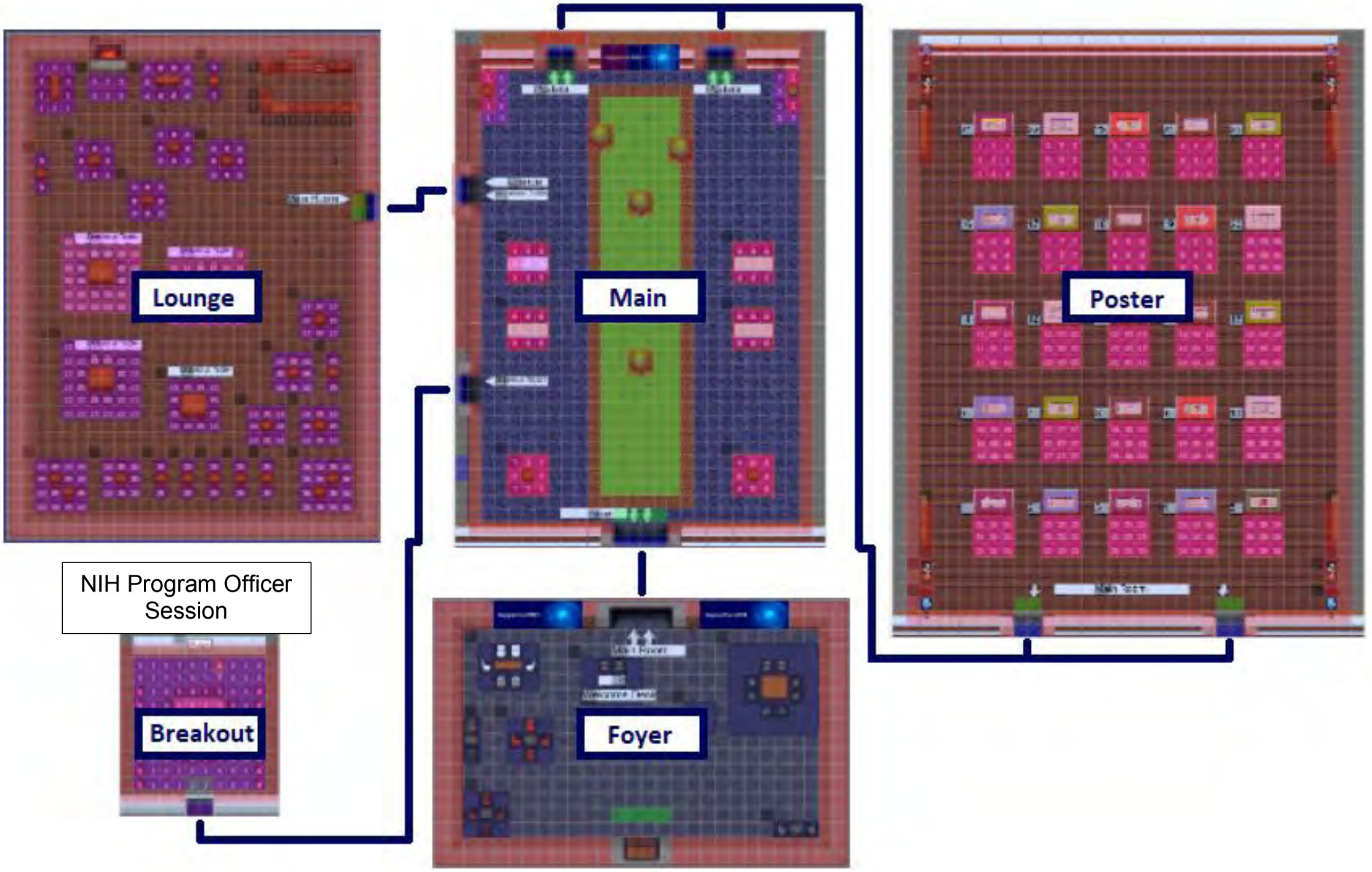
- Enter your first and last name (as it would appear on a nametag), select your camera and audio inputs, and click “Join the Gathering”.
- Upon entry a window will open with the message “Welcome to Gather”. It has a link to the tutorial, which you can skip or watch, as you prefer.
- On the right side of the screen, a list of participants will appear. You can search for the participants you would like to interact with. A direction line will show up, just follow that to reach the desired participant.
- Use your mouse or keyboard to walk around the space. You will be automatically connected by video and audio when you are in proximity to someone else.
- Posters are located in the poster room off of the main room. To view a poster, move your avatar to the poster of interest and press “x” to interact.
- Discussion tables for specified topics are located in the Lounge. To join these tables, simply move your avatar to one of the chairs or the space around the table, and you will be connected to others at the table.
- The chat function enables you to chat with anyone in our gather.town space.
- If you need assistance, please go to the welcome desk in the foyer and someone will be able to help you. Assistance will also be available via our Zoom link in the “technical assistance” breakout room for connectivity issues.
- The Kappa Therapeutics gather.town environment will be available 24 hr a day Apr 6-9, and we welcome people to utilize the platform for more spontaneous discussions, gatherings, and poster viewings during these days.

Instructions for connecting to the KappaCon Slack Channel

Slack is a messaging platform that facilitates both group and direct messaging, in “channels”, with additional features adapted for professional use. Slack is not required for any sessions planned for the meeting; we have created the KappaCon workspace on Slack to enhance and facilitate communications across our community, asynchronously and beyond the dates of the conference. For instance, this workspace can be used to continue discussions started during the meeting sessions. We have also created channels for job opportunity postings and technical assistance, and welcome suggestions for other channels to add.

Join us here:

https://join.slack.com/t/kappacon/shared_invite/zt-nz8pktci-pQ2Htvpa~0DUCKtBzDYblA


Lounge
Main
Poster
NIH Program Officer Session
Breakout
Foyer

Virtual Kappa Therapeutics, 2021
April 6-9

Zoom

Gather.Town

Tuesday April 6 (all times in PDT)

11:00 am – 11:10	Elyssa Margolis	Welcome
	Nora Volkow	
	George Koob	
Highlighting Sex as a Biological Variable in Kappa Research Moderator: Elena Chartoff		
11:10 – 11:30	Peng Huang	Agonist-induced KOR phosphorylation has sex-specific effects on KOR-mediated behaviors in mice
11:30 – 11:50	Keionna A. Newton	Regulation of kappa opioid receptor inactivation by NorBNI depends on sex and cellular site of antagonist action
11:50 – 12:10	Michael Nader	PET Imaging Studies of Kappa Opioid Receptors in Socially Housed Female and Male Monkey Models of Cocaine Use Disorder
12:10 – 12:25	Caroline A. Browne	KOR agonist induced avolition on a progressive ratio task in female and male rats
12:25 – 12:35	Discussion	
Short Break		
12:40 – 1:00	Brian Reed and colleagues	*Remembering Mary Jeanne Kreek*
Emerging Tools for Kappa Opioid Systems Neuroscience Moderator: Michael Bruchas		
1:00 – 1:20	Lin Tian	Imaging opioid peptides with genetically encoded indicators
1:20 – 1:40	Matthew R. Banghart	Toward an all-optical toolkit for probing dynorphin dynamics in the brain
1:40 – 1:55	Sineadh M. Conway	An electrochemical approach for rapid and sensitive detection of opioid peptides
1:55 – 2:15	Michael Placzek	Kappa opioid receptor agonist-mediated neural activation measured with functional MRI in rats
2:15 – 2:25	Discussion	

Wednesday April 7 (all times in PDT)

The Kappa System in Depression, Pain, and Analgesia Moderator: Jose Moron-Concepcion		
11:00 am – 11:15	Mariana Spetea	Antinociceptive efficacy of diphenethylamines, as selective kappa-opioid agonists, in inflammatory pain without causing aversion and sedation after subcutaneous administration in mice
11:15 – 11:35	Manish K. Madasu	Peripheral kappa opioid receptor activation drives cold hypersensitivity in mice

11:35 – 11:55	Moriah L. Jacobson	Changes in kappa opioid receptor signaling are required for ketamine's antidepressant action
Short Break		
Noon – 12:20	Prem N Yadav	Kappa opioid receptor mediates epigenetic silencing of BDNF in treatment resistant depression
12:20 – 12:40	Andrew D. Krystal	NIMH FAST-MAS Proof of Mechanism Study Evaluating if the Selective κ Opioid Antagonist JNJ-67953964 Engages Reward Circuitry in Anhedonic Patients with Mood and Anxiety Spectrum Disorders
12:40 – 12:55	Nicholas P. Massaro	Structure–Activity Relationships on Collybolide: Discovery of a Potent Kappa-Opioid Agonist with Enhanced Metabolic Stability
12:55 – 1:05	Discussion	
1:10 – 1:40	Poster Session 1	
1:40 –	Social	

Thursday April 8 (all times in PDT)

Novel Indications and Systems for KOR Translation Moderator: William Carlezon		
11:00 – 11:20	Bronwyn Kivell	Nalfurafine promotes remyelination and increases oligodendrocyte numbers in the augmented cuprizone model of multiple sclerosis
11:20 – 11:40	Andrew T. Luskin	Extended amygdala projections to parabrachial dynorphin neurons alter threat perception and encode feeding behaviors
11:40 – Noon	Leandra Mangieri	Decoding Dynorphin-Kappa Opioid Circuit Interactions During Stress-induced Binge Eating
Noon – 12:20	Antony D. Abraham	Prefrontocortical dynorphin opioids disrupt cognition through distinct pre- and postsynaptic mechanisms
12:20 – 12:30	Discussion	
Short Break		
Kappa Mechanisms and Translation for Itch Moderator: Sarah Ross		
12:35 – 12:55	Tayler D. Sheahan	Probing the cellular basis of kappa opioid receptor inhibition of itch and chemical pain
12:55 – 1:15	Eileen Nguyen	Medullary kappa opioid receptor neurons inhibit pain and itch through a descending circuit
1:15 – 1:25	Discussion	
1:30 – 2:30	Round Table Discussion: Short acting KOR antagonists: how do they stack up? Moderator: Charles Chavkin Panelists: Eduardo Butelman, Renata Marchette, Elyssa Margolis, Christoph Schwarzer, Sarah Simmons, Brian Trainor, Yan Zhou	

Friday April 9 (all times in PDT)

The Kappa System and Substance Use Disorder Moderator: Lee-Yuan Liu-Chen		
11:00 – 11:20	Ruby A. Holland	Activation of kappa opioid receptor-expressing neurons in the ventral tegmental area attenuates opioid withdrawal
11:20 – 11:40	Yan Zhou	Clinically utilized nalfurafine combined with naltrexone at low doses prevents excessive and "relapse" alcohol drinking in mice
11:40 – Noon	Jay P. McLaughlin	Refinement of kappa opioid activity in multifunctional cyclic tetrapeptides facilitates safer analgesics and treatments for substance abuse
Noon – 12:15	Vladana Vukojević	Effect of acute exposure to ethanol on kappa-opioid receptor antagonists binding. Live cell study using Fluorescence Lifetime Imaging Microscopy (FLIM)
12:15 – 12:25	Discussion	
Short Break		
New Advances in Understanding KOR at the Molecular Level Moderator: Bryan Roth		
12:30 – 12:50	Tao Che	Structural Studies Illuminate KOR Ligand Pharmacology
12:50 – 1:10	Manoj Puthenveedu	Compartment-specific signaling by kappa opioid receptors is selectively modulated by distinct Dynorphin subtypes
1:10 – 1:30	David Siderovski	Regulator of G protein Signaling-12 (RGS12): An accidental tourist in Kappa-Land
1:30 – 1:40	Discussion	
1:45 – 2:15	Poster Session 2	
2:15 –	Social and NIH Program Officer Session	

FEATURED SESSIONS

Round Table Discussion: Short acting KOR antagonists: how do they stack up?
April 8, 2021 1:30 pm PDT, on Zoom

Moderator: Charles Chavkin (University of Washington)
Panelists:
Elyssa Margolis (UCSF)
Sarah Simmons (Uniformed Services University)
Eduardo Butelman (Rockefeller University)
Christoph Schwarzer (Medical University of Innsbruck)
Brian Trainor (UC Davis)
Renata Marchette (NIAAA)
Yan Zhou (Rockefeller University)

Extensive preclinical research has provided compelling evidence that blocking KOR will have therapeutic efficacy for various indications including major depressive disorder, anxiety, substance use disorder, and pain. While bench scientists have had access to highly selective but long acting antagonists such as norBNI since 1987 and kappa opioid receptor knockout mice since 1998, more clinically viable short acting antagonists have only more recently become available. Whether or not these novel compounds have the desired pharmacological properties and will generate the desired pre/clinical outcomes are ongoing questions. Here we will discuss recent observations, both encouraging and challenging, for an objective perspective on the promise of short acting KOR antagonists in the clinic. **Dr. Elyssa Margolis** will describe *ex vivo* electrophyiological characterizations of BTRX-335140, BTRX-395750 (Blackthorn Therapeutics, Inc), Aticaprant (also known as JNJ-67953964, Johnson and Johnson (formerly CERC-501 (Cerecor, Inc.) and LY2456302 (Eli Lilly and Co.)), and PF-04455242 (Pfizer, Inc). Selectivity, reversal, and off target effects were probed in a ventral tegmental area brain slice preparation. Next, **Dr. Sarah Simmons** will present *ex vivo* electrophysiological observations in the lateral habenula comparing the impacts of norBNI, Aticaprant, BTRX-084266 (BlackThorn Therapeutics, Inc), and LY2444296 (Eli Lilly and Co.) on U50,488 actions. **Dr. Eduardo Butelman** will describe the rapid onset antagonist effects of LY2795050 (Ely Lilly and Co.) in mouse models including forced swim test and locomotor activity. **Dr. Christoph Schwarzer** will relate tests of the impact of BTRX-335140 and Aticaprant on pentylenetetrazol induced seizures. Aticaprant reduced seizure thresholds 1 hr after administration, similar to Pdyn knockout mice, while BTRX-335140 did not. **Dr. Brian Trainor** will describe work in the California mouse social defeat model: administering AZ-MTAB (AstraZeneca Pharmaceuticals) prior to the stressor blocked both short-term anxiety responses and long lasting increases in sucrose-anhedonia in both sexes. When AZ-MTAB was only administered several weeks prior to behavioral testing several weeks after the stressor, stress induced phenotypes were sustained. **Dr. Renata Marchette** will share data comparing the effects of Aticaprant to norBNI on vaporized fentanyl self-administration. Where Aticaprant failed to reduce fentanyl self-administration in dependent animals, norBNI significantly reduced fentanyl intake in dependent female mice. **Dr. Yan Zhou** will report on the effects of Aticaprant and norBNI on EtOH drinking in male and female mice during acute withdrawal. Aticaprant decreased EtOH intake in both sexes, to a greater extent in males than in females, while norBNI only decreased consumption in males. **Dr. Charles Chavkin** will then lead a discussion with the panelists and audience, including identifying the critical open questions regarding the clinical utility of the currently available short acting KOR antagonists.

COI: Work presented by EM was supported by a sponsored research agreement with UCSF from BlackThorn Therapeutics, Inc. All other presenters have no COI to disclose.

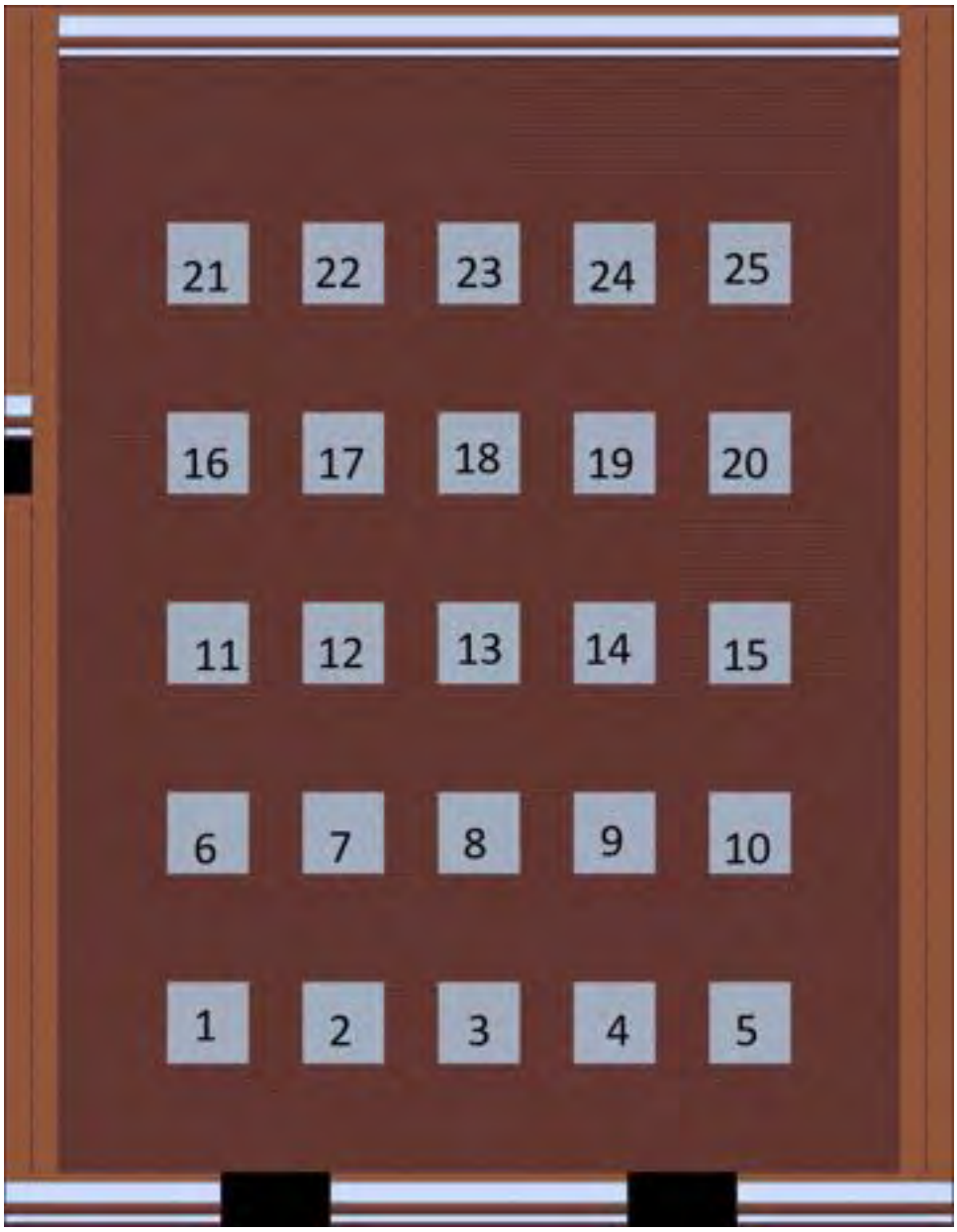
NIH Program Officers in Discussion

April 9, 2:15 pm PDT, during the social on gather.town

This discussion will be opportunity for trainees to learn more about the nuts and bolts of applying for NIH funding and for all to hear about grant mechanisms and new funding opportunities. **We encourage all attendees to bring their questions!** Breakout groups by topics and one-on-one meetings are also possible in our dedicated gather.town NIH PO event room. Participating program officers include:

- *Changhai Cui (NIAAA)*
- *Mark Egli (NIAAA)*
- *DP Mohapatra (NINDS/HEAL)*
- *Jenica Patterson (NIAAA)*
- *Roger Sorensen (NIDA)*
- *Ashlee Van't Veer (NIMH)*

Gather.town poster layout



Poster Sessions

Session 1: April 7, 1:10p – 1:40 PDT on gather.town; odd numbered posters
Session 2: April 9, 1:45p – 2:15 PDT on gather.town; even numbered posters

Behavioral Pharmacology

1. Joshua M.R. Bastacky
   VTA Kappa Opioid Receptor Stimulation Paired with CRF Elicits Conditioned Place Aversion, but Kappa Stimulation Alone Does Not
2. Chongguang Chen
   NCP, a dual mu and kappa opioid receptor agonist, is a potent analgesic without reinforcing or aversive properties
3. Bryan D. McKiver
   The short acting kappa opioid receptor antagonist aticaprant failed to reverse morphine physical dependence in male mice

Chemistry

4. Andrea Bedini
   CL39 is a novel, kappa opioid receptor (KOR)-selective partial agonist that displays limited activation of arrestin-3/p38MAPK signaling in vitro and induces antinociception without eliciting significant aversion in vivo
5. Jane V. Aldrich
   Structural modifications to Phe3 in [D-Trp]CJ-15,208 are well tolerated by kappa opioid receptors
6. Chase Webb
   Computational Design of Bifunctional MOR Agonists/KOR Antagonists for the treatment of Opioid Use Disorder (OUD)

New Diseases

7. Kendra Boyes
   The Salvinorin A analogue, EOM SalB, promotes remyelination in preclinical models of multiple sclerosis
8. Filippo Erli
   Selective kappa-opioid receptor partial agonist HS666 shows anticonvulsant efficacy in mouse models of temporal lobe epilepsy without inducing aversive behaviour
9. Galen Missig
   Kappa-opioid receptors (KORs) in microglia: expression and regulation in inflammatory and stress-related conditions
10. Melanie Widmann
    Does the kappa-opioid receptor derived DREADD have a therapeutical potential in temporal lobe epilepsy?

Pain

11. Kelly Paton
    Kappa opioid receptor agonists reduce paclitaxel-induced neuropathic pain in both male and female mice

Molecular/Intracellular

12. Selena S. Schattauer
    Characterization of JNK activation by KOR antagonists and partial agonists

Systems

13. Allison P. Manalo
    Kappa-opioid receptor-expressing spinal neurons mediate itch transmission and include projection neurons
14. C. Lebonville
    Activity of dynorphin-expressing neurons during voluntary alcohol consumption and dynorphin-expressing afferents in the central amygdala
15. Huikun Wang
    Medial prefrontal cortical dynorphin-containing neurons constrain emotional memories
16. Sanne M. Casello
    Unique morphological and electrophysiological characteristics define subpopulations of dynorphin-containing neurons within the medial prefrontal cortex (mPFC)
17. Valentina Martinez Damonte
    Nucleus accumbens GABAergic afferents to the ventral tegmental area display a stress-sensitive form of long-term plasticity
18. Thomas J. Cirino
    Acute Stress Shifts Kappa-Opioid Receptor Function from Inhibitory to Excitatory in a Subset of VTA Dopamine Neurons
19. Daniel C Castro
    Mu-opioid receptors potentiate appetitive behaviors via g-protein, but not beta-arrestin 2, signaling
20. Raajaram Gowrishankar
    Dynorphinergic control of amygdalo-striatal circuits for goal-directed action
21. Breanne Pirino
    Kappa-opioid receptor stimulation in the nucleus accumbens shell affects drinking in a subregion-, sex-, and substance-specific manner
22. Bart de Laat
    A role for the Kappa Opioid Receptor in how naltrexone changes drinking behavior
23. M.B. Spodnick
    Regional differences in kappa opioid receptor activation on reward seeking behaviors and monoaminergic transmission in the nucleus accumbens
24. Sarah C. Simmons
    Input-specific regulation of discrete populations of Lateral Habenula neurons by Kappa opioid receptors
25. Rodolfo J. Flores
    Examination of the kappa opioid receptor dynamics during acute stress tests in mice.

Tuesday April 6, 2021

Oral Session 1

Highlighting Sex as a Biological Variable in Kappa Research

Moderator: Elena Chartoff

Agonist-induced KOR phosphorylation has sex-specific effects on KOR-mediated behaviors in mice

Peng Huang[1], Chongguang Chen[2], Danni Cao, Melody Huang and Lee-Yuan Liu-Chen

Center for Substance Abuse Research and Department of Pharmacology, Temple University Lewis Katz School of Medicine, Philadelphia, PA, 19140, USA

[1,2] contributed equally

We reported previously that the selective agonist U50,488H promoted phosphorylation of the mouse κ opioid receptor (mKOR) *in vitro* at four residues in the C-terminal domain (S356, T357, T363, and S369). Rabbit antisera were generated against phospho-KOR peptides and purified antibodies were found to be specific for phosphorylated KOR in immunoblotting. We constructed a mouse KOR mutant cDNA with all the four residues mutated to alanine (K4A) and stably expressed the mutant and the wildtype KOR in neuro2A mouse neuroblastoma cells (N2A-K4A and N2A-KOR, respectively). K4A mutations did not affect binding affinity of [^{3}H]diprenorphine, U50,488H, nalfurafine, dynorphin (1-17) and norbinaltorphimine, nor did they change EC_{50} values of U50,488H, nalfurafine, dynorphin (1-17) in stimulating [^{35}S] GTPγS binding. We generated a K4A mouse line to examine the in vivo functional significance of agonist-induced KOR phosphorylation. Mice were treated with vehicle or U50,488H and immunoprecipitation of WT KOR and K4A from brains followed by immunoblotting with phospho-specific antibodies pT363 and pS369 confirmed that U50,488H promoted KOR phosphorylation in the WT, but not K4A, male or female mice. Autoradiography of [^{3}H] 69,593 binding to KOR performed on brain sections showed that WT and K4A mice had similar KOR distribution in both male and female mice and displayed similar expression levels in brain regions enriched in KOR. In K4A mice, U50,488H inhibited compound 48/80-induced scratching and attenuated novelty-induced hyperlocomotion, similar to WT mice and there were no sex differences. Interestingly, repeated pretreatment with U50,488H (80 mg/kg, s.c.) resulted in profound tolerance to the inhibitory effects of U50,488H (5 mg/kg, s.c.) on compound 48/80-induced scratching in WT mice of both sexes and female K4A mice, while in male K4A mice tolerance was attenuated. Moreover, U50,488H induced conditioned place aversion (CPA) in WT mice of both sexes and male K4A mice, but not in female K4A mice. Thus, K4A mutations decreased U50,488H tolerance in male, but not female, mice and abolished U50,488H CPA in female, but not male, mice, indicating sex-specific effects of K4A mutations. The mechanisms underlying these sex-specific effects of K4A mutations remain to be investigated.

(supported by NIH grants R01DA041359, R21DA045274, P30DA013429)

The authors declare no conflict of interest.

Regulation of kappa opioid receptor inactivation by NorBNI depends on sex and cellular site of antagonist action

Keionna A. Newton[1,2], Kathryn L. Reichard[1,2,3], Zeena M.G. Rivera[1,2], Paulo M. Sotero de Menezes[1,2], Selena S. Schattauer[1,2], Benjamin B. Land[1,2], Charles Chavkin[1,2,3]

[1]Center for Neurobiology of Addiction, Pain, & Emotion, [2] University of Washington, Department of Pharmacology, [3]University of Washington, Graduate Program in Neuroscience, Seattle, WA 98195

Kappa opioid receptor (KOR) antagonists have recently been developed as stress-resilience therapeutics with potential utility in the treatment of mood disorders and substance use disorders. However, there is still a lack of understanding surrounding the molecular mechanisms of how these novel therapeutics exert their biologic effects. Three different forms of KOR antagonists have been developed, and in this study, we focus on a selective, long-acting, receptor-inactivating compound known as norbinaltorphimine (norBNI). Our studies show that the actions of norBNI do not work as effectively in female mice as in male mice due to the activation of G-protein receptor kinase (GRK) by estrogen. However, long-duration effects of norBNI action were restored if female mice were pretreated with CMPD101, a selective inhibitor of GRK2/3. Previous studies suggested that *in vivo* treatment with norBNI does not produce long-lasting inhibition of KOR modulation of dopamine release in the nucleus accumbens. Fast-scan cyclic voltammetry experiments verified that presynaptic inhibition of dopamine release by KOR agonist U69,593 was not attenuated by prior *in vivo* norBNI pretreatment under conditions that blocked KOR mediated aversion and analgesia. In this study, we utilized a novel HyPerRed fluorescence imaging technique that can be used as a sensor for KOR activation of cJun Kinase. Results suggested that KOR activation produced JNK mediated ROS generation in VTA dopamine neurons, but did not activate ROS in dopaminergic terminals of the nucleus accumbens. This selective action observed in different subcellular components provides evidence to help describe how KORs expressed on somatic but not terminal regions of dopamine neurons are inactivated by norBNI. These studies are significant for advancing the molecular understanding and future development of the receptor-inactivating class of KOR antagonists.

This research was supported by the National Institute on Drug Abuse [RO1-DA030074, T32-DA07278, and P30-DA048736].

Conflicts of Interest: The authors have no conflicts of interests.

PET Imaging Studies of Kappa Opioid Receptors in Socially Housed Female and Male Monkey Models of Cocaine Use Disorder

Michael Nader[1,2], Bernard Johnson[1], Kiran Solingapuram Sai[2], Susan Nader[1] and Yiyun Huang[3]

[1]Department of Physiology and Pharmacology, [2]Department of Radiology, Wake Forest School of Medicine, Winston-Salem, NC, [3]Yale PET Center, Department of Radiology and Biomedical Imaging, Yale University School of Medicine, New Haven, CT

Cocaine use disorder persists as a worldwide public health problem for which there is no FDA-approved pharmacotherapy. A major target for understanding the mechanisms mediating the high abuse potential of cocaine has been the dopamine (DA) receptor system. Using positron emission tomography (PET) imaging in humans (all male subjects), investigators noted an inverse relationship between D2/D3 receptor (D2/D3R) availability and the subjective effects of the DA agonist methylphenidate (Volkow et al., 1999). Using socially housed male and female monkeys, we showed that dominant monkeys had higher levels of D2/D3R availability compared with subordinate monkeys. However, the inverse relationship between D2/D3R and cocaine vulnerability was only apparent in males (Morgan et al., 2002); for females, dominant monkeys with high D2/D3R were more sensitive to the reinforcing effects of cocaine compared with subordinates (Nader et al., 2012). The present study extended this characterization to include PET imaging of the kappa opioid receptor (KOR) system. KORs are implicated in the neurobiological regulation of aversive states. Recent PET imaging studies in humans have shown relationships between KOR availability and social status (Matuskey et al., 2019) and sensitivity to cocaine reinforcement (Martinez et al., 2019). For this study, subjects were cocaine-naïve male and female cynomolgus monkeys (N=8/sex) living in same-sex social groups of four/pen. First, T1-weighted whole brain images were used to anatomically define regions of interest (ROI). Following intubation and anesthesia maintained by 1.5% isoflurane, each monkey received a 120-min PET scan with the KOR radiotracer [^{11}C]EKAP; females were scanned in the follicular phase. For each PET scan, a binding potential (BP) was calculated for each region, with the cerebellum serving as the reference region. Overall, the results indicated that dominant females and subordinate males, the two most vulnerable phenotypes to cocaine reinforcement, had the lowest BPs; these differences were statistically significant regions critical for drug reinforcement including the insula cortex, ventral striatum and putamen. These findings suggest that the KOR is an important target for understanding the neurobiology associated with vulnerability to abused drugs. Future studies will examine how KOR availability change with chronic cocaine exposure, abstinence and re-exposure to better determine if there are sex and social rank-related differences in the plasticity of this receptor system. Supported by R01 DA017763

The authors declare no conflicts of interest

KOR agonist induced avolition on a progressive ratio task in female and male rats.

Caroline A Browne [1,] Amanda B. Namchuk[1], Mumeko C. Tsuda [2], Irwin Lucki [1,3],
[1] Department of Pharmacology & Molecular Therapeutics, Uniformed Services University, Bethesda MD 20814
[2] Rat Behavior Core, Uniformed Services University, Bethesda MD 20814
[3] Department of Psychiatry, Uniformed Services University, Bethesda MD 20814

Many neuropsychiatric disorders, including Major Depressive Disorder, are characterized by anhedonia and cognitive impairment that may be associated with dysfunction of kappa opioid receptor (KOR) signaling. There is a growing body of animal literature demonstrating the pronounced dysfunctional effect of KOR activation on components of rewarded behaviors, including motivation, negative hedonic value, and learning. Few studies have examined the impact of KOR agonists on goal-directed behavioral performance. As KOR agonists are known to produce dysphoria and anhedonia, a series of studies were performed to in male and female rats to **1)** establish an operant based model to investigate the avolitional effects of the KOR agonist U50,488 on reward-maintained behavior, and **2)** test the ability of the short acting KOR antagonist LY2444296 (3 mg/kg) and ketamine (20 mg/kg) pretreatment to prevent U50,488 induced behavioral deficits.

All studies were approved by the Institutional Animal Care & Use Committee. Eight-week-old Sprague Dawley rats (Charles River Laboratories) were housed two per cage, under a 12:12 reverse light cycle (lights off at 06:00). Water was available *ad libitum,* but a restricted meal was fed daily at 11:00 when rats were not in the testing apparatus (12-15 g of chow per rat). Male and female rats (n = 12 per sex) were trained to lever press for 45 mg sucrose pellets in sound attenuated operant conditioning chambers (Med Associates Inc.) under fixed ratio (FR) schedules of 1, 3 or 5 lever presses, 3-5 days per schedule, for 30 min each day. Drug treatments were then evaluated on performance during a 2 h progressive ratio (PR) test.

First, a dose-response curve for U50,488 (2.5 and 5 mg/kg) was conducted. Only the 5 mg/kg dose of U50,488 administered 15 min prior to testing decreased active lever presses, number of rewards received, breakpoint, and magazine head entries during the PR test. However, the 5 mg/kg dose of U50,488 did not alter total moving distance in a locomotor activity assay, suggesting that the effects of U50,488 on operant behavior were not induced by motoric deficits. Second, pretreatment with LY2444296 30 min prior to U50,488 blocked deficits in active lever pressing and reward acquisition. Third, a separate cohort of rats received ketamine 24 h prior to U50,488 treatment. In female rats, ketamine pretreatment prevented the reduction of active lever responses and reward acquisition produced by U50,488. In contrast, ketamine pretreatment did not block the effects of U50,488 in male rats. Furthermore, behavioral suppression by the KOR agonist was most apparent during the early phase of the PR test (first 15 min), which corresponded with the time when rats were most active in the test. Not only did U50,488 treatment reduce the number of active lever presses, U50,488 evoked longer latencies to acquire each subsequent reinforcer as the reinforcement requirement was increased. The protective effects of LY2444296 and ketamine specifically rescued these deficits.

Together, these data support a role for KORs in operant responding under progressively increasing reward requirements. Moreover, these data show suggest that ketamine pretreatment (24 h) may exert differential effects on protecting operant responding from the effects of KOR agonists in male and female rats.

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Disclaimer: The opinions and assertions expressed herein are those of the authors and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.

Tuesday April 6, 2021

Oral Session 2

Emerging Tools for Kappa Opioid Systems Neuroscience

Moderator: Michael Bruchas

Title: Imaging opioid peptides with genetically encoded indicators

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Opioid neuropeptides (NPs) modulate neuronal function through the three opioid receptors, kappa (κOR), mu (μOR), and delta(δOR) alongside small-molecule neurotransmitters. The release of endogenous peptides, including dynorphin, endorphin, and enkephalin, have been implicated in essential physiological responses and behavior. To understand the spatiotemporal dynamics of opioid NP release and how they control synaptic signaling, circuit function and behavior, we developed three genetically encoded indicators and color-variants using opioid receptors as scaffolds to enable optical measurement of endogenous release and receptor ligands. These indicators work by coupling ligand-induced conformational changes of receptors to fluorescence intensity changes in cpGFP. We characterized the affinity, dynamic range and ligand specificity of these indicators in mammalian cells and dissociated neuronal culture, and ex vivo in acute slice with two-photon imaging. We used these sensors to examine the distinct conformation changes of receptors induced by various synthetic ligands. Furthermore, we validated the utility of these indicators in detecting brain region specific endogenous dynorphyin release during fear-learning using fiber-photometry. Future work will focus on improving sensors' signal-to-noise ratio for in vivo recording and enabling simultaneous imaging of NPs with other neurotransmitters via dual-color imaging.

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Toward an all-optical toolkit for probing dynorphin dynamics in the brain

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The dynorphin/kappa opioid receptor (KOR) system is heavily implicated in the negative affective states associated with stress, anxiety, depression, pain, and drug addition. Due to a lack of sensitive methods for detecting dynorphin in brain tissue with high temporal resolution, many fundamental questions remain about the timing, location, and temporal dynamics of dynorphin signaling during these aversive states, as well as the electrical and biochemical signals that govern dynorphin secretion. Furthermore, both behavior pharmacology studies and brain slice electrophysiological studies have been limited by the inability to precisely control the timing and location of KOR activation. To address these limitations, we are developing all-optical toolkits that directly and precisely manipulate and measure KOR signaling in the form of photoactivatable peptides and drugs, as well as genetically-encoded optical sensors. Our initial efforts involve involve the development of several photoactivatable dynorphin analogues and a first-generation KOR sensor called kLight. To demonstrate the potential of photopharmacological probes for studying opioid signaling, I will highlight the implementation of reagents targeting mu and delta opioid receptors in the context of both brain slice electrophysiological studies and behavioral pharmacology experiments involving *in vivo* photorelease of opioid drugs. I will also discuss our characterization and implementation of kLight in brain slices. In addition to evaluating the sensitivity of several kLight variants, we established the kinetics of kLight activation and deactivation using the photoactivatable dynorphin analogue CYD8, evaluated potential confounds due to buffering by the overexpressed sensor, and obtained evidence supporting kLight's ability to detect endogenous dynorphin release.

This work was supported by grants R00DA034648 and R35GM133802 (to MRB), BRAIN Initiative grant U01NS113295 (to MRB and LT), and U01NS013522 (to LT).

The authors declare no conflict of interest.

An electrochemical approach for rapid and sensitive detection of opioid peptides

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Endogenous opioid peptide systems are critical for analgesia, reward processing, and negative affect; however research on the *in vivo* modulation of these behaviors has been challenging due to an inability to reliably and consistently detect dynamic changes in opioid peptides. There is little to no data directly measuring dynamic *in vivo* changes of endogenous opioids, in particular dynorphin. The ability to correlate changes in brain neuropeptide levels during circuit or behavioral manipulations will exponentially evolve our understanding of motivated and affective behaviors. Thus, our work aims to develop innovative approaches for rapid and sensitive detection of opioid peptide release *in vivo*. Here, we have developed microimmunoelectrodes (MIEs) for electrochemical detection of opioid peptides. All opioid peptides contain an electroactive tyrosine residue as part of their N-terminus sequence; therefore, square-wave voltammetry can be used to detect them. Briefly, a voltage is applied to the electrode to cause oxidation of the tyrosine residue, which is detected as current. To provide specificity to these voltammetric measurements, the carbon fiber surface of the MIE is coated with an antibody selective to the opioid peptide of interest, for example dynorphin, and any remaining binding sites are blocked with bovine serum albumin. To test the sensitivity of the MIEs, electrodes are immersed in solutions containing different concentrations of opioid peptides and oxidative current is measured. To confirm specificity, oxidative current is also measured from exposure to tyrosine and other opioid peptides such as metenkephalin in solution. Here, we show that dynorphin antibody-coated electrodes are sensitive and selective to increasing concentrations of dynorphin in the fmol range. Current work aims to demonstrate the utility of these MIEs both *in vitro* via brain slice preparation and *in vivo* for real-time, rapid detection of endogenous opioid peptide release in awake and behaving animals.

Support:
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Conflict of Interest: The authors declare no conflict of interest

Kappa opioid receptor agonist-mediated neural activation measured with functional MRI in rats

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In vivo imaging modalities such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are powerful translational tools for studying molecular interactions and neural activation in the living brain. While PET has been successful at measuring kappa opioid receptor (KOR) density the living brain, fMRI studies to measure KOR-mediated hemodynamic changes in the brain have not been reported. Given the rapid growth of simultaneous MR-PET, particularly for neuroimaging, there is an opportunity to characterize KOR-mediated neural signaling in the living human brain with fMRI. As a step towards that goal, we have studied the hemodynamic response (cerebral blood volume, CBV) in rats from salvinorin A and U50,488 (naïve and nor-BNI treated animals) to investigate fMRI as a tool to probe neural mechanisms of KOR activation in the living brain.

Naïve or nor-BNI treated (10 mg/kg, i.p., 24h prior) Sprague-Dawley rats (n = 12 male and n = 12 female) were anesthetized with isoflurane and placed head-first-prone in 4.7T Bruker MRI with custom rat MR-head coil. We employed multislice echo-planar imaging (EPI) with whole-brain coverage along with a T2-weighted MPRAGE for anatomical co-registration. Prior to fMRI, ferumoxytol was administered intravenously at 10 mg/kg to enhance fMRI detection power. Rats received low or high dose salvinorin A (i.v., 0.1 mg/kg (n = 6) or 1.0 mg/kg (n = 6)) or low or high dose U50,488 (i.v., 0.1 mg/kg (n = 6) or 1.0 mg/kg (n = 6)). Dynamic acquisition of fMRI images started ~20 min before KOR-agonist injection and total scan time was ~70 min. Maximum changes in fMRI signal were defined as peak magnitudes of the gamma-variate regressor scaled by the general linear model (GLM). These values were converted to changes in %CBV. Dynamic fMRI data was co-registered to the W. Schiffer rat atlas. %CBV for naïve animals following drug challenge (U50488 or Salv A) was measured across brain regions and compared to norBNI pretreated animals. The application of this imaging modality towards understanding KOR signaling in the living brain will be discussed along with preliminary data from these experiments.

Support:
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Disclosure:
The authors have no conflicts of interest to disclose.

Wednesday April 7, 2021

Oral Session 3

The Kappa System in Depression, Pain, and Analgesia

Moderator: Jose Moron-Concepcion

Antinociceptive efficacy of diphenethylamines, as selective kappa-opioid agonists, in inflammatory pain without causing aversion and sedation after subcutaneous administration in mice

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Despite the awareness of the current opioid crisis, opioids are still commonly prescribed for the management of acute and chronic pain. Optimal medical use of opioids is significantly limited by multiple, severe adverse effects, misuse and abuse. The central need for effective, safer and non-addictive pain therapies continues to drive the pursuit for novel lead molecules and new mechanism-based treatment strategies. The kappa-opioid receptor (KOR) has emerged as an alternative pharmacotherapeutic target to the mu-opioid receptor for relieving pain, without the risk of physical dependence or abuse liability. However, KOR agonists produce other unwanted side effects including dysphoria/aversion and sedation. In this study, we present the KOR activity in vivo profiles (i.e. nociception, aversive effects and sedation/locomotor activity) of several, selective KOR agonists from the class of diphenethylamines developed in our laboratory. Male adult CD-1 mice were used in the behavioral study with drugs administered subcutaneously. Antinociception was assessed in a mouse model of tonic inflammatory pain (the formalin test). Aversive effects were evaluated using the conditioned place aversion (CPA) paradigm. The rotarod and open-field tests were used to determine potential sedative effects and locomotor impairment. Subcutaneous administration of diphenethylamines significantly reduced pain-like behavior in the formalin injected paw in a dose-dependent manner. Antinociceptive effects were blocked by pre-treatment with the selective KOR antagonist nor-binaltorphimine, demonstrating a KOR-specific mechanism of action. None of the investigated diphenethylamines induced aversive behavior in the CPA paradigm nor caused sedation or altered locomotor activity at therapeutic doses. Analysis of the KOR in vivo activity profiles of targeted diphenethylamines, as selective KOR agonists, provides important structural and functional insights into the discovery of novel drugs targeting the KOR with improved pharmacological profiles for the treatment of pain.

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Conflict of interest:
The authors declare no conflict of interest.

Title: **Peripheral kappa opioid receptor activation drives cold hypersensitivity in mice**

Authors: Manish K. Madasu[1,2,3], Loc V. Thang[1,2,3], Priyanka Chilukuri[1,3], Sree Palanisamy[1,2], Joel S. Arackal[1,2], Tayler D. Sheahan[3,4], Audra M. Foshage[3], Richard A. Houghten[6], Jay P. McLaughlin[5,6], Jordan G. McCall[1,2,3], Ream Al-Hasani[1,2,3]

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Body of abstract:

Noxious cold sensation is commonly associated with peripheral neuropathies, however, there has been limited progress in understanding the mechanism of cold pain. Here we identify a role for kappa opioid receptors (KOR) in driving cold hypersensitivity. First, we show that systemic activation of KOR by the agonist U50,488 (U50), increases the latency to jump and the number of jumps on a cold plate at 3ºC. KOR antagonist, norbinaltorphimine (NorBNI), attenuates U50-induced cold hypersensitivity. However, the central administration of NorBNI does not block U50-induced cold hypersensitivity, suggesting that peripheral KORs may modulate this effect. To directly test this, we use the peripherally-restricted KOR agonist, ff(nle)r-NH2 and also show cold hypersensitivity. We also demonstrate that U50 and ff(nle)r-NH2 do not alter core body temperature or paw surface temperature, as compared to saline controls, using rectal probe and paw surface temperature using thermal imaging. To begin to understand how peripheral KORs drive cold hypersensitivity we investigated whether KORs interact with transient receptor potential ankyrin 1(TRPA1) channels, known to facilitate the perception of noxious cold, in dorsal root ganglion (DRG). Using fluorescent *in situ* hybridization, we show that KOR mRNA colocalizes with the transcripts for the cold-activated TRPA1 channels in DRG. Using calcium imaging, we also show a potentiation in intracellular calcium release in DRG during the simultaneous application of the TRPA1 agonist, mustard oil (MO), and a KOR agonist, U50, when compared to MO alone or U50 alone in wild type (WT) male mice. In TRPA1 knockout mice ($TRPA1^{-/-}$), MO application alone and together with U50 did not alter Ca^{+2} signaling. Together our data suggest that peripheral KORs may induce cold hypersensitivity through modulation of TRPA1 channels.

Conflict of interest: The authors declare no conflict of interest.

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Changes in kappa opioid receptor signaling are required for ketamine's antidepressant action

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Ketamine produces both rapid and enduring antidepressant effects in patients with major depressive disorder, but the complete mechanism of action is unclear. Emerging evidence suggests a role of the opioid system in mediating the protracted effects of ketamine. Specifically, pretreatment with the mixed opioid antagonist naltrexone attenuated ketamine's effects clinically in treatment-resistant patients with depression and preclinically in a congenitally learned helplessness rat model. Although naltrexone is an antagonist at both kappa (KOR) and mu opioid receptors (MOR), emphasis has been placed on the involvement of MOR activation by ketamine as being necessary for the beneficial effects of ketamine. However, KORs and their endogenous ligand dynorphin, are key regulators of responses to stress. We hypothesized that desensitization of KORs, through previous dynorphin release and KOR activation, mediates the long-lasting antidepressant effects of low dose ketamine.

Internalization of KORs was observed in HEK293 cells transfected with yPET-OPRK1 plasmids following stimulation with dynorphin, the KOR agonist U50,488 (U50), or ketamine. Serum dynorphin was increased at 1 h, but had dissipated by 24 h, following ketamine administration in mice, which could activate KORs. To demonstrate that activation of KORs by ketamine contributes to its protracted behavioral effects, mice were pretreated with naltrexone or the selective short-acting KOR antagonist LY2444296 30 min prior to ketamine, and were tested in the forced swim test 24 h later. Reductions in immobility scores by ketamine were prevented in mice pretreated with either naltrexone or LY2444296. The physiological impact of ketamine on KOR activity was tested ex vivo in the lateral habenula, a brain region related to reward. The number of action potentials in response to depolarization was increased with bath application of U50, but neuronal excitability was absent when ketamine was administered to mice 24 h previously. Further, LY2444296 given 30 min prior to ketamine partially restored U50-induced excitability assessed 24 h later. Behaviorally, ketamine given 24-48 h prior to testing blocked U50-induced alterations on nesting, prepulse inhibition, and antinociception. LY2444296 pretreatment also prevented the ability of ketamine to block U50-induced behavioral alterations.

Together, these studies provide evidence that KOR activation or acute dynorphin release by ketamine leads to a long-lasting reduction of KOR signaling, which likely contributes to the persistent clinical antidepressant effects of ketamine.

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The authors declare no conflict of interest.

Disclaimer:
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Kappa opioid receptor mediates epigenetic silencing of BDNF in treatment resistant depression

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Treatment-resistant depression (TRD) occurs in almost 50% of total depressed patients and underlying mechanism of TRD remains elusive. We previously reported that sustained activation of kappa Opioid receptor induced TRD like behaviors in mice and altered cortical BDNF expression appears as one of molecular determinants of antidepressant response. Considering the critical role of epigenetic factors in regulating expression of BDNF transcripts, the present study was designed to uncover the epigenetic mechanisms underlying the kappa opioid induced BDNF downregulation in the frontal cortex. Firstly, we evaluated the distinct epigenetic changes in the frontal cortex and found that sustained activation of kappa opioid receptor by chronic administration of its synthetic agonist U50488 leads to selective and specific down regulation H3K9ac and upregulation of SIRT1 in the frontal cortex, but not in hippocampus and striatum. Interestingly, this downregulation was absent in the frontal cortex of chronic unpredictable stress exposed mice. Further, we showed that KOR activation leads to specific downregulation of BDNF exon II, IV and VI in the frontal cortex. Using chromatin immunoprecipitation assay, we found decreased H3K9ac binding at BDNF promoter 2 and 4, and increased H3K27me3 binding at promoter 4 only, both of which were blocked by kappa selective antagonist norBNI. These observations suggest KOR induced region-specific epigenetic silencing of BDNF in TRD.

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Structure–Activity Relationships on Collybolide: Discovery of a Potent Kappa-Opioid Agonist with Enhanced Metabolic Stability

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Collybolide, a non-nitrogenous sesquiterpene natural product extracted from the mushroom *Collybia maculata* was found to be a highly selective and potent kappa opioid receptor (KOR) agonist (EC_{50} ~ 1 nM) having *in vivo* analgesic and antipruritic activities. Collybolide has a furyl-*δ*-lactone core similar to that of salvinorin A, however, differs from the latter in exhibiting biased agonistic activity. *Collybolide shows no conditioned place aversion unlike classical kappa agonists at doses at which it blocks non-histamine-mediated itch, suggesting the possibility of separating kappa mediated liabilities from its ability to block itch with this novel natural product-based template.* However, there are liabilities associated with use of collybolide as a drug candidate such as aqueous solubility, efficacy, and metabolic stability. These liabilities can be overcome by designing new collybolide analogues guided by the computational docking studies. We performed semi-synthesis of collybolides and discovered an analogue MN-074, which was found to have similar potency and selectivity to collybolide. Microsomal stability studies demonstrated that MN-074 was more metabolically resistant than collybolide. In addition, MN-074 had better drug-like properties than collybolide. The goal is to comprehensively examine the structure-activity relationships (SAR) of collybolide, which will yield a set of selective potent KOR ligands with the potential of becoming lead drug candidates for itch and pain with reduced side effects**.** Further, high-throughput screening at the NIMH-psychoactive drug screening program (PDSP) against 50 CNS receptors could find new hits, which could be optimized further to explore new therapeutic opportunities.

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Conflict of Interest: The Authors declare no conflict of interest.

Thursday April 8, 2021

Oral Session 4

Novel Indications and Systems for KOR Translation

Moderator: William Carlezon

Nalfurafine promotes remyelination and increases oligodendrocyte numbers in the augmented cuprizone model of multiple sclerosis.

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Multiple sclerosis (MS) is the most common demyelinating disease with 2.5 million sufferers worldwide. In MS, the body's own immune system attacks the protective myelin coating surrounding axons, inhibiting saltatory conduction. This results in a range of symptoms including as impaired locomotion, vision, impairment, and increased pain and fatigue which progressively worsen over time. Current treatments aim to modulate the immune attack through either supressing the inflammatory immune response, preventing immune cell migration or via lymphocyte depletion. These treatments slow disease progression but do not prevent the development of disability, and no currently available treatments function to repair damaged myelin.

The number of oligodendrocytes, the glial cells that lay down the myelin sheath, are reduced in MS and one strategy to promote repair and recovery is to identify drugs that increase the proliferation and/or differentiation of oligodendrocyte precursor cells into mature myelinating oligodendrocytes.

In the preclinical experimental autoimmune encephalomyelitis (EAE) model of MS, kappa opioid receptor (KOPr) agonist activation with U50,488 (1.6 mg/kg/i.p.), promoted disease recovery via remyelination when administered prior to disease onset. However, clinical development of U50,488 is not viable due to side effects.

Here we investigated the therapeutic potential of nalfurafine, a clinically safe KOPr agonist, in two pre-clinical models of MS. In addition to EAE we utilised an extended augmented cuprizone model of demyelination in 9 -10 week old C57BL/6J male mice. In this model, 0.2% cuprizone was administered in ground chow for 84 days in conjunction with daily administration of 10 mg/kg/i.p. rapamycin to prevent spontaneous remyelination. This model mimics demyelination and axonal loss seen in progressive forms of MS. On day 85 cuprizone and rapamycin treatment was withdrawn, and mice received daily injections of either nalfurafine (0.1 mg/kg/i.p. or 0.01 mg/kg/i.p.) or vehicle until day 126. At day 85 we found extensive demyelination of axons within the corpus callosum with a reduction in the proportion of myelinated axons, myelin thickness and significant disruption to the myelin sheath (65%) compared to healthy controls.

Daily therapeutic treatment with nalfurafine resulted in a dose-dependent increase in myelin thickness evaluated via transmission electron microscopic (TEM) analysis of g-ratios ($p=0.0015$, 0.1 mg/kg compared to vehicle-only controls). Nalfurafine treatment also restored the percentage of myelinated axons within the corpus callosum to levels seen in healthy control mice and significant increased the number of myelinated axons compared to control mice administered vehicle ($p=0.0324$). This was accompanied by an increase in the number of mature oligodendrocytes ($p=0.0468$). Collectively, these data demonstrates the ability of nalfurafine to promote remyelination and functional recovery in a pre-clinical model of MS.

Conflict of interest: Bronwyn Kivell, Thomas Prisinzano and Anne La Flamme, are inventors on patent applications that relate to this work and have been licensed to Rekover Therapeutics Ltd. ACL, BK and TP hold equity in Rekover Therapeutics Ltd. The authors declare no other financial interests.

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Extended amygdala projections to parabrachial dynorphin neurons alter threat perception and encode feeding behaviors

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In order to survive, an animal must seek and consume food while avoiding environmental threats. In mammals, food consumption is heavily influenced by the parabrachial nucleus (PBN), a pontine structure that integrates visceral information to encode metabolic needs. Peptidergic neurons in the PBN, including dynorphin-expressing neurons, receive top-down input from the bed nucleus of the stria terminalis (BNST), an extended amygdala structure that encodes affective and threat information. These dense projections to the PBN enable a circuit that may be involved in the complex integration of environmental threat evaluation with an animal's own feeding drive. Here we used complementary techniques to identify and characterize distinct BNST-PBN circuits. We performed projection-specific translating ribosome affinity purification (vTRAP) to determine the molecular expression profile of GABAergic (vGAT) and glutamatergic (vGLUT2) projections. To assess the role of these distinct circuits, we monitored and manipulated circuit activity during behaviors associated with affective motivation, such as hedonic and homeostatic feeding and threat perception. We found that vGAT- and vGLUT2-expressing BNST-PBN circuits have divergent roles in valence encoding, threat, and feeding behaviors. When activated, $BNST^{vGLUT2}$-PBN circuits cause aversion, operant negative reinforcement, anxiogenesis, and reduced feeding. $BNST^{vGAT}$-PBN circuits drive preference, operant positive reinforcement, anxiolysis/exploratory behavior, and increased feeding. Using fiber photometry, we uncovered divergent excitatory and inhibitory BNST-PBN circuit dynamics during feeding and threat-response behavior. We used *in situ* hybridization to show that dynorphin and CGRP neurons in the PBN form separate populations, and ChR2-assisted circuit mapping revealed that both populations receive excitatory and inhibitory input from BNST projections. Finally, we used fiber photometry and optogenetic and chemogenetic manipulation to show that dynorphin neurons in the PBN encode negative valence, decrease exploration, and reduce food consumption, consistent with both inhibitory and excitatory BNST input. Together, our findings characterize distinct circuitry involving dynorphin neurons in the PBN, revealing their modulation of threat perception and feeding behaviors to describe a mechanism by which animals evaluate environmental threat to enable feeding and promote survival.

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Disclosure:
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Decoding Dynorphin-Kappa Opioid Circuit Interactions During Stress-induced Binge Eating

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Binge eating disorder (BED) is characterized by a compulsive, frequent pattern of rapid overconsumption of food that is accompanied by subjective loss of control and negative emotional states. Similar to substance abuse and addictive disorders, encounters with acute stressors may act as precipitating factors leading to the maintenance of negative response patterns. Recruitment of the dynorphin/kappa opioid receptor system (Dyn/KOR) in response to stress plays an important role in dysphoric states and drug-seeking behaviors; however whether similar Dyn/KOR mechanisms underlie stress-driven symptomology of binge eating is unknown. To investigate this, we established a stress-responsive, binge-like eating behavioral model to high fat/high sugar food (HPD) in mice with 15 mins of forced swim (FS, 30 deg C), followed by time-limited (1h) access to HPD. Systemically blocking KORs (with i.p. norBNI) prior to FS and HPD access significantly reduced 1h HPD consumption following stress, and activation of KORs (via KOR agonist, U50,488) in non-stress (NS) mice was sufficient to increase 1h HPD intake compared to saline-injected mice. Using cFos as a surrogate marker for recent neuronal activation, we found that claustrum (CLS), a subcortical region with dense KOR expression, showed a significant and unique change (increase) in cFos compared to the CLS and other notable KOR-positive areas in controls. Blocking KORs directly in CLS (norBNI→CLS) with bilateral microinjections revealed that FS+norBNI→CLS injected mice significantly reduced consumption of HPD to NS levels compared to FS+saline→CLS mice. Further, targeted deletion of KORs in CLS (AAV-Cre-GFP→CLS) using conditional KOR knockout mice (cKO^{KOR}) blocked the increased HPD consumption in response to FS compared to injected, WT-control group. Last, bilateral inhibition of neuronal CLS activity using viral delivery of a promoter driven- (CAMKIIa, expressed in the majority of CLS cells) hM_4-Gi-DREADD and i.p. injection of DREADD ligand, CNO prior to experiments (FS+CAMKIIa-DREADD-Gi), confirmed cFos results that increased CLS activity is required for greater binge eating following stress. To begin reconciling the necessity of both simultaneous increases in CLS cell activity with inhibitory KOR activation to produce increased binge-like eating in response to acute stress, we assessed expression of CAMKIIa (which co-expresses with the majority of CLS-excitatory glutatmatergic cells), inhibitory (Vgat) and KOR populations in CLS cells using *in situ* hybridization. We found that KOR positive CLS cells overlap with both excitatory (CAMKIIa) and inhibitory (vGAT+) CLS neuronal types. To begin gaining granularity on a population level, we performed recordings using fiber photometry with the fluorescent calcium indicator GcaMP6f driven by CAMKIIa, in WT and pDyn KO mice. In WT mice, we compared calcium transients during free feeding assay in the following randomized trials: (a) NS; (b) NS/U50-488 ip. injected 15 mins prior to feeding assay; and (c) FS; and found that in NS trials, CLS neural activity increased shortly (<3 sec) following onset of new feeding bout, whereas U50-488 and FS treatment blocked this response to HPD, despite that mice in these groups showed elevated HPD kCal intake and feeding bouts compared to NS trials. Additionally, in controlled delivery of HPD (1 HPD pellet per minute), we observed that in NS condition, increase in neural activity occurred shortly prior to pellet retrieval, and was absent in pDyn KO mice. Further experiments to confirm these findings and further evaluate changes in neural activity in response to stress and HPD access are currently ongoing. In summary, dynorphin action on CLS-KOR cells play a unique role in modulating the response to HPD in situations when animals are stressed, possibly via disinhibition by inhibitory interneurons and overall excitation of excitatory projection neurons from the CLS.

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Prefrontocortical dynorphin opioids disrupt cognition through distinct pre- and postsynaptic mechanisms

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Release of dynorphin during periods of drug withdrawal or abstinence is hypothesized to increase escalation of drug intake and promote relapse in individuals with substance use disorders. Cognitive dysfunction contributes to relapsing behaviors in substance use disorders, and previous studies have shown that dynorphin suppresses activity in inputs to the prefrontal cortex (PFC). We first examined the conditions that led to KOR activation in the PFC by measuring immunoreactivity of a phosphorylation-selective antibody targeted to the Ser369 residue of the KOR. Administration of a systemic KOR agonist in male C57BL/6J mice increased KOR phosphorylation in local (postsynaptic) prefrontal cortical neurons. This increase could be blocked by pre-treatment with norBNI. Optogenetic stimulation of PFC prodynorphin-Cre (pdyn^{Cre}) neurons also produced KOR phosphorylation in the PFC that could be blocked by systemic norBNI pre-treatment. We found that morphine withdrawal or footshock stress produced KOR activation in PFC neurons. We next determined that KOR activation in the (PFC) produces multi-phasic disruptions of memory processing in an operant delayed alternation task. Mice were trained to press a lever to initiate a trial, wait during a delay period, then respond on the alternate lever for food pellet reinforcement. After establishing stable performance in the task, mice were tested for the effect of a systemic dose of the KOR agonist, U50,488. Cognitive disruptions manifest as reductions in response number and accuracy during early and late phases of an operant test session. Intracranial administration of norBNI into the PFC blocked the disruptive effect of a systemic KOR agonist. Viral Cre-mediated excision of postsynaptic PFC KOR in lox/lox mice blocked the early, but not late phase disruption produced by U50,488. This indicated that pre- and postsynaptic KORs produced distinct disruptive effects on behavior. Optical stimulation of *pdyn*^{Cre} PFC neurons or naloxone-precipitated withdrawal in morphine-dependent mice also disrupted performance in the delayed alternation task. Using a genetically encoded sensor based on inert KOR (kLight1.2a), we revealed the dynamics of endogenous dynorphin release in the PFC *in vivo* during opioid withdrawal. Our studies provide a basis for understanding how opioid withdrawal dysregulates cognition through dynorphin actions in the prefrontal cortex.

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Thursday April 8, 2021

Oral Session 5

Kappa Mechanisms and Translation for Itch

Moderator: Sarah Ross

Probing the cellular basis of kappa opioid receptor inhibition of itch and chemical pain

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The kappa opioid receptor (KOR, encoded by *Oprk1*) is a promising target for the treatment of chronic itch, with KOR agonists used clinically for the treatment of chronic itch in Japan. However, the cellular basis of KOR inhibition of itch remains unknown. Previous work from our lab demonstrated that KOR inhibition of itch occurs – at least in part – at the level of the spinal cord, raising the question: *which spinal neurons express KOR?* We are addressing this fundamental gap in knowledge through a combination of genetic, behavioral, and molecular approaches. First, we use a recently developed *Oprk1-Cre* allele to selectively express excitatory DREADDs in *Oprk1* spinal neurons. We found that chemogenetic activation of *Oprk1-Cre* spinal neurons elicited spontaneous nocifensive behaviors, and potentiated behavioral responses to chemical itch and pain, without affecting baseline nociceptive withdrawals. Next, we visualized *Oprk1-Cre* spinal neurons and discovered that *Oprk1-Cre* is expressed in local interneurons, as well as spinal projection neurons that target brainstem and thalamic structures that are critical for the supraspinal processing of somatosensory stimuli. Finally, we used RNAscope fluorescent in situ hybridization to determine the molecular identity of *Oprk1* spinal neurons that may drive pain and itch behaviors. We show that *Oprk1* is expressed within a heterogenous population of spinal neurons, primarily including those that express the neuropeptide substance P, which is known to drive both pain and itch behaviors. These data are the first in-depth look into which spinal neurons express KOR, and future studies will test the necessity of these neurons in KOR inhibition of itch and chemical pain.

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Conflict of interest
The authors have no conflicts of interest to declare.

Medullary kappa opioid receptor neurons inhibit pain and itch through a descending circuit

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Abstract

In perilous and stressful situations, the ability to suppress pain can be critical for survival. The rostral ventromedial medulla (RVM) contains neurons that can robustly inhibit pain processing in the spinal cord through a top-down modulatory pathway. Although much is known about the role of the RVM in the inhibition of pain, the precise identities and mechanisms of pain-inhibitory neurons in the RVM have never been identified. We now expose a cellular circuit that inhibits pain and itch in mice. Using a combination of molecular, tracing, and behavioral approaches, we found that spinally-projecting RVM neurons containing the kappa opioid receptor (KOR) inhibit itch and pain. With chemogenetic inhibition, we determined that these neurons are required for stress-induced analgesia. Furthermore, we found a dynorphinergic pathway arising from the PAG that modulates nociception within the RVM. These discoveries highlight a distinct population of RVM neurons capable of broadly and robustly inhibiting itch and pain.

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Conflict of interest

Authors declare no conflict of interests

Friday April 9, 2021

Oral Session 6

The Kappa System and Substance Use Disorder

Moderator: Lee-Yuan Liu-Chen

Activation of kappa opioid receptor-expressing neurons in the ventral tegmental area attenuates opioid withdrawal

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Opioid withdrawal is an excruciating and potentially life-threatening syndrome resulting from abrupt cessation of opioid use. Untreated opioid withdrawal frequently results in relapse and presents a major barrier to recovery from opioid use disorder (OUD). The kappa opioid receptor (KOR) has been shown to play complex roles in chronic pain and addiction through the modulation of dopamine neurons originating from the ventral tegmental area (VTA). However, the precise mechanisms through which KOR-expressing VTA neurons contribute to the development of opioid withdrawal remains elusive. In the present study, we used chemogenetic and optogenetic approaches in the recently developed KOR^{Cre} mouse to identify the role of KOR-positive VTA neurons in opioid withdrawal. Here, we find that KOR is expressed in the vast majority of dopaminergic VTA neurons, whereas it is seldom expressed in glutamatergic or GABAergic neurons. We also tested the role of KOR in real-time place aversion and conditioned place preference in opioid naïve mice. We then precipitated opioid withdrawal through administration of escalating doses of morphine followed by naloxone, and measured the effect of chemogenetic activation of KOR neurons in the VTA on opioid withdrawal-induced conditioned place aversion as well as withdrawal-induced anxiety-like and depressive-like behaviors. These studies have important implications for the role of VTA KOR neurons in the development of opioid withdrawal.

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The authors declare no conflict of interest.

Clinically utilized nalfurafine combined with naltrexone at low doses prevents excessive and "relapse" alcohol drinking in mice

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Nalfurafine [TRK-820] is a highly potent and selective Kappa full agonist. Nalfurafine has been clinically used in Japan as an anti-pruritic agent for more than 10 years. Recent post-marketing reports have shown that Remitch® [nalfurafine hydrochloride] is safe and efficacious without side effects associated with typical Kappa agonists [anhedonia and psychotomimesis]. In rodents, nalfurafine produces anti-pruritic and anti-nociceptive effects in a dose range lower that produce side effects, including conditioned place aversion (CPA), hypo-locomotion, motor incoordination, "anxiety" or "depression" behavior, consistent with the human data. Though both U50,488 and nalfurafine decrease dopamine release, they differentially modulate several signaling pathways, including the mTOR which contributes to U50,488-induced CPA. Because of its lack of side effects associated with typical Kappa agonists in humans or rodents, we have recently investigated potential effects of nalfurafine on excessive and "relapse" alcohol drinking behaviors in mice and found: [1] Nalfurafine [3-10μg/kg] dose-dependently reduced excessive alcohol consumption (by ~25%) and prevented "relapse" drinking, without causing sedation (spontaneous locomotion), "anhedonia" (sucrose preference), "anxiety" (elevated plus maze test), or "dysphoria" (CPA) behaviors; [2] Repeated daily nalfurafine administrations [10μg/kg] during 10 days decreased alcohol consumption without showing any blunted effects, suggesting tolerance did not develop to nalfurafine; [3] Activations of endorphin/mu and dynorphin/kappa transmissions prompt positive and negative reinforcing aspects of alcohol intake, respectively. We further tested nalfurafine [0.3-1μg/kg] combined with naltrexone [0.3-1mg/kg] both at low doses and found a greater reduction (by ~45%) than either drug alone, suggesting a new strategy of combining nalfurafine and naltrexone for therapeutic development for alcoholism with low risk of aversive effects. Consistently, nalfurafine has been investigated as a combination therapy for both pain treatment and oxycodone abuse; and [4] Using RNA-seq with the bioinformatics tool Ingenuity Pathway Analysis, we found that excessive alcohol drinking enhanced gene expression of key molecules in the mTORC1 pathway in mouse nucleus accumbens shell, and U50,488-induced increases in alcohol intake were blunted by mTOR inhibitor rapamycin, suggesting the mTOR pathway is involved in Kappa activation-induced drug taking. In contrast, nalfurafine decreased in alcohol intake and the decrease was not altered by rapamycin, suggesting differential signaling pathways between U50,488 and nalfurafine. Together, our *in vivo* results support the assumption that nalfurafine is an atypical Kappa agonist with a significantly improved side-effect profile relative to typical Kappa agonists, which needs further studies for its therapeutic potential for treating alcoholism.

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Refinement of kappa opioid activity in multifunctional cyclic tetrapeptides facilitates safer analgesics and treatments for substance abuse.

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We examined a trio of macrocyclic tetrapeptides with mixed kappa opioid receptor (KOR) antagonism and opioid agonism with differing ratios of mu opioid receptor (MOR) and KOR activity to determine the balance of mixed MOR/KOR agonism that would minimize liabilities while preserving the therapeutic ability to suppress pain and the rewarding effects of abused substances. Tetrapeptides CJ-15,208 (Ross et al., 2012) and [Ala[1],D-Trp[4]]CJ-15,208 (Aldrich et al., 2014) produced equipotent antinociception in the 55ºC warm-water tail-withdrawal test using wild-type C57BL/6J mice, with ED_{50} (and 95%CI) values of 1.74(0.62-4.82) and 3.03(2.16-4.59) nmol, i.c.v., respectively, whereas *cyclo*[Pro-Sar-Phe-D-Phe] (Ferracane et al., 2020) was 15-fold more potent (0.15(0.08-0.29) nmol, i.c.v.). All three compounds produced KOR antagonism. Tested with MOR knockout (MOR KO) and KOR knockout (KOR KO) mice, CJ-15,208 showed an equal mix of MOR and KOR agonism, whereas [Ala[1],D-Trp[4]]CJ-15,208 antinociception was predominantly KOR mediated and *cyclo*[Pro-Sar-Phe-D-Phe] antinociception was predominantly MOR mediated. Sedation, hyperlocomotion and depression of respiration rate were evaluated with the rotorod assay and CLAM system, with no compound producing sedation at doses up to 100 nmol i.c.v. CJ-15,208 produced initial conditioned place aversion (CPA) at a 3 nmol i.c.v. dose, but at higher doses demonstrated MOR-mediated conditioned place preference (CPP). In contrast, [Ala[1],D-Trp[4]]CJ-15,208 (3 nmol i.c.v.) was without effect in the conditioned place preference assay, but demonstrated CPA at higher doses. Both of these effects were absent in KOR KO mice. Notably, *cyclo*[Pro-Sar-Phe-D-Phe] demonstrated no place preference or aversion at any dose tested (0.1, 1 or 10 nmol i.c.v.). Of interest, only [Ala[1],D-Trp[4]]CJ-15,208 prevented acute morphine-CPP, but all three compounds blocked stress-induced reinstatement of extinguished morphine-CPP. In conclusion, the addition of even modest amounts of KOR to MOR agonism alleviates some liabilities attributed to receptor-selective agonists, validating further investigation of KOR activity in multifunctional opioid therapeutics for pain and substance abuse.

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Key words: Macrocyclic tetrapeptide, multifunctional ligand, kappa opioid receptor, analgesia, side effects, substance abuse, reinstatement

Conflict of Interest: none to declare.

Effect of acute exposure to ethanol on kappa-opioid receptor antagonists binding. Live cell study using Fluorescence Lifetime Imaging Microscopy (FLIM)

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The effect of acute exposure to ethanol on the binding of opioid receptor antagonists: naltrexone, nor-BNI, JDTic or LY-2444296, to the kappa-opioid receptor (KOP), was quantitatively characterized in live PC12 cells stably expressing KOP genetically fused at the C-terminal end with the enhanced Green Fluorescent Protein (eGFP). To this aim, an integrated massively parallel Fluorescence Correlation Spectroscopy and Fluorescence Lifetime Imaging Microscopy (mpFCS/FLIM) system was used that is especially designed by us to enable quantitative characterization in live cells of spatial variations in the local concentration, diffusion and interactions of biomolecules (*via* FCS) and their functional consequences (*via* local excited-state decay by FLIM). Our data show that for all antagonists tested, the IC_{50} values, determined as concentrations of the selected antagonists that results in 50 % change of eGFP fluorescence lifetime in the plasma membrane, are higher in live cells pre-treated for 1 h with 40 mM ethanol than in untreated cells. For naltrexone and JDTic, the difference was large, some two orders in magnitude, whereas it was several-fold for nor-BNI and CERC-501. Interestingly, the direction of change in eGFP fluorescence lifetime was opposite for JDTic as compared to the other antagonists tested – eGFP fluorescence lifetime increased for increasing JDTic concentrations, whereas it decreased for increasing concentrations of naltrexone, nor-BNI and LY-2444296.

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Friday April 9, 2021

Oral Session 7

New Advances in Understanding KOR at the Molecular Level

Moderator: Bryan Roth

Structural Studies Illuminate KOR Ligand Pharmacology

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The κappa-opioid receptor (KOR) agonists and antagonists have demonstrated potential as non-addictive analgesics and effective treatments as antidepressants, respectively. A complete understanding of KOR-ligand interaction is necessary to elucidate the pharmacological properties of this important drug target. Combining the structural, pharmacological and computational approaches, we revealed the structural basis for ligand-specific conformational states. The unique receptor conformation induced by different KOR agonists is consistent with their preference for differential intracellular transducers, which supports that the receptor's functional selectivity is dependent on its conformational plasticity. This study also provides insights into ligands' complicated *in vivo* pharmacology.

Disclosure:
The authors have no conflicts of interest to disclose.

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Compartment-specific signaling by kappa opioid receptors is selectively modulated by distinct Dynorphin subtypes

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Many signal transduction systems have an apparent redundancy built into them, where multiple endogenous agonists activate a given receptor. This redundancy is especially prominent in the opioid system, where over twenty different endogenous opioids activate three opioid receptors. Whether this represents true redundancy, or whether different opioids differ in aspects of downstream signaling, is just beginning to be addressed. We address this question using the kappa opioid receptor (KOR), a physiologically relevant G protein-coupled receptor that is activated by multiple members of the Dynorphin family of opioid peptides. We show that different Dynorphins bind and activate KOR comparably on the cell surface, but they drive different fates of KOR after activation. Dynorphin A localizes KOR to lysosomes and drives degradation, while Dynorphin B localizes KOR into recycling endosomes and drives recycling. Strikingly, KOR activated by Dynorphin A, but not Dynorphin B, remains in an active conformation on lysosomes and causes sustained cAMP signaling. Our study shows that different endogenous opioid peptides fine-tune KOR signaling by regulating receptor localization to and signaling from different endosomal compartments. These results suggest that physiological systems generate diversity in signaling by inducing different subcellular spatial and temporal profiles of receptors, and that these profiles should be an important consideration in drug development.

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Regulator of G protein Signaling-12 (RGS12): An accidental tourist in Kappa-Land

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The “Regulators of G-protein Signaling” (RGS proteins) serve to extinguish G protein-coupled receptor (GPCR) signaling by accelerating the GTP hydrolysis rate of the Gα subunit to return the G proteins to their inactive, heterotrimeric state. We previously showed that one particular member, Regulator of G protein Signaling type 12 (RGS12), is enriched within the ventral striatum (vSTR) and its genetic ablation in C57Bl/6J mice increases dopamine transporter (DAT) expression and dopamine uptake within the vSTR. The most likely direct targets for RGS12’s inhibitory action are striatal presynaptic kappa opioid receptors (KORs), the activation of which is established to attenuate striatal dopaminergic tone. We have found that *Oprk1* (*KOR*) and *Rgs12* mRNA expression levels overlap in the mouse CNS, and that KOR and RGS12 proteins coimmunoprecipitate as a complex from mouse vSTR extracts. This co-expression and complex formation suggests that the increased DAT expression/function seen in RGS12-null mice is likely caused by removing a critical negative influence on signaling downstream of KOR activation. We have found that RGS12 overexpression in cell culture markedly and selectively blunts G protein–dependent cAMP inhibition by KOR activation, yet greatly enhances β-arrestin recruitment to activated KOR. Consistent with these *in vitro* results, RGS12 loss in mice enhances the analgesic effect of U50,488, yet attenuates conditioned place aversion to U50,488 -- thought to be G protein- and β-arrestin-dependent behaviors, respectively. Given these data, we believe that RGS12 is a hitherto unappreciated key regulator of KOR signaling, acting on both G protein- and β-arrestin-dependent signals to modulate the output of dynorphin/KOR signaling to dopamine reuptake and to the behavioral responses of both analgesia and dysphoria.

Conflict of interest:
None of the above has any competing financial interest in relation to the work described.

Poster Abstracts

Unilateral VTA Kappa Opioid Receptor Stimulation Paired with CRF Elicits Conditioned Place Aversion, but Kappa Stimulation Alone Does Not

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Recent slice electrophysiology findings have demonstrated that kappa opioid receptor (KOR) agonism in Ventral Tegmental Area (VTA) dopamine neurons, which is typically inhibitory, becomes excitatory following foot shock stress, which also drives CRF release in the VTA. Previous work has suggested that kappa opioid receptors and stimulation by their endogenous ligand dynorphin are necessary for the aversive component of stress. In this work we examined the effects of unilateral VTA injections of the specific kappa opioid agonist U69593 (U69) with or without CRF in the same solution using a three chamber apparatus to assess effects on place conditioning in rats. Conditioned place preference or aversion was assessed by the difference of time spent in the drug-conditioned chamber before vs after four drug pairings over eight days. Cannula placements were confirmed via histology. U69 pairings elicited no effect, but U69 co-administered with CRF produced a conditioned place aversion effect. These data suggest that in the VTA CRF activity is required for KOR activation to be aversive, possibly involving the switch in KOR function to excitatory in some dopamine neurons.

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No conflicts of interest to report.

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

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While both MOR agonists and KOR agonists have analgesic effects, they produce opposite hedonic states, euphoria and dysphoria, respectively. Studies have shown that KOR agonists reduce the rewarding effects of MOR agonists. We hypothesize that compounds with dual MOR and KOR agonist activities may be effective analgesics with low likelihood of producing dysphoria or addiction. We found that in *in vitro* [^{35}S]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 in a dose-dependent manner with an A_{50} value of 47.6 μg/kg, indicating potent antinociceptive activity. In contrast, NCP did not inhibit compound 48/80-induced scratching, whereas U50,488H did. NCP did not 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.), unlike U50,488H at 2 or 5 mg/kg (s.c.). In intravenous self-administration, NCP did not function as a reinforcer at 1, 10, or 100 μg/kg/infusion in rats following substitution for 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.
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The authors declare no conflict of interest.

The short acting kappa opioid receptor antagonist aticaprant failed to reverse morphine physical dependence in male mice

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Opioid withdrawal syndrome is a symptom of opioid use disorder that manifests upon abstinence from opioids. These symptoms can cause severe discomfort which can contribute to opioid relapse. Current medications to manage opioid withdrawal include opioids and α2-adrenergic receptor agonists. Methadone and buprenorphine leave patients with residual withdrawal symptoms, while clonidine and lofexidine cause adverse side-effects and fail to completely suppress withdrawal symptoms in patients. There is a pressing need for new treatments for opioid withdrawal. Kappa opioid receptors (KORs) are a promising new therapeutic target for opioid withdrawal because disruption of KOR in KO mice or treatment with long-acting KOR antagonists reverse many signs of opioid physical dependence. The following study assessed whether treatment with the short acting selective KOR antagonist aticaprant (also known as JNJ-67953964, and previously LY-2456302 and CERC-501) was effective in reversing physical dependence in morphine-dependent mice.

Male ICR mice were rendered dependent on morphine (8 days of an ascending dosing protocol) and on day 9, six hours after the last morphine injection, mice were observed for physical dependence signs (including the total number of jumps, wet dog shakes, paw tremors, backing, ptosis and other signs) for 30 min. Mice were injected with vehicle or aticaprant (1 or 3 mg/kg, p.o.) 5 hours after the last dose of morphine and 60 min later withdrawal signs were observed.

During withdrawal, morphine-treated mice showed a significant increase in total somatic signs and various individual signs. Surprisingly, aticaprant significantly increased total somatic signs and various individual signs in morphine-dependent mice at the doses of 1 and 3 mg/kg. In the saline-treated group, the 3 mg/kg dose of aticaprant alone did not precipitate withdrawal somatic signs. In a separate group of animals, aticaprant at the dose of 3 mg/kg was found to block morphine-induced antinociception in male mice in the tail-flick test.

The present data do not support the use of aticaprant for the treatment of opioid withdrawal syndrome. They are also inconsistent with previous reported results on KOR KO mice and long-acting kappa antagonist JDTic.

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The authors have no conflicts of interest to disclose

CL39 is a novel, kappa opioid receptor (KOR)-selective partial agonist that displays limited activation of arrestin-3/p38MAPK signaling *in vitro* and induces antinociception without eliciting significant aversion *in vivo*.

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Recently we identified CL39, a novel kappa opioid receptor (KOR) selective partial agonist; here we investigate its G protein- versus arrestin-dependent signaling *in vitro*, and its antinociceptive, anti-allodynic, anti-hyperalgesic versus sedative/aversive effects and anti-addiction properties *in vivo*.
Classic KOR agonist U50,488 was employed as reference compound.
G protein activation and arrestin 3 recruitment at KOR were investigated by measuring adenylyl cyclase inhibition and by performing arrestin complementation assay in U2OS and BRET assay in HEK-293 cells. Activation of distinct MAPKs over others and the subsequent functional selectivity on related cellular responses were studied in HEK-293, U87-MG astrocytoma cells and primary cultures of human astrocytes. Antinociception was assessed in the warm-water tail withdrawal test; anti-allodynic and anti-hyperalgesic effects were studied in oxaliplatin-induced neuropathy in mice; U50,488- and CL39-mediated effects on place aversion and on cocaine-induced place preference were evaluated in mice.
Similarly to U50,488, CL39 inhibited adenylyl cyclase, albeit displaying partial agonism. Conversely to U50,488, CL39 weakly recruited arrestin 3 at KOR and induced early (5-15 min), G protein-dependent ERK1/2 phosphorylation but neither late (60 min), arrestin-dependent ERK1/2 nor p38MAPK phosphorylation. U50,488, but not CL39, significantly increased U87-MG and primary human astrocytes cell proliferation in arrestin 3, p38MAPK-dependent fashion.
In vivo both CL39 and U50,488 (0-30 mg/kg; 0-60 min; i.p.) induced a significant, KOR-mediated, dose-dependent antinociception in warm-water tail-withdrawal test, displaying CL39 partial agonism. Conversely to U50,488, CL39 counteracted oxaliplatin-induced hyperalgesia and allodynia but did not determine any significant motor incoordination or aversion. In preliminary experiments, CL39 showed a trend to counteract cocaine-induced conditioned place preference; further studies at this regard are currently ongoing and will be discussed at the conference
Our findings show that CL39 is a KOR selective partial agonist preferentially activating G protein-dependent over arrestin-mediated signaling; as a consequence, CL39 fails to activate p38MAPK, that in turn is related to astrocyte activation and other adverse effects as sedation, motor incoordination, anhedonia. Consistently, CL39 determines a significant, aversion-free antinociception in animal model of acute and chronic pain and a trend to counteract cocaine-induced place preference; thus emerging as a promising candidate to be further investigated as potential innovative analgesic and anti-addiction therapeutic.

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Conflict of interest:
The authors have no conflicts of interest to disclose.

Structural modifications to Phe^3 in [D-Trp]CJ-15,208 are well tolerated by kappa opioid receptors

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The macrocyclic tetrapeptide [D-Trp]CJ-15,208 (*cyclo*[Phe^1-D-Pro-Phe^3-D-Trp]) is an orally active kappa opioid receptor (KOR) antagonist (*Eans* et al., *Br. J. Pharmacol.* 2013) and promising lead compound for development of potential treatments for substance abuse. In this study we examined the structure-activity relationships (SAR) of this peptide in order to improve its pharmacokinetic properties and *in vivo* potency. As part of these studies we explored the scope of modifications to Phe^3 in the peptide that were compatible with KOR activity, incorporating a substitution into the aromatic ring as well as replacing this residue with aliphatic amino acids. The effects of modifications on metabolic stability were examined using mouse liver microsomes. The stability to metabolism varied substantially among the analogs examined, with half-lives from 5 min to > 3 h. *In vivo*, except for [His^3,D-Trp^4]CJ-15,208, all of the analogs significantly antagonized the KOR agonist U50,488 in the 55ºC warm-water tail-withdrawal assay following intracerebroventricular (i.c.v.) administration, although antagonist potencies varied. As was found for [Ala^3,D-Trp^4]CJ-15,208 (Aldrich *et al.*, *Br. J. Pharmacol.* 2014), most of the analogs also exhibited antinociception in this assay following i.c.v. administration. Thus analogs with a variety of structural modifications in this position retain activity via KOR, although the specific modification influences the metabolic stability and the *in vivo a*ctivity profile observed following i.c.v. administration.

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Conflict of interest:
Patent applications have been filed on these analogs.

Computational Design of Bifunctional MOR Agonists/KOR Antagonists for the treatment of Opioid Use Disorder (OUD)

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Opioids relieve pain by stimulating their cognate receptors on the surface of neurons. Although these effects are primarily mediated by the mu-opioid receptor (MOR), there is a growing appreciation that actions at the other opioid receptor subtypes, namely delta (DOR) and kappa (KOR), contribute to the pharmacology of so-called MOR agonists. Indeed, compounds like buprenorphine, that partially activate the MOR while simultaneously inactivating the KOR produce effective analgesia while mitigating drug seeking behavior, opioid cravings, and opioid-cessation related depression. For this reason, buprenorphine is one of the first line therapies for Opioid Use Disorder (OUD). This project seeks to build on the success of buprenorphine, a molecule conceived in the 1970's, to develop novel next generation bifunctional MOR agonist/KOR antagonist molecules. To this end, a structure-based virtual screen of nearly 300 million molecules was prosecuted against the active MOR and inactive KOR crystal structures. 13,500 compounds that scored well in both screens were assessed for similar poses resulting in 4,700 molecules, which were manually examined for productive contacts with both binding sites as well as high internal energy. Ultimately, 64 molecules were selected and assayed, 16 of which were active at either receptor. Further testing and analoging the active compounds could lead to novel opioid analgesics for treatment of OUD with improved safety/efficacy profiles.

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The Salvinorin A analogue, EOM SalB, promotes remyelination in preclinical models of multiple sclerosis

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Multiple sclerosis (MS) is a devastating autoimmune disease that affects an estimated 2.5 million people worldwide. MS is caused by the immune system attacking and destroying the protective myelin coating on nerve cells. There is no cure for MS and current disease-modifying treatments target the immune system and reduces damage by limiting the immune attack of myelin. Our approach is different, we aim to evaluate the ability of our novel kappa opioid receptor drugs to repair and restore myelin levels. We have discovered that the Salvinorin A analogue, ethoxymethyl ether Salvinorin B (EOM SalB), is highly effective at promoting functional recovery and remyelination in two models of MS. Following therapeutic administration in the experimental autoimmune encephalomyelitis (EAE) model, mice treated with EOM SalB significantly reduced disease score and increased the percentage of mice recovered. This effect was reversed with the kappa opioid receptor antagonist nor-binaltorphimine. In the cuprizone toxin-induced model of demyelination, administration of EOM SalB lead to improved health measures, as evidence by a restoration of body weight. Transmission electron microscopy analysis of the corpus callosum showed that EOM SalB increased the number of myelinated axons and significantly reduced g-ratios compared to vehicle controls. Overall, our findings show the potential of EOM SalB to promote functional recovery and remyelination in two preclinical models of MS, and that the kappa opioid receptor is a therapeutic target for remyelination.

Conflict of interest: Anne La Flamme, Bronwyn Kivell and Thomas Prisinzano are inventors on patent applications that relate to this work and have been licensed to Rekover Therapeutics Ltd. ACL, BK and TP hold equity in Rekover Therapeutics Ltd. The authors declare no other financial interests.

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Selective kappa-opioid receptor partial agonist HS666 shows anticonvulsant efficacy in mouse models of temporal lobe epilepsy without inducing aversive behaviour

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Mesial temporal lobe epilepsy (mTLE) is one of the most common and severe types of epilepsy. The high incidence of resistance and numerous adverse effects of currently available drugs highlight the need of new mechanism-based and safer treatments. Accumulated evidence established the importance of the dynorphin, and its primary target, the kappa-opioid receptor (KOR) in epileptogenesis and seizure control. Modulation of the dynorphin/KOR system emerges as a prominent avenue in the pursuit of novel therapies for mTLE. Along with promising antiepileptic effects of full KOR agonists, detrimental side effects of dysphoria and sedation limit their potential clinical use. A behavioral study on the anticonvulsant efficacy in mouse models of mTLE and KOR-mediated adverse effects of the selective KOR partial agonist, HS666, after intraperitoneal administration is presented. Pentylenetetrazole (PTZ)-induced seizures were used to model acute seizures in prodynorphin-knockout mice. The intrahippocampal injection of kainic acid (KA) in C57BL/6N mice was used as a model for drug-resistant TLE. Conditioned place aversion (CPA) and locomotor activity were assessed in C57BL/6N mice. Intraperitoneal administration of HS666 produced dose-dependent (0.3-10 mg/kg) and significant increase in the threshold for PTZ-induced seizures, and reduced paroxysmal activity in the KA model in mice. The anti-seizure/anticonvulsant effects of HS666 were reversed by pre-treatment with the selective KOR antagonist nor-binaltorphimine indicating a KOR-mediated mechanism of action. The central site of action of HS666 with a KOR-specific effect was also demonstrated by the blockade with the KOR antagonist 5′-GNTI after intracisternal administration. Moreover, HS666 did not induce aversive behavior in the CPA paradigm nor did it cause locomotor impairment at therapeutic doses. These results indicate that HS666 has the prerequisite pharmacological characteristics of an effective drug in experimental epilepsy by activating central KORs to produce anticonvulsant effects with reduced KOR-mediated liabilities.

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The authors declare no conflict of interest.

Kappa-opioid receptors (KORs) in microglia: expression and regulation in inflammatory and stress-related conditions

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There is growing appreciation that neuropeptide receptors are expressed in microglia and can have important roles in the regulation of microglia function. Previous studies suggest that kappa-opioid receptors (KORs) are expressed in microglia cells, and that activation of KOR receptors on microglia suppresses pro-inflammatory signaling. Beyond this small number of studies, however, the potential roles for microglia KORs in inflammatory and stress-related conditions have not been thoroughly investigated. Here, we describe our preliminary investigations into the expression and function of KORs on microglia. Using single-cell RNA sequencing (scRNAseq) of the nucleus accumbens (NAc) in mice, we determined that *Oprk1* is expressed in both neurons and microglia. These findings are consistent with those reported previously, although our new work indicates that KORs are expressed in some but not all NAc microglia. To investigate whether KOR might play a role in regulation of inflammatory signaling, we used knockout mice that lack KORs (KOR-/-) and activated the innate immune system by intraperitoneal (IP) administration of lipopolysaccharide (LPS). Three hours after LPS, we isolated microglia for rt-qPCR analysis using magnetic cell sorting. In the microglia (CD11B+) fraction, LPS substantially upregulated the proinflammatory cytokines *IL1ß* and *TNF*, with larger increases in microglia samples from KOR-/- mice. These results are consistent with the possibility that KOR signaling in microglia act to regulate (inhibit) proinflammatory signaling. In addition, since it has been established that chronic social defeat stress (CSDS) activates microglia and upregulates proinflammatory cytokines, we investigated how CSDS might affect KOR expression on microglia. Preliminary data in a small cohort of mice indicate that after a 10-day CSDS regimen, microglia KOR expression was decreased compared to microglia from control mice, and this effect was accompanied by increases in *IL1ß* and *TNF* in microglia. Considered together, these early data raise the possibility that KOR signaling in microglia plays an important role regulating proinflammatory states, and that during chronic stress a downregulation of microglia KORs might contribute to immune activation.

Support:

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Conflict of interest:

Dr. Carlezon has a patent (US 6,528,518; Assignee: McLean Hospital) related to the use of kappa-opioid antagonists for the treatment of depressive disorders.

Does the kappa-opioid receptor derived DREADD have a therapeutical potential in temporal lobe epilepsy?

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Background:
Epilepsy, with a prevalence of 0.5 – 1%, represents one of the most common neurological diseases. Among the different types of epilepsy, temporal lobe epilepsy (TLE) is the most frequently diagnosed. Since up to 80% of TLE patients do not achieve seizure-freedom with currently available pharmacotherapies, novel treatment strategies are urgently needed.
Targeting anatomically restricted regions such as epileptic foci with gene therapy tools has shown encouraging results. Designer receptors exclusively activated by designer drugs (DREADDs) may be well-suited candidates for such a gene therapy approach offering the additional advantage of suppressing neuronal excitability in a controllable way.

Aim:
In this study we investigated the therapeutic potential of a viral-vector delivered DREADD based on the kappa-opioid receptor (KORD) in the kainic acid mouse model of chronic TLE.

Methods:
Using mice after unilateral intrahippocampal kainic acid application, we injected an AAV6 vector construct encoding the KORD under the control of the neuron-specific hSyn promoter into the epileptic focus in the hippocampus. Upon selective activation of the KORD by Salvinorin B (SALB) an inhibitory Gi-cascade is initiated. In order to evaluate the ensuing effect on epileptic activity, we analyzed in-vivo EEG recordings regarding spike trains and hippocampal paroxysmal discharges (hpds, representing drug-resistant focal seizures).

Results:
SALB did not show a statistically significant effect on spike trains and hpds in comparison to the vehicle DMSO, whereas DMSO alone, intriguingly, resulted in a significant reduction of spike trains and hpds compared to saline. Regarding the spreading of the AAV6-KORD, we observed a remarkable discrepancy between non-epileptic animals with robust expression of the AAV6-KORD in the whole hippocampus and epileptic mice with pronounced hippocampal sclerosis showing only limited spreading.

Discussion:
In conclusion, the observed effect of the solvent DMSO on epileptic activity interferes with the evaluation of the KORD/SALB pressing the quest for alternative solvents.

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Conflict of interest statement:
The authors declare no conflict of interest.

Kappa opioid receptor agonists reduce paclitaxel-induced neuropathic pain in both male and female mice

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Current pain medications targeting the mu opioid receptor are highly addictive. As an alternative, kappa opioid receptor (KOPr) agonists have proven antinociceptive effects without rewarding properties. 16-ethynyl salvinorin A (16-ethynyl SalA) is a potent analogue of salvinorin A (SalA) and has been shown to attenuate cocaine-prime induced drug seeking behaviour in rats without causing sedative, anxiogenic, aversive or pro-depressive side effects. Here, we investigated the ability of 16-ethynyl SalA to modulate pain behaviours in a preclinical model of chemotherapy-induced neuropathic pain. The paclitaxel-induced neuropathic pain model was used to assess the cumulative dose response effects of 16-ethynyl SalA, SalA, morphine and prototypical KOPr agonist U50,488, on mechanical and cold allodynia in C57Bl/6J mice. Paclitaxel-induced neuropathy was evaluated at baseline and every second consecutive day, with dose-response effects evaluated on day 15. 16-ethynyl SalA (ED_{50}: 0.65 mg/kg in males; ED_{50}: 1.08 mg/kg in females) was significantly more potent at reducing mechanical allodynia compared to morphine (ED_{50}: 0.65 mg/kg in males; ED_{50}: 1.08 mg/kg in females) in both male and female mice. SalA (ED_{50}: 0.60 mg/kg in males; ED_{50}: 1.03 mg/kg in females) and 16-ethynyl SalA (ED_{50}: 0.31 mg/kg in males; ED_{50}: 0.71 mg/kg in females) were more potent at reducing cold allodynia than morphine (ED_{50}: 2.06 mg/kg in males; ED_{50}: 3.64 mg/kg in females). In a chronic model, with drug administration daily for 22 days, morphine (10 mg/kg), U50,488 (10 mg/kg) and 16-ethynyl SalA (3 mg/kg), reduced both cold and mechanical allodynia. The KOPr agonist U50,488, was the most effective, reducing mechanical and cold allodynia to baseline levels in both male and female mice. This study shows that KOPr agonists have antinociceptive effects in both sexes in a paclitaxel-induced neuropathic pain model, indicating that KOPr agonists are a potential therapeutic avenue for this debilitating condition.

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Characterization of JNK activation by KOR antagonists and partial agonists

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c-Jun N-terminal Kinase (JNK) MAPK activation by the Gi/o protein coupled kappa opioid (KOR), mu opioid (MOR) and D2 dopamine receptors stimulates peroxiredoxin 6 (PRDX6)-mediated production of reactive oxygen species (ROS). This mechanism results in ROS-mediated inactivation of G-protein signaling and other c-Jun-Kinase mediated effects. JNK/ROS receptor regulation is not a unique property of the long-acting KOR antagonists norBNI and JDTic; but rather low efficacy partial agonists including morphine (acting at MOR), quinpirole (acting at D2 dopamine receptors), and THC (acting at CB1 receptors) also produce JNK-dependent desensitization.

Initial characterization of this mechanism used exclusively male mice. We have since demonstrated that while norBNI is an effective competitive antagonist in blocking U50,488-mediated analgesia in female mice, the duration of a single injection of norBNI is not long lasting unless female mice are ovariectomized or pretreated with the GRK inhibitor CMPD101. We previously reported that CMPD101 increased analgesic efficacy of KOR agonists in female mice. Furthermore, immunoblots reveal norBNI does not stimulate JNK phosphorylation in spinal cord of female mice unless pretreated with CMPD101. The present studies confirm that the duration of action of norBNI in female mice may be dependent on cellular context including hormonal regulation, as predicted since the prolonged effects of norBNI are signaling-dependent.

We have recently extended these published studies to assess JNK possible activation by other opioid antagonists and partial agonists in by characterizing the effects of BTRX-335140 and LY2456302 (also known as CERC-501) which are in development for depressive disorder, the MOR/KOR antagonists, naloxone and naltrexone which are used for opioid overdose and abuse and for alcohol or opioid dependence respectively, and by the MOR antagonist/KOR partial agonist nalmefene which is in use for alcohol dependence. First, agonist and antagonist activities were confirmed in KOR-expressing HEK293 cells by immunoblot analysis of induced ERK activation relative to U50,488. Next phospho-JNK-IR was analyzed in transfected HEK293 cells by immunoblot. As we previously demonstrated, LY2456302 and naloxone did not induce JNK phosphorylation. Naltrexone did not induce JNK phosphorylation, which was somewhat unexpected, as norBNI is structurally related to naltrexone. In contrast, BTRX-335140 induced modest JNK phosphorylation, although to a lesser extent than norBNI. Nalmefene induced a modest increase in JNK phosphorylation, corresponding to the early phase of JNK phosphorylation observed after U50,488 treatment.

These findings will be extended by assessing the ability of these ligands to induce ROS generation and by assessing their effects on KOR function when administered daily *in vivo*. These data will provide mechanistic insights into clinically relevant KOR ligands.

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Conflicts of Interest: The authors have no conflicts of interests.

Kappa-opioid receptor-expressing spinal neurons mediate itch transmission and include projection neurons

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Previous studies have shown that a subpopulation of spinal inhibitory neurons mediate itch sensation through the release of dynorphin, the endogenous ligand of the kappa-opioid receptor (KOR). Later studies found that KOR bidirectionally modulates itch at the level of the spinal cord. It is evident that KOR signaling plays a role the neurotransmission of itch, and KOR agonists, in particular, have yielded promising results as potential therapies for chronic pruritis. However, the population of spinal neurons that express KOR (encoded by *Oprk1*) has yet to be comprehensively studied. Here we use the *Oprk1*^{cre} knockin allele and a Cre-dependent excitatory DREADD to visualize and manipulate this population of neurons in the spinal cord. We provide evidence that chemogenetic activation of *Oprk1*^{cre} expressing spinal neurons increases nocifensive spontaneous behavior as well as acute itch and chemical pain sensitivity in mice. Immunohistochemistry and RNAscope fluorescence in situ hybridization revealed widespread *Oprk1* expression in both superficial and deeper laminae of the spinal cord dorsal horn. Moreover, we found that *Oprk1*-expressing spinal neurons comprise a heterogeneous population of both excitatory and inhibitory cell types, observing co-expression of *Oprk1* with VGLUT and VGAT. Finally, *Oprk1*^{cre} spinal neurons were found to include projection neurons with central targets that are consistent with a role of KOR in the circuitry conferring itch: the inferior olivary nucleus (ION), parabrachial nucleus (PBN) and periaqueductal gray (PAG), and ventral posterolateral nucleus of the thalamus (VPL).

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Conflict of interest
The authors have no conflicts of interest to declare.

Title:
ACTIVITY OF DYNORPHIN-EXPRESSING NEURONS DURING VOLUNTARY ALCOHOL CONSUMPTION AND DYNORPHIN-EXPRESSING AFFERENTS IN THE CENTRAL AMYGDALA

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Abstract:
Dynorphin signaling within the central amygdala (CeA), has been implicated in responses to both stress and alcohol. Disrupting CeA dynorphin-expressing neurons or kappa opioid receptor signaling attenuates aberrant drinking behavior that results from the interaction of stress and prolonged alcohol drinking. Yet, how cellular activity of dynorphinergic neurons in the CeA relates to active alcohol drinking is not well-understood. Furthermore, brain regions providing dynorphinergic input to the CeA that would activate kappa opioid receptors, other than the CeA itself, have not been explored. The goals of the current studies were twofold: 1) probe the engagement of CeA dynorphin-expressing neurons during voluntary alcohol consumption using fiber photometry and 2) to explore dynorphinergic inputs to the CeA using retrograde viral tracing. To accomplish the first goal, a virus allowing expression of a Cre-dependent calcium sensor, GCaMP7f, was infused into the CeA of prodynorphin-Cre (Pdyn-Cre) transgenic mice. Simultaneously, a fiber optic ferrule was implanted into the CeA to allow for recording calcium-dependent neuronal activity from dynorphin-expressing neurons in the CeA during alcohol drinking. Using a modified drinking-in-the-dark paradigm, 3 hours into the dark cycle Pdyn-Cre mice were given access to 20% alcohol in their home cages for 2 hours/day, 5 days/week for 3 consecutive weeks. During these sessions, lickometer circuitry in the home cage allowed for time-locked GCaMP7f activity in CeA dynorphin neurons to bouts of licking for alcohol. Preliminary analysis of GCaMP7f activity from CeA dynorphin neurons indicates a trend toward an increase in calcium transients during bouts of licking for alcohol, which differed from activity around bouts of drinking water or sucrose, indicating these neurons are uniquely engaged during alcohol consumption. To accomplish the second goal of probing dynorphinergic input to the CeA, a retrograde virus driving the expression of a Cre-dependent fluorescent marker, eGFP, was infused into the CeA of Pdyn-Cre transgenic mice. Brain tissue was surveyed for eGFP expression outside the CeA using confocal microscopy. Preliminary analysis of eGFP expression in dynorphin-expressing inputs to the CeA indicate that one potential source of dynorphin to the CeA is the intermediate-posterior insula, a region known for its involvement in negative affective behaviors, which can be induced by both stress and prolonged alcohol exposure (i.e. withdrawal). These findings begin to shed light on fundamental cell population- and circuit-level mechanisms that could underlie the complex interactions between stress and alcohol in AUD.

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Medial prefrontal cortical dynorphin-containing neurons constrain emotional memories.

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The medial prefrontal cortex (mPFC) is implicated in modulation of stress and anxiety. Dysfunction of mPFC circuitry is associated with several common symptoms in neuropsychiatric disorders, including cognitive deficits, anhedonia, and anxiety. The dynorphin (DYN) / kappa-opioid receptor (KOR) system has been implicated in mediating stress-induced adaptive and mal-adaptive behavior and is highly expressed within mPFC. However, our understanding of how the DYN / KOR system is embedded in mPFC networks and regulates circuit function is extremely limited. Here, we first utilized *in situ* hybridization and comprehensive anatomical analysis to describe the cell type and layer organization of mPFC DYN / KOR system. Prodynorphin (PDyn) mRNA is expressed in subpopulation of mPFC glutamatergic and GABAergic somatostatin (SST) neurons, and is not overlapped with KOR mRNA expression. We then utilized *in-vivo* fiber photometry and single cell imaging to demonstrate that mPFC DYN-expressing neurons are recruited during processing of threats and during retrieval of these memories. mPFC DYN neurons are not only activated by the threat itself but also tuned to respond to the cues that predict the threat upon associative learning. By using intersectional transgenic and viral approaches, we examined the activity of glutamatergic DYN neurons, GABAergic SST DYN neurons, and their DYN-lacking counterparts, and demonstrated that distinct DYN-containing cell types differentially encode threats and the cues that predict them. Moreover, we used a novel, fluorescence-based KOR sensor to probe the *in-vivo* dynamics of DYN / KOR transmission during fear conditioning. Similar to the activity of PDyn neurons, the KOR sensor signal is increased by threats and the cues that predict them, while responses to on-going threats are modulated in a dynamic manner. These results demonstrate the involvement of local DYN / KOR signaling during the Pavlovian threat conditioning. Finally, we demonstrated that manipulating DYN / KOR signaling by knocking-down DYN expression via viral-mediated expression of PDyn small hairpin RNA (shRNA) modifies the acquisition and expression of Pavlovian threat memories. We find that mice exhibit enhanced freezing to threat associated cues compared to control group during Pavlovian threat conditioning, without impacting expression of long-term memories or their extinction. In summary, we address the anatomical organization of DYN / KOR system in mPFC and demonstrate the role of mPFC DYN neurons and DYN / KOR signaling in formation of emotional memories. As dysregulation of mPFC circuits and DYN / KOR signaling therein have been implicated in various neuropsychiatric disorders, these results may provide a framework to inform the development of therapeutic targets focused on these systems.

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The authors declare no conflict of interest.

Unique morphological and electrophysiological characteristics define subpopulations of dynorphin-containing neurons within the medial prefrontal cortex (mPFC)

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The medial prefrontal cortex (mPFC) plays an essential role in cognitive processing and regulation of emotion, sociability, and motivation. Dysfunction of the mPFC is associated with numerous neurological and psychiatric disorders including depression, anxiety disorder, schizophrenia, and addiction. These psychiatric disorders are characterized by overlapping symptom clusters, including anhedonia, cognitive deficits, and anxiety, which suggests common neurological circuitry is involved. The dynorphin/KOR system is highly expressed in the mPFC and is a prominent modulator of motivated behavior as well as highly implicated in stress-induced dysphoria and vulnerability to drug abuse. Although the dynorphin/KOR system has been investigated in subcortical regions, our understanding of how it is embedded in mPFC networks is limited. Intricate organization of mPFC networks is crucial for proper function of these circuits, and characterizing dynorphin-containing neuron morphology and organization within in the mPFC is a necessary step to uncovering how the dynorphin/KOR system shapes cortical circuits that underlie neuropsychiatric disorders. In this study, we used a combination of transgenic mice, viral tracing approaches, ex-vivo electrophysiology, and reconstruction of electrophysiologically-characterized dynorphin-containing neurons to anatomically and electrophysiologically characterize the neuronal populations that express dynorphin in the mPFC. *In-situ* hybridization revealed dynorphin is predominately expressed in glutamatergic neurons and a subpopulation of GABAergic interneurons. Investigation of excitatory dynorphin-containing neuronal long-range projections through use of both anterograde- and retrograde-viral tracing reveal outputs to the nucleus accumbens, paraventricular nucleus of the thalamus, and ventral tegmental area. The layer in which these neurons reside is congruent with their output target as neurons in L2/3 tend to project to striatal regions and neurons in deeper layers tend to project to thalamic and midbrain regions. Further, neuronal arborization and branching patterns were investigated to identify morphological features of dynorphin-containing mPFC cell types. Uncovering the identity of mPFC dynorphin-containing neurons and the anatomical architecture of the dynorphin/KOR system within the mPFC is a critical step in elucidating how dynorphin peptides shape cortical circuits that underlie neuropsychiatric disorders. Understanding this system within the mPFC may reveal therapeutic targets to treat symptoms associated with various neuropsychiatric disorders including depression, anxiety disorder, and schizophrenia.

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Nucleus accumbens GABAergic afferents to the ventral tegmental area display a stress-sensitive form of long-term plasticity.

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Dopamine (DA) neurons in the ventral tegmental area (VTA), a brain region known to be necessary for drug reinforcement, play an essential role in the reward pathway. Acute stress modulates plasticity at both excitatory and inhibitory synapses onto DA neurons. We previously reported that inhibitory postsynaptic currents (IPSCs) on VTA DA neurons undergo nitric oxide-dependent LTP (LTP_{GABA}). LTP_{GABA} is lost after acute stress that activates kappa opioid receptors (kORs). This loss depends on altered activity of kappa opioid receptors (kORs), since nor-BNI, a long-lasting kappa selective receptor-inactivating antagonist, given either in vivo after acute stress or bath applied during post-stress electrophysiological recordings rescues LTP_{GABA}. The loss of LTP_{GABA} and its rescue by nor-BNI after stress correlates with stress-induced reinstatement of cocaine-seeking (Polter et al., 2014).

To understand how acute stress modifies brain circuitry relevant to drug-seeking, it is critical to know which GABAergic afferents to VTA are involved. Our previous work indicated that GABAergic afferents from the rostromedial tegmental nucleus do not undergo LTP_{GABA}, while local VTA GABAergic afferents only exhibit modest LTP_{GABA} (Polter et al., 2018). The nucleus accumbens medium spiny neurons also project strongly to the VTA. Here, using optogenetics, we report that the nucleus accumbens (NAc) is a source of GABAergic inputs to VTA DA neurons that exhibits robust LTP_{GABA}.

Our experiments with nor-BNI indicate that kOR activation is sufficient for stress to block $LTP_{GABA,}$ and hence deleting kORs from the relevant cell type should prevent this. Using a conditional knock-out approach, we also report that deletion of kORs on postsynaptic DA cells does not prevent stress-induced loss of LTP_{GABA}. Instead, kORs on the presynaptic terminals of relevant GABAergic afferents may be those relevant to stress-induced loss of LTP_{GABA}. When kORs were genetically deleted in NAc neurons, LTP_{GABA} was expressed normally even after acute stress.

Our data indicate that 1) specific GABAergic afferents to the VTA from cells in the NAc are a likely target of stress, and 2) kORs in these terminals but not kORs on DA cells mediate loss of LTP_{GABA} during acute stress. These results highlight the importance of dissecting the specific subcircuits that converge in the VTA and the subcellular circuit elements that modulate them.

Polter, A.M., Bishop, R.A., Briand, L.A., Graziane, N.M., Pierce, R. C., and Kauer, J.A. (2014) Post-stress block of kappa opioid receptors rescues LTP_{GABA} and prevents reinstatement of cocaine seeking. Biol. Psychiatry 76:785-93.

Polter AM, Barcomb K, Tsuda AC, Kauer, JA. Synaptic function and plasticity of inhibitory inputs onto VTA dopamine neurons. (2018) Eur. J. Neurosci. 47:1208-1218.

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Acute Stress Shifts Kappa-Opioid Receptor Function from Inhibitory to Excitatory in a Subset of VTA Dopamine Neurons

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Aversive stressors are a major driver of relapse to drug taking in people with SUD; interventions that boost resilience in the face of stressors will help patients stay sober. The corticotrophin releasing factor (CRF) system is activated by aversive stress, which in turn induces kappa opioid receptor (KOR) activation downstream (Land et al., 2008). Blocking either CRF receptors or KORs in the VTA diminishes stress induced reinstatement of cocaine seeking (Vranjkovic et al., 2018). Here we investigated how KOR modulates VTA circuits following stress. In naïve animals, in whole cell *ex vivo* recordings, KOR activation inhibits a subset of VTA dopaminergic neurons (Margolis et al., 2003), in particular those that project to prefrontal cortex (Margolis et al., 2006) and the amygdala (Margolis et al., 2008). Surprisingly, here we found that a single footshock stress session induced a change in responses to U69593 (1 μM) to depolarizations in a subset of VTA dopaminergic neurons. This switch persisted for several days after the single stress exposure. Pretreating naïve slices with CRF (200 nM) for 5 min was sufficient to drive this switch, and this switch occurred specifically in prefrontal cortex-projecting VTA dopamine neurons. The p38 inhibitor SB203580 (10 μM) prevented the CRF induced KOR switch in signaling. Since dopamine signaling in the prefrontal cortex is required to drive stress responses such as reinstatement to drug seeking (McFarland et al., 2001), these observations raise the possibility that the behavioral impact of KOR activation in the VTA after stress is due to an increase, rather than a decrease, in dopamine release in this circuit.

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Mu-opioid receptors potentiate appetitive behaviors via g-protein, but not beta-arrestin 2, signaling.

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Opioid peptide stimulation alters respiration, analgesia, and reward behavior, and can induce addiction and drug overdose. Though complex, evidence over the last several decades has indicated that many of the rewarding effects of opioids primarily occur via the mu opioid peptide receptor (MOPR), though many fundamental neurobiological questions remained. Recently, our lab has discovered that endogenous MOPR regulation of appetitive behavior specifically involves a dorsal raphe to nucleus accumbens projection. While these results provide important functional and anatomical information, they do not address how MOPR stimulation engages subsequent molecular signaling mechanisms. Here, we show bourgeoning evidence that MOPRs on dorsal raphe to nucleus accumbens terminals primarily use G-protein, but not arrestin, signaling cascades to modulate downstream reward behaviors. Using constitutive MOPR, or beta-arrestin 2, knockout mice we show that loss of MOPRs reduces reward behaviors, but loss of arrestin does not. Furthermore, selective genetic rescue of a G-protein biased mutant MOPR via targeted viral injection is sufficient to restore deficits in motivation. Ongoing work include fluorescent in situ hybridization approaches to isolate specific G-proteins of interest in dorsal raphe nucleus. This will aide in the development and use of CRISPR-Cas9 viruses for cell-type selective deletion of specific G-protein subunits. Altogether, these experiments will delineate a molecular-to-behavioral pathway underlying MOPR control of appetitive motivated behaviors.

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Dynorphinergic control of amygdalo-striatal circuits for goal-directed action

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Elegant work spanning decades has implicated the striatum at large, and especially the dorsomedial (DMS) in how animals learn, perform and update outcome-dependent or goal-directed actions. Yet, the circuit- and molecular-level substrates involved in these behaviors remain largely unknown. Given the amygdala's role in encoding value, neural projections from specifically the basolateral amygdala (BLA) to the DMS are uniquely poised to sculpt action-outcome strategies. ~50% of the neurons in the DMS express dynorphin; but whereas prior research has focused on the role of dynorphinergic neurons in action learning and performance, how dynorphin (dyn) itself coordinates these strategies via the control of circuits projecting to the striatum is yet to be elucidated. Interestingly, ours and prior studies have shown that BLA neurons project exclusively to dynorphin neurons in the DMS and that DMS-projecting BLA neurons express kappa-opioid receptors (KOR). ***Hence, we hypothesize that BLA projections to the DMS coordinate outcome-based action learning and maintenance, under the control of dyn-KOR signaling.*** Here, using fiber photometry, we show that BLA-DMS terminals exhibit a biphasic pattern in activity during the performance of an action-outcome strategy with an increase in activity specifically during goal-directed action, followed by inhibition during outcome. We also find that BLA-DMS activity during action is informed by outcome, changing dynamically and reversibly to manipulations in outcome-probability or outcome-value. Notably, we find that this engagement is sensitive to disruptions in opioidergic signaling via naloxone. In parallel, we observe that time-locked photo-activation of BLA-DMS terminals during outcome, disrupts action and conversely, photoinhibition during outcome using a light-activatable Gi/o-coupled GPCR enhances actions. Furthermore, we find that local deletion of dyn in the DMS, or KOR in the BLA disrupts the incentive value for the outcome, negatively impacting reward-processing, goal-directed action learning and maintenance. ***Collectively, our data suggest that BLA-DMS activity informs outcome-dependent action and is regulated by dyn-KOR signaling at these terminals during outcome.*** Current work is focused on how BLA-DMS terminal activity is affected during action-outcome strategies by local dyn deletion in the DMS, local KOR deletion in the BLA and the time-locked, local manipulation of dyn-KOR signaling in the DMS. Future work will delineate how BLA-DMS axons differentially engender action or outcome using *in-vivo* 2-photon Ca^{2+} imaging of individual axons and how their activity is differentially controlled by local dynorphin signaling in the DMS.

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Kappa-opioid receptor stimulation in the nucleus accumbens shell affects drinking in a subregion-, sex-, and substance-specific manner

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The kappa-opioid receptor (KOR) has been investigated as a potential therapeutic target for alcohol use disorder, but studies using stimulation of the KOR in a major limbic region, the nucleus accumbens (NAc) shell, have thus far failed to show an effect on ethanol drinking. While these studies have typically targeted the middle NAc shell, a growing body of evidence demonstrates that behavioral effects of KOR stimulation in the NAc shell depend on the rostro-caudal location examined. Thus, we sought to determine if KOR stimulation of specific subregions of the NAc shell could affect ethanol intake, and if these effects extended to other reinforcing substances. We first trained adult male and female Long-Evans rats to drink 20% v/v ethanol under an intermittent access two-bottle-choice paradigm until daily levels became stable. We then bilaterally microinjected them, prior to daily ethanol access, in the rostral, middle, or caudal NAc shell with the selective KOR agonist U-50,488H (0.008 - 8.0 nmol) or saline vehicle (0.3 µl), using a within-subject Latin-square design across ethanol access days. In male rats (n = 7-9/group), consistent with prior studies, KOR stimulation in the middle shell had no significant effect on ethanol drinking; however, stimulation in the rostral shell significantly decreased ethanol drinking, and stimulation in the caudal shell significantly increased it ($p < 0.05$). In female rats (n = 5-8/group), in contrast, our preliminary results indicate that KOR stimulation failed to significantly affect ethanol drinking ($p > 0.05$). Next, to determine if the subregion-specific effect of KOR agonism in male rats is also substance-specific, we trained a separate group of adult male Long-Evans rats to drink 2.5% w/v sucrose under the same intermittent access paradigm and then microinjected them in either the rostral or caudal NAc shell with U-50,488H (0.8 or 8.0 nmol) or saline vehicle (0.3 µl). Importantly, accumbal KOR stimulation in neither subregion (n = 8/group) significantly affected sucrose drinking. These results demonstrate that KOR stimulation in the NAc shell can affect ethanol drinking, but that this effect is subregion-, sex-, and substance-dependent. Thus, while the KOR may represent a promising target for pharmacotherapies for alcohol use disorder, such treatments may be more appropriate for specific populations and under specific conditions.

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Disclosure
The authors have no conflicts of interest to disclose.

Title: A role for the Kappa Opioid Receptor in how naltrexone changes drinking behavior.

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Abstract:

Purpose: Our group recently demonstrated that among individuals with AUD, occupancy of the kappa opioid receptor (KOR) by naltrexone was negatively associated with naltrexone-associated decreases in drinking. We now seek to understand the association between changes in drinking behavior and the availability of the KOR and its occupancy by naltrexone.

Methods: Non-treatment seeking heavy drinkers meeting criteria for AUD participated in two alcohol drinking paradigms (ADP); before and after a week of 100 mg/day naltrexone. During each ADP participants consumed a priming drink (0.03 g/dl) followed by three self-administration periods during which they could consume up to 12 additional drinks (0.015 g/dl). Subjects also underwent [^{11}C]-LY2795050 PET scans to measure KOR availability in the amygdala, hippocampus, pallidum, striatum, cingulate, and prefrontal cortex prior to starting naltrexone, and a second PET scan to measure KOR occupancy following one week of naltrexone treatment. Primary outcomes during the ADP self-administration period were: *time to first sip* of the first drink and mean *drink duration* of all drinks consumed.

Results: The forty-nine (16F) heavy drinkers with AUD were balanced on family history of alcoholism (FH+/-, 29 FH+) and consumed 47 ± 16 drinks per week at baseline. Overall, participants increased time to first sip ($p = 0.007$), but not drink duration ($p = 0.15$) at ADP2 (after naltrexone) compared to ADP1. KOR availability was not associated with drinking behaviors outcomes. KOR occupancy by naltrexone was inversely associated with time to first sip at ADP2 ($p = 0.008$).

Conclusion: In this study, we observed that higher occupancy of KOR by naltrexone was associated with shorter times to first sip of the first drink, suggesting that higher KOR occupancy may decrease how long it takes people on naltrexone to continue drinking following a lapse. These findings are in line with our previous observation that higher KOR occupancy was associated with smaller reductions in drinking during the ADP after a week of naltrexone. Taken together, these observations further support the hypothesis that alcohol-induced KOR-activation might curb drinking. Therefore, lower doses of naltrexone that do not fully block KOR and which have been demonstrated to have greater efficacy in clinical trials, merit further exploration.

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Title: **REGIONAL DIFFERENCES IN KAPPA OPIOID RECEPTOR ACTIVATION ON REWARD SEEKING BEHAVIORS AND MONOAMINERGIC TRANSMISSION IN THE NUCLEUS ACCUMBENS**

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Kappa opioid receptor (KOR) activation in rostral and caudal nucleus accumbens (NAc) shell can produce opposing behavioral responses: KOR activation in the rostral NAc shell has been found to be anxiolytic and elicit hedonic orofacial expressions, while KOR activation in caudal NAc shell is anxiogenic and suppresses hedonic orofacial expressions. We recently showed that the aversive effect of KOR activation in caudal shell correlates with greater inhibition of dopamine by KORs in this subregion compared to the rostral NAc shell. However, the lower dopamine inhibition in the rostral shell may not be sufficient to explain the hedonic responses observed following rostral KOR activation. Activation of KORs also results in inhibition of serotonin release in the NAc. Because serotonin transmission in this region is implicated in reward learning, this project aimed to examine the impact of KOR activation on serotonin transmission and its effect on reward-seeking behavior in the rostral versus caudal NAc shell.

Male and female Long-Evans rats were trained on an operant fixed-ratio 1 schedule of reinforcement to self-administer a 10% w/v sucrose solution. After establishing stable responding, rats began a progressive-ratio schedule to assess motivation for sucrose. To assess the effect of KOR activation on sucrose self-administration, we then either systemically administered the KOR agonist U50,488 (0, 2.5 mg/kg; i.p.) or, in a separate cohort, locally microinjected U50,488 (0, 0.8, 8 nmol) into the rostral or caudal NAc shell. In separate rats, we used ex vivo fast-scan cyclic voltammetry with tonic (1 pulse) and phasic (5 pulses, 10-100 Hz) stimulations to examine serotonin kinetics in the rostral and caudal shell. We then bath applied U50,488 (0-1.0 uM) and measured serotonin release.

Both systemic administration and caudal microinjection of U50,488 decreased motivation for sucrose in both sexes. To our surprise, rostral microinjection of U50,488 had no effect on motivation for sucrose. Neither the varying stimulation parameters used nor the bath-application of U50,488 revealed differences in serotonin release across the rostro-caudal axis of the NAc shell; however, KOR-induced inhibition of serotonin was greater in males than in females.

These data suggest that KOR activation-driven aversive effects result in diminished motivation to acquire a natural reward. Because we found no differences in KOR control over serotonin release across the rostro-caudal axis in the NAc shell, serotonergic action may not be directly involved in mediating the different subregional effects on behavior. More research is needed to clarify the neurochemical processes that ultimately lead to the opposing behavioral effects of rostral versus caudal KOR activation in the NAc shell.

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Input-specific regulation of discrete populations of Lateral Habenula neurons by Kappa opioid receptors

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The lateral habenula (LHb) is an epithalamic brain region associated with value-based decision making and stress evasion through its modulation of dopamine (DA)-mediated reward circuitry. Increased activity of the LHb is associated with drug addiction and stress-related mood disorders. Dynorphin (DYN)/Kappa opioid receptor (KOR) signaling is an endogenous mediator of stress response in reward circuitry. Previously, we have shown a novel functional role of a kappa opioid receptor (KOR) signaling within Lhb from adult and adolescent rats. Specifically, KOR agonist (U50,488) has distinct effects on LHb neuronal excitability in distinct neuronal populations identified by the presence of hyperpolarizing current (Ih). Namely, U50,488 significantly decreases excitability of Ih negative (Ih-) LHb neurons while simultaneously increasing excitability of Ih positive (Ih+) LHb neurons. Additionally, KOR induced alteration in excitability is dependent on both presynaptic glutamatergic and GABAergic signaling and therefore it is likely that U50,488 induced effects rely on presynaptic KOR expression on LHb inputs. Entopeduncular nucleus (EP) has been implicated in stress-induced mood disorders, such as depression, and anxiety and may contribute to aberrant LHb excitability in depressive-like phenotypes. We hypothesize that our previously identified presynaptic-KOR driven effect on LHb neuron activity may be driven by EP inputs. Here we determined that KORs differentially effect EP presynaptic strength and optically evoked action potentials (oAP) across Ih- and Ih+ LHb subpopulations using input-specific expression of channel rhodopsin in rat LHb slice electrophysiology. Specifically, EP input-specific oEP of Ih- Lhb neurons were significantly reduced following U50,488 application while oAP of Ih+ neurons remained unchanged. Ih- and Ih+ neurons molecular identification were further assessed following electrophysiological recordings by combining ionophoresis labeling during electrophysiology experiments and post-hoc immunohistochemistry to identify the distribution of Ih across glutamatergic (VGLUT+) and GABAergic (somatostatin, SST+) neuronal populations within the LHb. Collectively, these studies show evidence for input- and cell-specific regulation of LHb neurons by KOR signaling and highlights a future hotspot for pharmacological intervention of KOR-mediated mood disorders and resulting aberrant neural circuitry.

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Conflict of Interest: None to declare

Examination of kappa-opioid receptor dynamics during acute stress tests in mice.

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Dynorphin/Kappa-opioid receptor (DYN/KOR) signaling in different brain regions is implicated in the pathophysiology of various psychiatric disorders, highlighting the importance of understanding DYN/KOR dynamics within microcircuits. The development of fluorescence-based GPCR sensors has expanded our ability to determine neuromodulator dynamics with a sub-second temporal resolution, ex-vivo, and in-vivo. Here, we sought to validate kLight, a KOR-based fluorescence-based sensor, to study KOR dynamics in behaving mice. kLight virus was stereotaxically injected unilaterally into the dorso-or ventro-medial NAcc, and an optic fiber was implanted above the virus infusion site. Mice then underwent a battery of stress-inducing stimuli. First, mice underwent fear conditioning procedures involving the presentation of 10 tone cues that co-terminated with a mild footshock (2 s duration; 0.6 mA). The following day a group of mice was introduced into a novel context and presented with ten tone cues but no shock to test for tone-induced fear memory retrieval. A separate group of mice was kept on fear conditioning for three days to examine the kLight signal expression over repeated testing sessions. On separate days, mice were then exposed to five presentations of an aversive auditory stimulus (22.5 kHz tone), a cold temperature environment, or received a challenge of the alpha-2-adrenoceptor antagonist, yohimbine. To pharmacologically validate the kLight sensor, mice received an injection of the highly specific opioid receptor agonist, U50-488, following pretreatment with saline or the kappa-opioid receptor antagonist, naloxone. These imaging experiments revealed that the magnitude of kLight dynamics was most robust during the fear conditioning versus the other tests. Interestingly, the magnitude of the kLight fluorescence was greater in the ventromedial versus dorsomedial NAcc, suggesting that footshock-stress promotes dynorphin release in the NAcc in a region-specific manner. Furthermore, U50-488 increased the kLight fluorescent signal, an effect that was antagonized by naloxone. Together, these results demonstrate that the fluorescence-based kLight sensor allows for imaging and detecting sub-regional differences in KOR dynamics in freely moving mice.

Support
This work was supported by the National Institute of Mental Health Intramural Research Program, the Center on Compulsive Behaviors at the National Institutes of Health, and the NARSAD Young Investigator's Award.

Conflict of interest
The authors declare no conflict of interest.

Job Opportunities

Postdoctoral Fellow opening at City of Hope: Super-Resolution Microscopy - NIH Funded
Position Summary
A Postdoctoral Fellow Position is available to probe the mechanisms of alcohol use disorders. In the U.S., this chronic relapsing brain disorder is estimated to occur in 15 million people. The resulting pattern of long-term alcohol use can lead to cancer and diabetes; it may limit the life span of an individual by more than 20 years. Unfortunately, current treatments for alcohol use disorders are inadequate. Jovanovic-Talisman laboratory has been probing the disorder at the molecular level: We develop new methods to perform quantitative single molecule localization microscopy (qSMLM). This super-resolution imaging technique allows us to detect individual molecules in cells and biospecimens. We have been using qSMLM to assess how alcohol perturbs the dynamic organization of signaling domains that harbor opioid receptors (ACS Chemical Neuroscience, 2019, Tobin et al.). The NIH funded Postdoctoral Fellow will probe the nano-organization of kappa and mu opioid receptors in cultured cells and rat brains.
Key Responsibilities include:
Acute exposure to ethanol affects both the clustering and nano-organization of opioid receptors. However, this effect is largely impeded by the drug naltrexone. To probe this mechanism further and to assess new therapeutics, we seek a Postdoctoral Fellow to design and execute studies that comprise some of the following activities: cell culture, plasmid transfection, antibody labeling and validation; immunostaining of cells and rat brain slices; qSMLM imaging and data analysis; development of new codes (MATLAB) to process qSMLM data.
Basic education, experience and skills required for consideration:

- A Ph.D. degree in biomedical sciences, biophysics, chemical engineering or a related discipline is required with no more than 3 years of postdoctoral training.
- Experience in quantitative microscopy, cell biology, and MATLAB programing.
- Ability to carry out independent research and prepare manuscripts for publication.
- Super-resolution microscopy experience is highly preferred.

Additional Information:

About Dr. Jovanovic-Talisman: Tijana Jovanovic-Talisman, PhD is an Associate Professor in the Department of Molecular Medicine. Dr. Talisman and team are investigating biological processes that are critical to the progression of cancer and other diseases by using quantitative single molecule localization microscopy (qSMLM).

For more information on the Jovanovic-Talisman Lab, please click here.

About City of Hope

City of Hope, an innovative biomedical research, treatment and educational institution with over 6,000 employees, is dedicated to the prevention and cure of cancer and other life-threatening diseases and guided by a compassionate, patient-centered philosophy.
Founded in 1913 and headquartered in Duarte, California, City of Hope is a remarkable non-profit institution, where compassion and advanced care go hand-in-hand with excellence in clinical and scientific research. City of Hope is a National Cancer Institute designated Comprehensive Cancer Center and a founding member of the National Comprehensive Cancer

Network, an alliance of the nation's leading cancer centers that develops and institutes standards of care for cancer treatment.

City of Hope is committed to creating a diverse environment and is proud to be an equal opportunity employer. All qualified applicants will receive consideration for employment without regard to race, religion, color, national origin, sex, sexual orientation, gender identity, age, status as a protected veteran, or status as a qualified individual with disability.

Postdoctoral Fellow Opportunity at Temple University

Postdoctoral Fellow in Neurobiology. A Postdoctoral osition is available in the Center for Substance Abuse Research (CSAR) at Temple University Lewis Katz School of Medicine to study cellular and molecular determinants of addiction-related behaviors. The mission of CSAR is to support innovative interdisciplinary research that will lead to the development of novel treatments of substance use disorders and pain. CSAR is located in a state-of-the-art medical school building on the health sciences campus of Temple University in Philadelphia, PA.

Working under the direction of Dr. Ellen Unterwald, the postdoctoral fellow will be part of a multidisciplinary team investigating how drugs of abuse induce neuroplasticity in the central reward pathway using preclinical models of addiction. Examples of research topics include identification of molecular and cellular circuitry involved in the maintenance of drug-associated memories, molecular basis of susceptibility to drug-seeking behaviors following traumatic stress exposure, and the intersection of immune factors and drugs of abuse. Techniques employed in the research include viral mediated gene transfer, confocal fluorescent microscopy, chemogenetics, in vivo microscopy, and molecular analysis, all applied to rodent models of drug-seeking and relapse behaviors.

The fellow will be supported on their pathway to independence through participation in many career-development activities offered by CSAR, by closely interacting with a highly successful and diverse group of investigators, and by pursuing novel research questions.

A Ph.D. with experience in neurobiology, pharmacology, cell biology, molecular biology or a related field is desired. The position offers the opportunity to work with a dynamic group of investigators in a supportive, collaborative atmosphere. US citizenship or permanent residency status is required. The position is supported by the NIH.

Interested candidates should send their cv, statement of research interests, and names of three references to Dr. Ellen Unterwald, Ellen.Unterwald@temple.edu. *Temple University is an EEO/AA employer and strongly encourages applications from individuals traditionally under-represented in science. Further information about the Lewis Katz School of Medicine at Temple University School is available at* https://medicine.temple.edu/

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