
8Th Conference on the Therapeutic Potential of Kappa Opioids
March 19-21, 2025
Washington University in St. Louis, MO
KappaCon 2025 Program Committee
Ream Al-Hasani (Chair)
Jordan McCall
Tao Che
Aaron Norris
Annushree Karkhanis

8th Conference on the "Therapeutic Potential of Kappa Opioids"
March 19-21, 2025
Washington University in St. Louis, Missouri, USA
University of Health Sciences and Pharmacy in St. Louis, Academic and Research Building, 2 Pharmacy Place Missouri, USA

Wednesday, March 19th

5 - 7 PM Registration (2nd Floor Lobby ARB)

5 - 7 PM Opening Reception (2nd Floor Lobby ARB)

(Dinner: suggestions of local restaurants provided in the program)

Thursday, March 20th

7 - 8 AM Breakfast, Registration, and Poster Setup (2nd Floor Lobby ARB)

8:00 AM Welcome: Ream Al-Hasani

8:15 – 9:55
Oral Session 1: 2nd Floor Lecture Hall 212, ARB

Kappa/Dynorphin system interactions with fentanyl and misuse implications (Chair: Dr. Niko Massaly)

8:15 AM High-throughput Engineering of Genetically Encoded Fluorescent Sensor for Detecting Opioids in vivo. Yuxuan Wang (Univ Wash)

8:35 AM Engineering a genetically encoded fluorescent sensor for in vivo fentanyl detection. Lily Torp (Univ Wash)

8:55 AM Fentanyl exposure and withdrawal modulate opioid peptide dynamics in the nucleus accumbens shell. Aidan Evans-Strong (Wash U)

9:15 AM Characterizing xylazine exposure among pregnant people who use non-prescribed fentanyl. Cassandra Trammel (Wash U)

9:35 AM The role of dynorphin in the periaqueductal grey in the vulnerability to fentanyl addiction-like behaviors. Renata Marchette (NIDA) (ZOOM)

9:55 AM Discussion

10:05 AM Coffee Break (Posters, corridor outside Lecture Hall 212, ARB**)**

10:15 AM – 12:05 PM
Oral Session 2: 2nd Floor Lecture Hall 212, ARB

Molecular pharmacology of kappa opioid receptor activation and signaling (Chair: Dr. Tao Che)

10:15 AM Molecular mechanisms of inverse agonism via κ-opioid receptor–G protein complexes. Cornelius Gati (Univ Southern Cal)

10:35 AM How kappa opioid receptor efficacy and functional selectivity is modulated. Nokomis Ramos-Gonzalez (Wash U)

10:55 AM Pharmacological Characterization of the Novel Selective Kappa Opioid Receptor Agonists 10-Iodo-Akuammicine and 10-Bromo-Akuammicine. Kathryn Bland (Temple)

11:15 AM Iboga-inspired Analogs with Modulated Kappa Opioid Activity Display Potential Therapeutic Applications. Vaclav Havel (Columbia)

11:35 AM Treatment of Diuretic Resistance with a Kappa Opioid Agonist, an Inhibitor of Central Vasopressin Secretion. Daniel R. Kapusta (LSU)

11.55 AM Dissecting the Neural Circuits and Signaling Pathways Underlying Kappa Opioid Receptor-Mediated Psychotomimetic Effects. Aaron Norris (Wash U)

12.15 Discussion

12:30 PM -1:30 PM **Buffet Lunch (**2nd Floor Lobby ARB**)**

1:30 PM – 3:30 PM
Oral Session 3: 2nd Floor Lecture Hall 212, ARB

Kappa opioid dynamics in motivated behaviors I (Chair: Dr. Aaron Norris)

1:30 PM Neuropeptidergic Control of Paraventricular Thalamic Circuitry: Opposing Roles of Orexin and Dynorphin in Arousal and Aversion. Marta Trzeciak (Univ Wash)

1:50 PM A preoptic neuronal population regulates energy balance and expenditure. Juan Liu (Wash U)

2:10 PM Decoding Dynorphin-KOR control of stress-induced binge eating in Claustrum. Jingyi Chen (Univ Wash)

2:30 PM A dorsal hippocampus-prodynorphinergic dorsolateral septum-to-lateral hypothalamus circuit mediates contextual gating of feeding. Travis Goode (Mass Gen)

2:50 PM The role of dorsal nucleus accumbens shell dynorphin neurons in reward seeking. Graydon B. Gereau (Wash U)

3:10 PM Rapid endogenous dynorphin dynamics in the dorsal striatum promote goal-directed action. Raaj Gowrishankar (Univ Wash)

3.30 pm Discussion

3:30 PM – 5:00 PM Poster session (Posters, corridor outside Lecture Hall 212, ARB**)**

6:00 PM – 9:00 PM Networking Mixer. The Boathouse Annex, Forest Park, 6101 Government Dr, St. Louis, MO 63110

Friday, March 21st

7 – 8.15 AM Breakfast & Registration (2nd Floor Lobby ARB)

8:15 – 9:55 AM
Oral Session 4: 2nd Floor Lecture Hall 212, ARB

Kappa opioid receptor and dynorphin in alcohol use disorder
(Chair: Dr. Anushree Karkhanis)

8:15 AM Altered dynorphin signaling in the nucleus accumbens mediates social deficits after chronic alcohol use in male mice. Ruixiang Wang (Univ Iowa)

8:35 AM Effects of CRISPR/CAS9-mediated ventral tegmental area Oprk1 mRNA knockdown on maladaptive behavioral regulation in alcohol use disorder. Gaetan Lepreux (Univ S Florida)

8:55 AM The Role of Central Amygdala Dynorphin Neurons in Mediating Chronic Alcohol Induced Changes in Threat Responsivity. Christina Lebonville (MUSC)

9:15 AM Sex- and subregion-specific changes in kappa opioid receptors in regulating dopamine and pain after adolescent alcohol exposure. Abigail M. Kelley (Binghamton Univ)

9:35 AM Ethanol and Antagonists' Effects on the Dynamic Kappa-Opioid Receptor-Lipid Interface in the Plasma Membrane: Nanoscale Insights Using Fluorescence Methods with Single-Molecule Sensitivity. Vladana Vukojević (Karolinska Institutet)

9:55 AM The Kappa Opioid Receptor Antagonist Aticaprant Reduces Elevated Dependence-Related Alcohol Drinking and Stress-Enhanced Alcohol Consumption in Mice. Sam Gottlieb (MUSC)

10:15 AM Discussion

10:30 AM Coffee Break (2nd Floor Lobby ARB)

10:50-12:00 PM
Oral Session 5: 2nd Floor Lecture Hall 212, ARB

Emerging pharmacology of kappa opioid receptor antagonism and functional interactions
(Chair: Dr. Lee-Yuan Li-Chen)

10:50 AM Exploring the functional crosstalk between and kappa-opioid and cannabinoid 2 receptors. Christian W. Gruber (Medical University of Vienna)

11:10 AM Tissue selectivity of KOR inactivation by norBNI-like antagonists. Carlie Neiswanger (Univ Wash)

11:30 AM Development of macrocyclic tetrapeptide kappa opioid receptor antagonists for potential clinical use to prevent relapse to cocaine abuse. Jane Aldrich (University of Florida, Gainesville)

11:50 Discussion

12:00 PM -1:30 PM Buffet Lunch (2nd Floor Lobby ARB**)**

1:30-3:20PM
Oral Session 6: 2nd Floor Lecture Hall 212, ARB
(In memorium of Elena Chartoff, PhD 1969-2024)

Kappa opioid dynamics in motivated behaviors II
(Chair: Dr. Jordan McCall)

1:30 PM Associations between kappa opioid receptor availability and hoarding behaviors: Preliminary evidence using [11C]EKAP PET. Emily Weiss (Yale)

1:50 PM Positron emission tomography (PET) imaging of kappa opioid receptor binding in major depression. Elizabeth Bartlett (New York State Psychiatric Institute)

2:10 PM VTA Kappa opioid control of Stress-Induced Dopamine Dynamics in the dmPFC. Alex J. Keip (University of California San Francisco)

2:30 PM Serotonin and dopamine dynamics during KOR activation. Micaela V. Ruiz (Univ Wash)

2:50 PM Prefrontal cortical opioid receptor regulation of recurrent inhibitory microcircuits: from mice to man. Hugo Tejeda (NIDA) (ZOOM)

3:10 PM Discussion

Coffee Break 3:20 PM (2nd Floor Lobby ARB)

3:30 PM – 4:30 PM
Oral Session 7: 2nd Floor Lecture Hall 212, ARB

Deciphering the role of kappa opioid receptors in pain
(Chair: Dr. Manish Madasu)

3:30 PM Dynorphinergic extended amygdala circuitry regulates nociception and negative affect. Jessica A. Cucinello-Ragland (Wash U)

3:50 PM Surgical incision engages endogenous kappa opioid receptor (KOR) activity in spinal KOR-expressing neurons to keep chronic postsurgical pain in remission. Paramita Basu (Univ Pitts)

4:10 PM Opioid modulation of contralateral effects of brain and spinal cord injury: a potential for pharmacological treatment. Igor Lavrov (Mayo)

4:30 PM Peripheral kappa opioid receptor activation drives cold hypersensitivity in the oxaliplatin-dependent model of cold allodynia. Manish K. Madasu (Wash U)

4:50 – 5:10 PM **Where do we go from here?**

An open discussion on how to advance the field and increase translational impact.
(Topic suggestion: Aticaprant's journey through clinical trial)
(Moderator: Charley Chavkin)

5.10PM-7 PM Closing Reception (2nd Floor Lobby ARB)

6:00 PM Presentation of the *2025 Young Investigator Awards* (Ream Al-Hasani)

Mentors may nominate trainees by e-mail (al-hasanir@wustl.edu) to Ream Al-Hasani. Awards will be selected by vote of the Program Committee.

Dinner (suggestions of local restaurants provided in the program)

Saturday, March 22nd

Checkout & Departure

Poster Presentations

(Corridor outside Lecture Hall 212, ARB; 4ft high x 6ft wide, pins provided)

Poster Presentations (The full listing of authors and affiliations will be available in the abstracts section.)

1. Kappa opioid receptor (KOR) induced analgesia and aversion modulated by R7 Regulator of G protein signaling (RGS) Family. Alyson Blount (University of Maryland)

2. KOR in Paraventricular nucleus of the thalamus (PVT). Chongguang Chen (Temple University)

3. Characterizing the role of Mu opioid receptors in metabolism. Diego De Gregorio (Washington University)

4. Ligand Recognition and G Protein Signaling Diversification in the Kappa Opioid Receptor. Qianru Jiang (Washington University, Saint Louis)

5. Reproducible interrogation of opioid withdrawal using novel machine-learning enabled approaches in mice. Marwa O. Mikati (Washington University in St. Louis)

6. Second generation SAR studies on Akuammicine guided by cryoEM structure of an AKC complex in the κOR. Joseph Noel-Torres (University of Illinois Chicago)

7. Efforts to Define the Mechanism of Action of Ibogaine. Danielle C. Quindel (University of Illinois Chicago)

8. The role nociceptin opioid peptide in dorsal raphe nucleus on pain and motivation. Laura N. Massó Quiñones (Washington University, St. Louis)

9. Study of conformational dynamics of k-Opioid Receptor to probe ligand efficacy using single-molecule FRET. Susovan Roy Chowdhury (Washington University in Saint Louis)

10. JWT-101 acts as a long-lasting KOR antagonist. Micaela V. Ruiz (University of Washington)

11. Structural Basis of Inverse Agonism via Kappa Opioid Receptor: G protein Complexes. Aaliyah S. Tyson (University of Southern California)

12. NAc-VTA dynorphin modulation of dopamine dynamics in associative learning. Marcelina Wezik (University of Minho, Portugal)

13. Kappa opioid receptor availability and interoceptive awareness: Investigating links across disordered eating behaviors. Xiaoyuan Li (Yale)

14. Central amygdala dynoprhin neurons in inflammatory pain and negative affect. Lea Becker (Washington University in Saint Louis)

15. Ugi Multicomponent Reaction Derived Kappa Opioid Receptor Modulators. Alexis Knoll (Washington University in Saint Louis)

ORAL ABSTRACTS

High-throughput Engineering of Genetically Encoded Fluorescent Sensor for Detecting Opioids *in vivo*

Yuxuan Wang[1,4], Lily Torp[1,4], Sarah Wait[2,4], Lila Gin[1,4], Mikayla Gargantiel[1,4], Catalina Zamorano[3,4], Mary Loveless[3,4] Marta Soden[3,4], Michael Bruchas[3,4], Andre Berndt[1,2,4]

[1]Department of Bioengineering, University of Washington, Seattle, WA [2]Molecular Engineering and Sciences Institute, University of Washington, Seattle, WA [3]Department of Pharmacology, University of Washington, Seattle, WA [4]Center of Neurobiology of Addiction, Pain and Emotion, University of Washington, Seattle, WA

Genetically encoded fluorescent indicators (GEFIs) are powerful tools for real-time monitoring of neural activity and enable high sensitivity, specificity, and spatiotemporal resolution. Engineering GEFIs has become a primary goal for many seeking to understand neuromodulation. However, it is challenging to study opioid signaling due to the vast mutational landscape of large G protein-coupled receptors (GPCRs) like the μ-opioid receptor (μOR). To address this problem, the Berndt Lab developed the optogenetic microwell array throughput screening system (Opto-MASS), a high-throughput screening platform capable of screening thousands of μOR-based GEFIs in a single day. Using Opto-MASS, we previously leveraged Opto-MASS to identify an improved opioid sensor μMASS, which displays an increased fluorescence response to endogenous (met-enkephalin) and exogenous (fentanyl) opioid ligands. To further improve the baseline fluorescence, signal-to-noise ratio, and opioid selectivity of μMASS, we tested μ-opioid receptors from different species (Zebrafish and Killifish), leading to μMASS1.5(Zebrafish) and μMASS1.7(Killifish). These sensor variants display enhanced responses to met-enkephalin (~1.5 fold and ~3 fold, respectively), providing improved sensitivity to endogenous opioid signaling.
Additionally, to engineer a μMASS variant with optimal neuronal expression, we engrafted protein expression tags to μMASS1.5 and μMASS1.7. We found the HA-FLAG and PRC tags resulted in greater fluorescence response (~1.5 fold) to met-enkephalin in neurons compared to μMASS. These advancements establish a framework for engineering next-generation opioid sensors with improved sensitivity and specificity. Taken together, we leveraged the high-throughput capabilities of Opto-MASS to engineer novel tools to detect opioids in real-time. Future studies will apply these sensors *in vivo* to explore the spatial and temporal regulation of opioid peptides in freely behaving animals, providing critical insights into the neuromodulatory roles of endogenous opioid signaling across different brain regions.

Support:
Work supported by NIGMS R01 GM139850-01 and NINDS U01NS128537

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

Engineering a genetically encoded fluorescent sensor for *in vivo* fentanyl detection

Lily Torp[1,4], Yuxuan Wang[1,4], Sarah Wait[2,4], Lila Gin[1,4], Mikayla Gargantiel[1,4], Catalina Zamorano[3,4], Mary Loveless[3,4] Marta Soden[3,4], Michael Bruchas[3,4], Andre Berndt[1,2,4]

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Opioid-based analgesics are the most widely used treatments for chronic pain. However, opioid use and misuse have resulted in a national overdose crisis fueled by untreated opioid use disorder (OUD). Within the past decade, fentanyl has rapidly infiltrated the drug supply, leading to a significant rise in OUD cases and subsequent opioid overdose deaths. Fentanyl is a potent synthetic opioid and, like other exogenous opioids, binds to the μ-opioid receptor (μOR), a G protein-coupled receptor (GPCR). The μOR is expressed throughout many subcortical brain regions and regulates pain perception, reward, stress, and emotion. However, the pharmacological and spatiotemporal profile of fentanyl distribution in the brain is currently poorly understood. Traditional tools to monitor opioid accumulation in the brain fall short in terms of sensitivity, specificity, and real-time kinetics. To address this need, the Berndt Lab developed μMASS, a genetically encoded μOR-based fluorescent opioid sensor that couples real-time opioid detection to an increased fluorescence response. However, μMASS detects many opioids, including endogenous met-enkephalin and exogenous fentanyl, making it difficult to discern the presence of specific ligands. We hypothesized this was due to μMASS recapitulating the native μOR's promiscuous ligand binding. As a solution, we tested whether structural analysis of μMASS-fentanyl binding may elucidate residues within the binding pocket governing specificity. We generated AlphaFold2 μMASS models and docked fentanyl within the binding pocket using AutoDock4, and observed fentanyl did not maintain a critically conserved salt bridge with amino acid D147$^{3.32}$. Altering this residue to glutamate (D147$^{3.32}$E) rendered μMASS selective only for fentanyl while greatly reducing affinity for endogenous opioid peptides, and we dubbed this fentanyl specific sensor FentMASS1.0. This tool detects fentanyl *in vitro* in HEK293 cells with nanomolar affinity. Importantly, the fluorescence signal is rapid and reverses upon application of μOR antagonist naloxone. To enhance FentMASS1.0 signal and neuronal expression we introduced μOR receptors from alternative species (zebrafish, killifish) and applied homologous mutations (D139E, D124E) to each sensor. Both variants demonstrated increased response to fentanyl in HEK293 cells and primary cortical neurons while maintaining fentanyl selectivity over met-enkephalin. Ultimately, this sensor will enable cell-type specific fentanyl pharmacokinetics measurements to determine if there are appreciable differences in the timing, accumulation, and potency of fentanyl across μOR-positive brain regions, such as the VTA, NAc, and PAG. In future *in vivo* experiments we can determine fentanyl pharmokinetics in the brain during various stages of fentanyl use and fentanyl-OUD development (fOUD), including initial drug consumption, repeated administration, and withdrawal, to directly link real-time fentanyl pharmacology to behavioral phenotypes in freely behaving animals.

Support:
Work supported by NIGMS R01 GM139850-01 and NINDS U01NS128537

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

Fentanyl exposure and withdrawal modulate opioid peptide dynamics in the nucleus accumbens shell

Aidan Evans-Strong[1,3,] Marwa O. Mikati[1,3,] Ream Al-Hasani[1,2,3,4]

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Negative affect in withdrawal from opioids is a significant barrier to long-term abstinence. Dynorphin, the endogenous kappa opioid receptor ligand, is thought to increase during withdrawal and drive affective symptoms. Endogenous opioid peptides such as dynorphin and enkephalin are dysregulated by opioid use, but real-time dynamics of endogenous opioid peptides across different behavioral models are unknown. We hypothesized withdrawal from fentanyl would increase activation of dynorphin expressing neurons in the ventral nucleus accumbens shell (vNAcSh). To test this, we measured calcium activity and peptide levels in the vNAcSh in two different models of fentanyl administration. We found that acute fentanyl (0.15mg/kg) briefly suppresses dynorphin activity, while enkephalin peptide levels increase. vNAcSh dynorphin activity is similarly suppressed in a chronic fentanyl model (0.15mg/kg/day for 14 days). Interestingly, we found dynorphin transient frequency remains decreased for up to a week of withdrawal, while amplitude increases. Taken together, our data highlight an important yet complex role for endogenous dynorphin and enkephalin together in the nucleus accumbens during fentanyl exposure and withdrawal.

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NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation, grant no. 28243 (RA)

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

Characterizing xylazine exposure among pregnant people who use non-prescribed fentanyl

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[1]Department of Obstetrics & Gynecology, Washington University in St Louis; [2]Department of Laboratory Medicine, Washington University in St Louis

Xylazine, an alpha-2 adrenergic and kappa receptor agonist used as a veterinary sedative, is a known adulterant in the non-prescribed fentanyl supply in the United States associated with prolonged loss of consciousness, amnesia, and necrotic wounds. Incidence of xylazine exposure around the country has been estimated from toxicology testing in fatal overdoses, but little is known about exposure in pregnancy. We sought to characterize the frequency of xylazine exposure, identify potential risk factors, and to determine rate of fetal transmission. First, we performed a retrospective cross-sectional study of all urine drug screen (UDS) results from our inpatient obstetric unit from December 2022, when liquid chromatography-tandem mass spectrometry xylazine testing began, through July 2023 to characterize the frequency of xylazine positivity in our patient population. We then performed a case-cohort study on all patients who underwent inpatient drug screening in 2024 with a positive fentanyl result, comparing xylazine-negative controls with xylazine-positive patients to evaluate demographic characteristics associated with an increased odds of xylazine exposure. Finally, using the same cohort of patients from 2024, we performed a retrospective cohort study to assess rates of neonatal xylazine positivity at delivery. On initial review of 2022-2023 results, 47.2% of UDS positive for non-prescribed fentanyl were also positive for xylazine. Month by month, the rate of xylazine positivity significantly increased from 0% in December 2022 to 100% in July 2023 (p=0.03). When examining patients positive for xylazine exposure, only concomitant stimulant use was significantly associated with increased odds of xylazine positivity (100% in xylazine group versus 75% in non-xylazine, OR 27.33 [1.45-515.86]). Other variables, including insurance, housing status, use of non-opioid substances, prenatal care, and medication for opioid use disorder, were not found to be statistically significant. Finally, 73.3% of neonates born to persons who had evidence of xylazine exposure tested positive for xylazine at delivery. Our findings demonstrate that xylazine is present in the local fentanyl supply available to our patients, and obstetric patients in St. Louis who use non-prescribed fentanyl are at high risk of exposure. Because few risk factors were identified to help stratify patients at higher risk of xylazine exposure, and given the high rate of subsequent neonatal exposure, we recommend all patients using non-prescribed fentanyl receive counseling regarding the potential risks of xylazine. Further research is needed to characterize the effects of xylazine on birthing dyad outcomes.

Support: none

Conflict of Interests: none

The role of dynorphin in the periaqueductal grey in the vulnerability to fentanyl addiction-like behaviors

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Background: Chronic opioid use leads to hyperalgesia, increased pain sensitivity and lower pain tolerance during withdrawal, that is hypothesized to contribute to continued opioid intake and escalation of use. Hyperalgesia is mediated by the descending inhibitory system and its reciprocal connections with cortical and limbic brain structures. The periaqueductal grey matter (PAG) is a midbrain structure that is part of the descending inhibitory system and integrates negative emotions with autonomic and neuroendocrine responses. The dynorphin/κ-opioid receptor (DYN/KOR) system is highly expressed in the PAG but its role in the neurobiology of hyperalgesia remains to be elucidated.

Methods: To further understand the role of DYN on the emergence of addiction-like behaviors, we gave DYN-Cre mice escalating doses of fentanyl over 4 days and tested for hyperalgesia 8 days after the last injection. We then trained the mice on fentanyl vapor self-administration with extended access, which produces addiction-like opioid seeking. To further characterize the anatomical distribution of the DYN/KOR system in the PAG and its functional involvement in the mediation of hyperalgesia, we used a combination of transgenic animals, molecular techniques, chemogenetics, and cell-type specific retrograde tracing.

Results: We found that mice that were more vulnerable to hyperalgesia during protracted fentanyl abstinence showed higher fentanyl intake and higher sensitivity to capsaicin-punished drug-seeking without changes in motivation or on a second hyperalgesia test. Using RNAscope we found that the highest density of DYN and KOR-positive cells was in the PAG's ventrolateral portion. We also found that the majority of the DYN- and KOR-positive cells are glutamatergic: 44.2% of DYN-positive cells were also positive for VGLUT2 while 19.2% were positive for VGAT; 50.9% of the KOR-positive cells were VGLUT2 positive and 29.1% VGAT-positive. Combining a DYN-Cre transgenic line and viral vectors we determined the inputs and outputs of DYN in the ventrolateral PAG. Additionally, using the DYN-Cre mouse line and cre-dependent chemogenetics, we found that inhibition of DYN cells in the vl-PAG decrease baseline nociceptive responses in females and protracted fentanyl-withdrawal hyperalgesia in males whereas chemogenetic stimulation increases acute fentanyl withdrawal nociceptive responses.

Conclusion: Collectively, our data suggests that hyperalgesia is a predictor of addiction-like behaviors in male and female mice and that fentanyl withdrawal-induced hyperalgesia may be modulated by DYN in the ventrolateral PAG.

Molecular mechanisms of inverse agonism via κ-opioid receptor–G protein complexes

Cornelius Gati[1,2,3], Aaliyah S. Tyson[1,2], Saif Khan[1,3], Zenia Motiwala[1,3*], Gye Won Han[1], Zixin Zhang[1,4], Mohsen Ranjbar[1,2], Daniel Styrpejko[1,3], Nokomis Ramos-Gonzalez[5], Stone Woo[6], Kelly Villers[1], Delainey Landaker[1], Terry Kenakin[7], Ryan Shenvi[6], Susruta Majumdar[5]

Affiliations
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Opioid receptors, a subfamily of G protein-coupled receptors (GPCRs), are key therapeutic targets. In the canonical GPCR activation model, agonist binding is required for receptor–G protein complex formation, while antagonists prevent G protein coupling. However, many GPCRs exhibit basal activity, allowing G protein association without an agonist. The pharmacological impact of agonist-free receptor–G protein complexes is poorly understood. Here we present biochemical evidence that certain κ-opioid receptor (KOR) inverse agonists can act via KOR–G_i protein complexes. To investigate this phenomenon, we determined cryo-EM structures of KOR–G_i protein complexes with three inverse agonists: JDTic, norBNI and GB18, corresponding to structures of inverse agonist-bound GPCR–G protein complexes. Remarkably, the orthosteric binding pocket resembles the G protein-free 'inactive' receptor conformation, while the receptor remains coupled to the G protein. In summary, our work challenges the canonical model of receptor antagonism and offers crucial insights into GPCR pharmacology.

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

How kappa opioid receptor efficacy and functional selectivity is modulated

Nokomis Ramos-Gonzalez[a], Balazs R. Varga[a], Alexis Knoll[a], Kevin Appourchaux[a], Vipin Rangari[a], Sarah Bernhard[a], Antonina Nazarova[b], Saheem A Zaidi, Vsevolod Katritch[b], Tao Che[a], Susruta Majumdar[a]

[a] Center for Clinical Pharmacology, University of Health Sciences & Pharmacy at St. Louis and Washington University School of Medicine; Department of Anesthesiology, Washington University Pain Center, Washington University School of Medicine, St. Louis, Missouri 63110, United States. [b]Department of Quantitative and Computational Biology, Department of Chemistry, Bridge Institute, Michelson Center for Convergent Bioscience, University of Southern California, Los Angeles, United States.

Introduction: Chronic pain affects 1 in 5 Americans and is largely treated with agonists that act on the μ opioid receptor (MOR); misuse of MOR drugs has led to the opioid epidemic in the US. Drugs targeting the κ opioid receptor (KOR) may be a viable alternative to MOR agonists as analgesia can be achieved without respiratory depression. As KOR is a viable target for new analgesics it is important to understand how functional selectivity and efficacy can be achieved at KOR on a structural and molecular level.

Methods: Using the previously solved structure of MP1104 (a 6β-arylamido morphinan and a non-selective opioid full agonist which robustly recruits arrestin) we designed ligands with differential pharmacology and efficacy, we optimized linker length and head group to target different areas of the orthosteric pocket, leading to VRB37 and MP1404. Activity was characterized using bioluminescence resonance energy transfer (BRET) assays in HEK293T cells. Cryo-EM structures were solved by expressing KOR-Gαβγ-ScFv16 in Sf9 cells and purifying in the presence of agonist by His-tag and size exclusion chromatography.

Results: VRB37, designed by varying the head group, was determined to potently activate KOR through Gi1 with around 80% efficacy relative to standard agonist U50,488H and potent, low efficacy β-arrestin2 recruitment. Our cryo-EM structure of VRB37 bound to KOR revealed that VRB37's amidophenyl arm was positioned towards transmembrane (TM) domain 5 and extracellular loop 2 (ECL2), forming potential interactions here, a series of analogues were designed and characterised to probe this interaction. Overlay with the crystal structure of KOR-MP1104 highlighted that VRB37's arm binds in a distinct subpocket that MP1104 does not occupy. MP1404, designed by varying the linker length, activated KOR through Gi1 with very low efficacy (25% of U50,488H), the cryo-EM structure revealed that MP1404's long linker is positioned towards TM4/5/ECL2. We have synthesized a series of MP1404 analogues and show a relationship between increasing linker length and decreasing efficacy. We are now using molecular dynamics simulations to further explore the cryo-EM structures.

Conclusions: We have used chemistry, pharmacology and structural biology to determine key interactions and subpockets in KOR that allow for the determination of ligands with functional selectivity and a range of efficacy. We propose distinct receptor regions can be used in the same receptor to drive bias and efficacy at KOR. We anticipate our methodology can be extended to other GPCRs where these parameters currently are optimized using iterative approaches.

Support: This work is supported by a PhRMA Foundation Postdoctoral Fellowship in Drug Discover (Crossref Funder ID: 100001797) and NIH/NIDA grants (R01DA057790, R01DA059978, R61NS136307, R01DA036246)**.**

Conflict of Interest: Dr Majumdar is a co-founder of Sparian biosciences.

Pharmacological Characterization of the Novel Selective Kappa Opioid Receptor Agonists 10-Iodo-Akuammicine and 10-Bromo-Akuammicine

Kathryn Bland[#,1], Chongguang Chen[#,1], Peng Huang[#,1], Conrad Ho[1], Theodora Howe[1], Katrina Ocampo[1], Pingwei Zhao[1], Simone Creed[2], Joseph Noel-Torres[2], Andrew P. Riley[2], and Lee-Yuan Liu-Chen[1] ([#]contributed equally)

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Akuammicine (AKC), a naturally occurring indole alkaloid, is a kappa opioid receptor (KOR) full agonist with a moderate affinity. 10-Iodo-akuammicine (I-AKC) and 10-Bromo-akuammicine (Br-AKC) showed higher affinities for the KOR with K_i values of 2.4 and 5.1 nM, respectively, and high selectivity for the KOR over other opioid receptors. Both were KOR full agonists. As AKC and derivatives have distinctly different chemical structures from other KOR agonists, herein we investigated whether Br-AKC and I-AKC produced similar pharmacological effects as typical KOR agonists. Br-AKC and I-AKC inhibited compound 48/80-induced scratching in a dose-dependent manner, with ED_{50} values of 1.3 and 3.0 mg/kg (s.c.), respectively, indicating anti-pruritic activities. Side effects of I-AKC and Br-AKC and their promotion of KOR phosphorylation and internalization were examined using doses in the effective anti-scratch dose range, at 1.9-3.8x ED_{50} and 1.7-3.3x ED_{50}, respectively. At 5 mg/kg, Br-AKC and I-AKC produced profound conditioned place aversion (CPA). Br-AKC (10 mg/kg), but not I-AKC (5 mg/kg), reduced novelty-induced hyperlocomotion, and Br-AKC impaired rotarod performance more profoundly than I-AKC. Br-AKC, but not I-AKC, caused KOR phosphorylation at S369 in the mouse brain and KOR internalization in the ventral tegmental area. These results indicate that Br-AKC and I-AKC produce anti-scratch effect and CPA, similar to typical KOR agonists. However, there are some differences between the two. In addition, KOR phosphorylation and internalization in mouse brains are not associated with CPA but may be related to hypolocomotion and impaired rotarod performance. This is the first *in vivo* pharmacological characterization of AKC derivatives.

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Conflict of interests
None.

Iboga-inspired Analogs with Modulated Kappa Opioid Activity Display Potential Therapeutic Applications

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Iboga alkaloids are indole-containing natural products, commonly found in many plant species native to central Africa. The most notable example ibogaine, abundantly occurring in *Tabernanthe iboga* root bark, has a long and storied history of underutilized therapeutic potential in treatment of opioid use disorder (OUD) and more recently other neurological pathologic states (traumatic brain injury, post-traumatic stress disorder). Ibogaine is rapidly metabolized *in vivo* to noribogaine, a long-lasting metabolite, which displays low potency and efficacy kappa opioid receptor (KOR) agonist activity - which was proposed as one of the targets contributing to its observed *in vivo* pharmacological and therapeutic activity.

We have recently reported a class of iboga-inspired analogs, termed “oxa-iboga”, that show remarkably enhanced KOR potency while maintaining the partial efficacy profile, compared to noribogaine, after the indole core was replaced with benzofuran (Havel V, Kruegel AC, Bechand B, et al. **2024** *Nat Commun*. 15: 8118). This modification of iboga scaffold not only improved its therapeutic activity in rodent models of OUD (self-administration, reinstatement, alleviation of opioid induced hyperalgesia), but also reduced the risk of inducing cardiac arrhythmia, a major side effect of iboga alkaloids, linked to their inhibitory activity of the hERG ion channel.

In the next stage of our research, we used a structure-activity relationship approach to identify the key pharmacological unit that encodes the KOR activity of oxa-iboga analogs. This reduced-complexity system retains many aspects of the iboga pharmacological profile, while further separating the undesirable hERG off-target activity and can serve as a modular discovery platform for further development.

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Conflict of interest:
Drs. Havel, Sames and Hemby are named inventors on several patent applications (US11840541B2, US20230382919A1, US20230102206A1, US20230348465A1 and US20240150372A1) related to the preparation and use of iboga analogs with therapeutic potential. D. Sames is the co-founder and board observer of Gilgamesh Pharmaceuticals, which licenses the iboga assets from Columbia University.

Treatment of Diuretic Resistance with a Kappa Opioid Agonist, an Inhibitor of Central Vasopressin Secretion

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Prolonged use of furosemide for treatment of congestive heart failure is associated with potential life-threatening adverse effects including hypokalemia, hyponatremia, and diuretic resistance. We hypothesized that due to loss of water, increased vasopressin (AVP) secretion may be a key mechanism contributing to diuretic resistance. Since kappa opioid receptor (KOR) agonists act centrally to inhibit AVP secretion and produce water diuresis, we predicted that KOR agonist administration will reverse the diuretic resistance to furosemide without enhancing urinary sodium/potassium excretion. To test this, changes in 5-hr urine output (metabolic cages; no water) were measured daily in Sprague-Dawley rats following injection (9:00am) of saline (control day) and furosemide (10 mg/kg, i.p.; days 1-5). Over days 6-10 rats were then administered either, 1) furosemide, 2) furosemide + difelikefalin (F+D, 20 µg/kg, i.p.; days 6-10) a KOR agonist, or 3) furosemide + tolvaptan (F+T, 1 mg/kg, i.p.) an AVP V2 receptor antagonist. After the 5-hr urine collection, rats received a 2nd drug treatment (2:00 pm) and were placed in home cages. The results showed that initial treatment (day 1) of rats with furosemide evoked an expected and marked increase in urine output (V), urinary sodium excretion (UNaV), and urinary potassium excretion (UKV), with the magnitude of each greatly reduced by day 5 (day1:day5; V, 10.5±0.5 vs 5.5±0.35 ml/5hrs; UNaV, 971±55 vs 396±23 µEq/5hrs; UKV, 250±14 vs 193±20 µEq/5hrs). Over days 6-10, 5-hr urine output remained reduced during continued furosemide treatment. In contrast, co-administration of difelikefalin or tolvaptan both reversed the diuretic resistance as noted by increased diuresis to furosemide over days 6-10 (day 10, V; F+D, 10.1±0.77 ml/5hrs; F+T, 11.1±0.9 ml/5hrs) without further increasing UNaV. However, over days 6-10, tolvaptan but not difelikefalin significantly increased UKV. These findings demonstrate that AVP plays a significant role in mediating diuretic resistance to furosemide. Further, we show that combination therapy of a KOR agonist (difelikefalin) and a loop diuretic can reverse and potentially prevent diuretic resistance and improve loop diuretic induced hyponatremia and hypokalemia. (LSUHSC Research Enhancement Program)

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Conflict of interest: Drs. Kapusta, Gao, and Meariman have a patent application related to kappa opioids and water retaining disorders.

Dissecting the Neural Circuits and Signaling Pathways Underlying Kappa Opioid Receptor-Mediated Psychotomimetic Effects

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Kappa opioid receptor (KOR) agonists produce dissociative hallucinogenic effects, including sedation, sensory disturbances, dissociation, and motor impairment, limiting their clinical utility. Understanding how to selectively modulate these effects is critical for the development of novel KOR-targeted therapeutics. We are investigating the neural circuits and signaling mechanisms underlying KOR-mediated psychotomimetic effects to determine whether these effects can be pharmacologically disentangled. We employ novel EEG and wide field Ca^{2+} imaging modalities. We are examining (1) the role of KOR expression in claustrum neurons in mediating psychotomimetic and motor effects, (2) the functional impact of KOR-expressing neurons in the claustrum, and (3) the contribution of β-arrestin signaling to KOR-induced psychotomimetic effects. Our findings provide key insights into the role of the claustrum in KOR-mediated effects and offer potential pathways for developing more selective KOR-targeted therapeutics. This work advances our understanding of KOR pharmacology and its relevance to psychiatric disorders and psychedelic research.

Support: Washington University Department of Anesthesiology

Conflicts of interest: None

Neuropeptidergic Control of Paraventricular Thalamic Circuitry: Opposing Roles of Orexin and Dynorphin in Arousal and Aversion

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The paraventricular thalamus (PVT) serves as a critical interface between hypothalamic arousal systems and limbic circuits that regulate motivation and stress-related behaviors. The lateral hypothalamic area (LHA) provides a major peptidergic input to the PVT, releasing both orexin and dynorphin, which have opposing effects on behavioral state regulation. While orexin is associated with arousal and reward seeking, dynorphin is linked to aversion and stress responsivity. However, the receptor distribution, physiological impact, and behavioral consequences of these neuropeptidergic signals within the PVT remain poorly understood. To define the spatial organization of orexinergic and dynorphinergic modulation, we used hybridization chain reaction *in situ* hybridization (HCR) to map the expression of kappa opioid receptor 1 (*Oprk1*) and orexin receptor 1 (*Hcrtr1*) across the anterior-posterior axis of the PVT. Two-photon calcium imaging in acute brain slices revealed that bath application of orexin increased neuronal excitability, whereas dynorphin suppressed calcium activity, indicating distinct functional effects on PVT neurons. Retrograde viral tracing confirmed that the LHA serves as a major source of both orexinergic and prodynorphinergic inputs to the PVT.

To assess the effects of stimulation of prodynorphinergic terminals PVT we combined optogenetics during real-time place preference (RTPP) assays. Terminal stimulation of LHA-PVT fibers induced place avoidance, demonstrating that activation of prodynorphinergic projections to the PVT drives aversive behavior.

Additionally, to investigate endogenous dynorphin release dynamics, we utilized the KLight neuropeptide sensor in a multi-spout consummatory task, revealing state-dependent fluctuations in dynorphin levels during fluid consumption. These findings establish a direct link between LHA prodynorphinergic inputs to the PVT and aversive state processing while highlighting the broader opposing influences of orexin and dynorphin on PVT circuit function. Future studies will dissect the cellular and synaptic mechanisms through which these neuropeptides modulate PVT activity and influence motivated behavior.

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

A preoptic neuronal population regulates energy balance and expenditure

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Energy homeostasis and tissue metabolism are coordinated through the hypothalamus. Dysregulation of hypothalamic circuits is a key contributor to obesity-associated metabolic disorders. Compared to circuits regulating energy intake, those regulating energy expenditure remain poorly understood. Prior studies implicated signaling through the kappa opioid receptor (KOR) in modulating energy expenditure. Here, we identify a newly appreciated population of GABAergic neurons in the preoptic area, marked by expression of KOR (POA^{KOR+}) as a key regulator of energy expenditure and balance. POA^{KOR+} neurons were unresponsive to the cold or warm thermal challenge; but were recruited following a circadian pattern. They were recruitment during the light phase, when mice naturally expend less energy. POA^{KOR+} neurons were not recruited during food deprivation but were suppressed with refeeding during the light phase. Chemogenetic activation of POA^{KOR+} neuron suppressed energy expenditure including brown adipose tissue (BAT) thermogenesis leading to lower body temperatures. Acute inhibition of POA^{KOR+} neurons promoted energy expenditure. Chronic silencing of POA^{KOR+} neurons in lean mice drove marked body weight loss by increasing energy expenditure without suppression of feeding. Lean body mass and brow adipose tissue mass were preserved. Additionally, in high fat diet induced obese mice synaptic silencing of POA^{KOR+} neurons significantly decreased body weight and improved glucose metabolism. POA^{KOR+} neurons exerted a broad influence on adipose tissues, inducing loss and browning of white adipose tissue and preservation brown adipose tissue. Together, these findings demonstrate POA^{KOR+} neurons incorporate circadian timing signals and leverage thermogenesis and beiging process on peripheral adipose tissue to improve energy expenditure and body weight regulation. Their dysfunction may contribute to metabolic disorders and are novel therapeutic target for obesity and metabolic disorders.

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A dorsal hippocampus-prodynorphinergic dorsolateral septum-to-lateral hypothalamus circuit mediates contextual gating of feeding

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Adaptive regulation of feeding depends on the context-dependent linkage of external cues, internal states, and food outcomes. Human brain imaging has identified dysregulation in a dorsal hippocampus (DHPC)-lateral hypothalamic area (LHA) network in overweight and binge eating individuals, but mechanistic instantiation of a pathway bridging these structures is lacking. Here, we identify an evolutionarily conserved, genetically distinct, and topographically discrete Prodynorphin (*Pdyn*)-expressing subpopulation of Somatostatin (*Sst*)-expressing inhibitory neurons in the dorsolateral septum (DLS) that receives primarily dorsal, but not ventral, HPC inputs. In turn, DLS(*Pdyn*) neurons project to and inhibit GABAergic populations in the LHA. *In vivo* calcium imaging of DLS(*Pdyn*) neurons reveals context-dependent encoding of food-seeking and consummatory actions, and optogenetic manipulations implicate the DHPC-DLS(*Pdyn*)-LHA pathway in distinct facets of context- and internal state-dependent calibration of feeding. Viral deletion of *Pdyn* in the DLS mimicked effects seen with optogenetic silencing of DLS(*Pdyn*) cells, suggesting a potential role for DYNORPHIN-KAPPA OPIOID RECEPTOR signaling in contextual regulation of food-seeking. Together, our findings illustrate how the DHPC has evolved to recruit an ancient LHA feeding circuit module through DLS(*Pdyn*) inhibitory neurons to link contextual information with regulation of food consumption.

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The role of dorsal nucleus accumbens shell dynorphin neurons in reward seeking

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The nucleus accumbens (NAc) expresses endogenous opioids and their receptors, and is implicated in consumption, reward, and substance use. Activation of mu opioid receptors in the NAc drives palatable food consumption, but little is known about how dynorphin (Dyn) and the Kappa opioid receptor (KOR) modulate consummatory behavior in the NAc. Prior work has shown that optogenetic stimulation of Dyn neurons in the dorsal NAc shell (dNAcSh) is rewarding and drives a real time place preference. However, it is unknown whether or how these neurons modulate reward seeking. To investigate whether dNAcSh Dyn neurons are involved in reward seeking, we used a combination of approaches. First, we optogenetically stimulated NAc shell Dyn neurons in pDyn cre mice and evaluated the effect this activation had on feeding behaviors. In a free feeding paradigm, mice consumed less chow food during dNAcSh Dyn neuron photostimulation. In an operant task, sated mice consumed less sucrose reward pellets during dNAcSh Dyn neuron photostimulation compared to controls. This effect of decreased sucrose pellet consumption in an operant task during photostimulation of dNAcSh Dyn neurons in mice was present following an overnight food restriction. Following an overnight food deprivation, mice also showed a decrease in sucrose pellet consumption compared to controls in an operant task when dNAcSh Dyn neurons were activated, and dNAcSh Dyn neuron stimulation lowered sucrose consumption in a sucrose preference test. Next, we investigated how DREADD activation of dNAcSh Dyn neurons via hM3Dq modulates fentanyl seeking in a 2-bottle choice home cage fentanyl drinking paradigm. We found that dNAcSh Dyn neuron activation lowers fentanyl consumption in this model. We are currently working to determine whether decreased sucrose and fentanyl consumption is dependent on kappa opioid receptor activation, by blocking these receptors with the antagonist norBNI, prior to behavioral testing. Together, these data help to understand a novel role for dynorphin expressing cells in the dNAcSh in modulation of reward seeking.

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Title:
Rapid endogenous dynorphin dynamics in the dorsal striatum promote goal-directed action

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Abstract:
Targeting the endogenous opioid dynorphin, via the kappa-opioid receptor (KOR) has shown promise in the treatment of multiple neuropsychiatric disorders including substance use disorder (SUD). A hallmark of SUDs is maladaptive goal-directed behavior, which is our ability to make causal associations between actions and rewarding outcomes. The dorsomedial striatum (DMS), where dynorphin is abundant, is critical for goal-directed behavior. Furthermore, enhanced dynorphin-KOR signaling and aberrant striatal activity are associated with the transition of recreational to persistent drug-seeking. Yet, how DMS dynorphin neuron activity, dynorphin release, and subsequent dynorphin-KOR signaling promote goal-directed behavior is unknown. Here, using *in vivo* two-photon calcium imaging of thousands of dynorphin neurons when mice learn to perform actions to obtain rewarding outcomes, we observe an increase in dynorphin neuron recruitment that evolve discrete patterns of activity during behavior upon learning. Importantly, using an artificial recurrent neural network trained to predict engagement in goal-directed behavior, we show that dynorphin neuron activity specifically during the anticipation of the outcome promotes subsequent goal-directed action. Next, we assayed *in vivo* DMS dynorphin dynamics during goal-directed behavior using a novel genetically-encoded dynorphin biosensor. We observe that dynorphin tone increases upon learning, and is released specifically in anticipation of reward delivery upon learning. Furthermore, using a generalized linear model, we show that the magnitude of dynorphin release strongly correlates with subsequent goal-directed action vigor. In support of these findings, mimicking DMS dynorphin release during anticipation using optogenetics enhances goal-directed action without being inherently reinforcing, and is sensitive to KOR antagonism. Conversely, we find that conditional deletion of dynorphin production from the DMS decreases the learning and performance of goal-directed behavior, without affecting the innate preference for sucrose. Altogether, we reveal that dynorphin-KOR signaling in the DMS shapes goal-directed behavior, by dynamically promoting future goal-directed action at strikingly fast timescales.

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

Altered dynorphin signaling in the nucleus accumbens mediates social deficits after chronic alcohol use in male mice

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Abstract:

Loss of social connectedness is a common symptom of alcohol use disorder (AUD) that can delay recovery and promote relapse to alcohol drinking. The goal of the present study was to help elucidate neural circuits underlying social deficits following chronic alcohol use.

After 8 weeks of chronic intermittent ethanol (CIE) exposure, male, but not female, mice exhibited social deficits in a 3-chamber test, associated with increased excitability of serotonin (5-HT) neurons in the dorsal raphe nucleus as well as enhanced 5-HT transients in the nucleus accumbens (NAcc) while mice were engaging in social interaction. In male mice, we also observed an increase in excitability of dynorphin neurons in the NAcc, which change could be abolished by bath-applying 5-HT2c-receptor antagonist RS102221, indicating that tonic 5-HT release is crucial for enhanced NAcc dynorphin signaling after CIE through a 5-HT2c receptors-dependent mechanism. Associated with this physiological alteration by CIE, our *in situ* hybridization study showed that CIE upregulated expressions of 5-HT2c (but not 5-HT1b or 5-HT2a) receptors and prodynorphin, as well as expression of 5-HT2c receptors in dynorphin neurons in the NAcc of male mice. Importantly, such anatomical changes were also observed in post-mortem human brains from male subjects with AUD. Moreover, genetic ablation of prodynorphin or 5-HT2c receptors in dynorphin neurons, or chemogenetic inhibition of dynorphin neurons in the NAcc, could rescue social deficits in CIE mice. Finally, using fiber photometry, we showed that chemogenetic activation of NAcc dynorphin neurons inhibited local dopamine release during social interaction.

Taken together, our results indicate that CIE activates 5-HT2c signaling in dynorphin neurons in the NAcc, which then inhibits the mesolimbic dopamine transmission. That may reduce the motivation to engage in social behavior.

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

Effects of CRISPR/CAS9-mediated ventral tegmental area *Oprk1* mRNA knockdown on maladaptive behavioral regulation in alcohol use disorder.

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Alcohol use disorder (AUD) is a chronic relapsing condition that affects 28.9 million individuals in the USA and is characterized by compulsive alcohol use despite adverse social, occupational, or health consequences. Dysregulation of the dynorphin (DYN)/kappa opioid receptor (KOR) system has been implicated in the development of AUD, contributing to escalation of alcohol self-administration in an attempt to self-medicate dysphoric or negative affective-like states. Our lab previously demonstrated that alcohol-dependent rats (compared to non-dependent controls) showed increased *Oprk1* (KOR gene) mRNA expression in the ventral tegmental area (VTA), and that overexpressing *Oprk1* mRNA in the VTA of non-dependent rats recapitulated key phenotypes of alcohol dependence such as escalated alcohol self-administration. Here, we investigated whether blocking dependence-induced increases in *Oprk1* mRNA in the VTA could prevent these maladaptive behaviors. To do so, Wistar rats were trained to self-administer alcohol on a fixed-ratio (FR1) schedule before progressive-ratio (PR) testing to assess the animal's motivational state for alcohol. Animals were then chronically and intermittently exposed to alcohol vapor (or air for control) to induce dependence. Four weeks after vapor exposure, a viral CRISPR/CAS9 construct (or control construct) was infused into the VTA to knock down *Oprk1* mRNA expression. Following surgical recovery and viral incubation for 4 weeks while continuing in the exposure paradigm, animals resumed alcohol self-administration on FR1 and PR schedules during acute withdrawal in limited-access sessions. Subsequently, animals were tested for negative affective-like behaviors using the elevated plus-maze and forced swim test. We found dissociable effects of CRISPR/CAS9-mediated mRNA knockdown in the VTA between non-dependent and alcohol-dependent animals. Dependence-induced escalation of alcohol consumption and motivation for alcohol during withdrawal was prevented by *Oprk1* knockdown. Interestingly, this knockdown exhibited an anxiolytic effect in non-dependent animals but not in alcohol-dependent animals. These findings suggest that DYN/KOR signaling in the VTA plays a crucial role in regulating motivation for alcohol in both the context of non-dependence and dependence and may represent a promising target for therapeutic strategies aimed at treating AUD.

Key Terms: alcohol use disorder, CRISPR/CAS9, dynorphin, kappa-opioid receptor, *Oprk1*, fixed-ratio, progressive-ratio, self-administration, ventral tegmental area.

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Title: The Role of Central Amygdala Dynorphin Neurons in Mediating Chronic Alcohol Induced Changes in Threat Responsivity

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Abstract: Altered threat responses during stress are hallmarks of alcohol use disorder (AUD) and posttraumatic stress disorder (PTSD), which are often comorbid. Our investigations of the stress-alcohol relationship have shown that the central amygdala (CeA) plays a key role in mediating stress-potentiated alcohol drinking, particularly CeA neurons expressing the peptide dynorphin (Dyn). In mice, Dyn-expressing CeA (CeA^{Dyn}) neuronal activity is uniquely increased during male and female voluntary alcohol consumption and chronic alcohol has been shown to induce neuroadaptations in this population. Further, behavioral responses to stressful threatening stimuli rely on the CeA more generally and stress/negative affect stimulates CeA Dyn expression. CeA^{Dyn} neurons, therefore, may be a key node in alcohol-stress interactions that becomes dysregulated after chronic alcohol exposure and thus may underlie aberrant threat responding. Yet, how cellular activity of CeA^{Dyn} neurons, specifically, relates to threat responses is not well-understood. The looming sweeping disk task (LSDT) is an ethological behavioral assay that mimics a surveying aerial predator (sweeping disk stimulus) or a fast-approaching aerial predator (looming disk stimulus). Behavior in this model is evolutionarily relevant to rodents (i.e. predation threat) and lends itself to nuanced analysis. Importantly, task stimuli evoke distinct threat responses known to be gated by specific neuronal populations within the CeA. We hypothesized: 1) male and female mice would show distinct innate and chronic alcohol-induced behavior in the LSDT; 2) inhibition of CeA^{Dyn} neurons during the task would sex-dependently alter chronic alcohol-induced changes in behavior. We expressed a Cre-dependent inhibitory chemogenetic construct in the CeA of male and female (pro)Dyn-Cre mice (N=24) and exposed mice to chronic intermittent ethanol vapor (CIE) or control air for two weeks. We administered the chemogenetic agonist (CNO, 3 mg/kg), or vehicle (control), prior to testing in the LSDT during protracted withdrawal. Behavior was analyzed using an automated machine learning pipeline. Outcome associations (e.g. latency to flee to safety) were analyzed using linear mixed models with Sex, CIE condition (CIE vs air), Drug (CNO vs vehicle), and interactions as fixed effects and subject as a random intercept. A priori comparisons of innate sex effects (controls) were conducted. Other significant interactions were probed using Tukey's HSD post-hoc tests. Latency to flee to safety during imminent threat showed a significant effect of Sex (p= .0395) and CIE by Sex interaction (p= .0210). Planned comparisons revealed that within controls, females were faster to flee than males, suggesting increased innate threat reactivity in females (p= .0395). Analyzing within sex, we show specifically in males, not females, that CIE-exposed mice flee quicker than air-exposed control mice (CIE, p= .0148) and that CNO-induced inhibition of CeA^{Dyn} neurons reverses this effect (CIE*CNO, p= .0463). These results establish promising utility of LSDT to probe cellular and biological sex mechanisms of alcohol-induced dysregulated threat responding.

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Sex- and subregion-specific changes in kappa opioid receptors in regulating dopamine and pain after adolescent alcohol exposure.

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Alcohol consumption in adolescence promotes pain hypersensitivity. In rats, adolescent chronic intermittent ethanol (aCIE) exposure facilitates dopamine release in the NAc shell with an associated augmentation in pain sensitivity, an effect reversed by chemogenetic inhibition of dopamine. Like dopamine, kappa opioid receptors (KORs) are involved in regulating alcohol use and pain sensitivity. Here we examine the effect of aCIE exposure on the KOR-dopamine interaction in the NAc shell and the associated impact on pain sensitivity during protracted abstinence. Male and female Long-Evans rats were exposed to air or ethanol vapor from PD28–65. Von Frey filaments were used to assess tactile sensitivity and accumbal dopamine kinetics were measured using *ex vivo* fast-scan cyclic voltammetry during protracted abstinence. Global activation of KORs with U50,488 attenuated tactile sensitivity in male and female aCIE-exposed rats at both the low (1.25 mg/kg) and high (2.5 mg/kg) doses, while only the high dose was effective in air-exposed controls, indicating a hyperresponsivity of KORs in aCIE-exposed animals. In the NAc shell, the impact of aCIE exposure on KOR-mediated inhibition of dopamine was both sex- and subregion-specific, such that, aCIE exacerbated KOR-mediated dopamine inhibition in the rostral shell in females, but the caudal shell in males. Interestingly, acute ethanol treatment-associated potentiation in KOR-mediated dopamine inhibition was blunted in aCIE- compared to air-exposed rats. Similarly, this effect was specific to the rostral shell in females and the caudal shell in males. Ultimately, aCIE exposure-associated augmentation in tactile sensitivity was mitigated by KOR activation at higher potency. This effect may be driven by a KOR-dependent mechanism that controls dopamine, specifically in the rostral shell in females and the caudal shell in males. These sexually dimorphic data highlight the importance of developing sex-specific pain management treatment options.

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Abigail Kelley's work has no conflicts of interest.

Ethanol and Antagonists' Effects on the Dynamic Kappa-Opioid Receptor-Lipid Interface in the Plasma Membrane: Nanoscale Insights Using Fluorescence Methods with Single-Molecule Sensitivity

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Using complementary methods with single-molecule sensitivity: fluorescence correlation spectroscopy (FCS), massively parallel FCS integrated with fluorescence lifetime imaging microscopy (mpFCS/FLIM) and single-molecule localization microscopy (SMLM) and Ca^{2+} imaging, we have characterized the effects of ethanol, general opioid receptor antagonist naltrexone (NTX), and the kappa-opioid receptor (KOP)-selective antagonist LY2444296, on the lateral organization and dynamics of KOP [1], and cholesterol- and sphingomyelin-enriched domains, a subclass of lipid-enriched plasma membrane domains that often harbor opioid receptors [2]. Our data show that ethanol changes the dynamic lateral organization of plasma membrane lipids, affecting cholesterol- and sphingomyelin-enriched domains that harbor opioid receptors; that NTX wards against these effects through a multifaceted action mechanism, involving its potent antagonistic action on opioid receptors but also a hitherto unknown effect on plasma membrane lipids dynamics and lateral organization in the plasma membrane; the selective short-acting KOP antagonist LY2444296 at concentrations tested (100 nM) perturbed plasma membrane nanoscale lateral dynamics but did not exhibit protective actions against ethanol-induced changes.

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The Kappa Opioid Receptor Antagonist Aticaprant Reduces Elevated Dependence-Related Alcohol Drinking and Stress-Enhanced Alcohol Consumption in Mice

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Comorbidity of stress-related disorders (e.g., PTSD) is as high as 15-30% in individuals with alcohol use disorder (AUD). The causal relationship between these conditions may stem from engagement of common brain mechanisms and circuitry, and one of particular interest is the dynorphin/kappa opioid receptor (DYN/KOR) system. Stress exposure activates the DYN/KOR system and elevates DYN immunoreactivity in brain regions involved in alcohol addiction. Additionally, DYN/KOR signaling has been shown to contribute to chronic alcohol related increased drinking. Here we evaluated the effect of systemically administering the short-acting KOR antagonist aticaprant (0, 5, 10, 20 mg/kg) in a mouse model of stress-enhanced alcohol drinking. The CIE+FSS Drinking model involves combined chronic intermittent ethanol (CIE) and repeated forced swim stress (FSS) exposure. After establishing stable limited access (1-hr/day) alcohol (15% ethanol) intake, adult male C57BL/6J mice were divided into 4 groups: CTL, FSS-alone, CIE-alone, CIE+FSS (N= 8-9/group). Weekly cycles of CIE (or air) exposure were alternated with weekly 5-day test drinking cycles, with FSS-alone and CIE+FSS groups exposed to FSS (10-min) 4-hr prior to drinking sessions. Over the course of 4 Test drinking cycles, CIE+FSS mice showed the expected robust escalation in alcohol drinking compared to CIE-alone mice and the FSS-alone and CTL groups which did not differ. Aticaprant given 30-min prior to the drinking sessions reduced alcohol intake in a dose-related manner. The 5 mg/kg dose had no effect on alcohol drinking, treatment with 10 mg/kg aticaprant showed a potential effect of decreasing drinking in CTL ($p=0.07$). and 20 mg/kg aticiparant significantly decreased drinking in all experimental groups compared to vehicle-treated animals ($p<0.05$). These data provide support for the potential utility of a KOR antagonist in decreasing alcohol consumption, especially elevated dependence-related drinking and stress-enhanced alcohol consumption.

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Tissue selectivity of KOR inactivation by norBNI-like antagonists

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Extensive preclinical studies have underscored the therapeutic potential of kappa opioid receptor (KOR) antagonists for treating drug addiction, depression, and psychosis. Schattauer et al., (2017) reported that KOR-inactivation by long-acting antagonists results from a realignment of the KOR signaling complex caused by cJun-kinase / peroxiredoxin 6 (JNK/PRDX6) mediated depalmitoylation of Gαi. Utilizing a novel peroxide sensor, oROS, we demonstrate that both nalfurafine and nalmefene activate JNK/PRDX6 to stimulate peroxide production in mouse brain. Notably, KOR inactivation is sex-dependent, and we show that nalfurafine causes peroxide production only during estrus (low-estrogen state) or following progesterone treatment of female mice. Daily microdosing with nalfurafine or nalmefene blocked KORs responsible for antinociceptive effects, blocked KORs mediating stress-induced aversion, mitigated KOR-mediated dysphoria during acute and protracted withdrawal in opioid-dependent mice, and blocked KOR-induced prolactin secretion. In contrast, KORs mediating the diuretic and anti-pruritic effects were not regulated by JNK/PRDX6. Our findings highlight several potential advantages of KOR inactivation over competitive antagonism: microdosing minimizes off-target effects typically associated with high drug doses needed for competitive inhibition; stable antagonism is achieved through slow accumulation of inactivation; and drug effects can be targeted to tissues where KOR regulation involves JNK/PRDX6. The endogenous dynorphin/kappa opioid receptor (KOR) system in the brain mediates the dysphoric effects of stress, and KOR antagonists have therapeutic potential for the treatment of stress disorders. This study identifies nalfurafine and nalmefene as two medications that cause selective KOR inactivation, and the evidence presented in this study suggests that daily administration of these drugs at microdoses may be a novel approach to effectively promote stress resilience in people. Importantly, KOR inactivation by JNK-dependent mechanisms shows tissue selectivity: KOR inactivation does not occur in 1) dopamine terminals of VTA neurons projecting to the nucleus accumbens; 2) neurons controlling diuresis; or 3) in cells controlling the pruritic response.

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Development of macrocyclic tetrapeptide kappa opioid receptor antagonists for potential clinical use to prevent relapse to cocaine abuse

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Kappa opioid receptor (KOPr) antagonists have potential application in the treatment of substance abuse by preventing stress-induced relapse to drug seeking behavior. Macrocyclic tetrapeptides (MTPs) are a promising class of peptides for development because of their stability to proteases and activity after systemic, including oral, administration. The MTP KOPr antagonist [D-Trp]CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-D-Trp]) prevents stress-induced reinstatement of cocaine-seeking behavior in mice after oral administration (Eans et al, 2013) and thus is a promising lead compound for the development of treatments to prevent relapse to substance abuse in abstinent individuals. Recently we have evaluated >90 analogs of the lead peptide for KOPr affinity, with promising analogs undergoing additional evaluation for KOPr selectivity and antagonist potency *in vitro*. KOPr affinities ranged from <1 nM to >10 μM, with >30 analogs exhibiting high KOPr affinity (K_i <20 nM). In a cAMP assay multiple analogs exhibited comparable or better KOPr antagonist potency than the lead MTP and the small molecule KOPr antagonist navacaprant (IC_{50} = 66 and 69 nM, respectively). Advancing these peptides for potential therapeutic application involves optimizing their structure to increase brain levels following systemic administration while maintaining KOPr affinity and antagonist potency. The ADME (absorption, distribution, metabolism, and elimination) properties of the peptides, including aqueous solubility, metabolism and permeability, are being evaluated to determine the effects of structural modification on these properties and prioritize analogs for further evaluation; these properties varied widely among the analogs examined. Promising analogs are evaluated in the mouse 55 °C warm-water tail-withdrawal assay for antinociception and antagonism of the KOPr-selective agonist U50,488. Five analogs have already been identified that antagonize centrally (intracerebroventricular) administered U50, 488 after oral administration with ID_{50} values <10 mg/kg, p.o. Further, we have identified three analogs to date that prevent stress-induced reinstatement of extinguished cocaine-seeking behavior in a mouse conditioned place preference assay following pretreatment at 3 or 10 mg/kg, p.o., doses which are >5-fold lower than required for the lead peptide [D-Trp]CJ-15,208 (Eans et al., 2013). In both the *in vitro* and *in vivo* assays promising analogs have comparable KOPr potency, selectivity and duration of action to that of the small molecule KOPr antagonist aticaprant which is undergoing clinical trials for treatment of major depression. Thus these are promising peptides for advanced preclinical development and for potential therapeutic application to prevent relapse to substance abuse.

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Conflict of interest: Drs. Aldrich and McLaughlin are inventors on patent applications on analogs of [D-Trp]CJ-15,208 and their potential application for treating substance abuse.

Associations between kappa opioid receptor availability and hoarding behaviors: Preliminary evidence using [^{11}C]EKAP PET.

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Background: Hoarding disorder (HD) is associated with compulsive saving and acquiring behaviors leading to excessively cluttered living spaces and profound functional impairment. HD has also been extensively associated with emotion dysregulation. Alarmingly, pharmacological interventions for HD are extremely limited, and fewer than 1/3 of psychotherapy patients demonstrate clinically significant change. There is a critical need for the development of biological treatment options that can act alongside psychotherapy and improve prognoses for patients. The kappa opioid receptor (KOR) may be a promising treatment target due to involvement in compulsive behavior and emotion regulation. **Objectives:** Relationships between hoarding and KOR have not yet been examined *in-vivo*. Using positron emission tomography (PET), this pilot study examines KOR availability in psychiatric subjects with hoarding behaviors (HB) relative to healthy controls (HC) matched for age and biological sex. **Methods:** 18 subjects (borderline personality disorder or post-traumatic stress disorder [n=16]; HC [n=2]) participated in an [^{11}C]EKAP PET scan and clinical assessment. The PET outcome measure was volume of distribution (V_T) in grey matter regions of the cingulo-opercular network (anterior-cingulate cortex and insula). These regions were selected due to their involvement in the pathophysiology of HD and summed to create a composite score. Measures included Saving Inventory-Revised (SI-R) and Difficulties in Emotion Regulation Scale (DERS) to assess HB and emotion dysregulation, respectively. V_T values were available for 16 psychiatric subjects and 2 HCs. Out of the 18 subjects scanned, six displayed subclinical HB (SI-R >24; M_{age} = 29; 83% female). Four additional, matched HC were selected from an existing PET repository to facilitate comparison between HB and HC. No age or biological sex differences were observed between HB and HC (p's=.99-1.0). **Results:** Individuals in the HB group demonstrated 36.58% lower cingulo-opercular KOR availability relative to HC, with large effect sizes (t(11)=-3.01, p=.011, d=1.7). In the full sample, difficulties discarding were negatively associated with KOR availability (r(16)=-.51, p=.045), such that lower KOR availability was observed among individuals with more severe compulsive saving behaviors. Links between HB and emotion dysregulation were also observed; difficulties discarding (r=(18)=.48, p=.042) and excessive acquisition were both associated with emotion dysregulation (r(18)=.65, p=.003) in the full sample. **Conclusions:** Findings show novel evidence for a role of KOR in the pathophysiology of hoarding behaviors. Specifically, psychiatric subjects displaying hoarding behaviors evidenced significantly lower KOR availability relative to age- and sex-matched HC, and the severity of difficulties with discarding was significantly associated with KOR availability in the full sample. Consistent with past research, hoarding behaviors were also associated with difficulties with emotion regulation. The severity of excessive acquisition and discarding problems were both associated with higher rates of emotion dysregulation. Future studies should aim to confirm findings in a larger sample of individuals with formal diagnoses of HD.

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Positron emission tomography (PET) imaging of kappa opioid receptor binding in major depression

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Background: Preclinical work finds that kappa opioid receptor (KOR) activation mediates stress-induced development of depression symptomatology. However, few human studies have probed KOR signaling *in vivo*. A pilot study by our group used the KOR agonist positron emission tomography (PET) tracer [^{11}C]GR103545 to study KOR binding *in vivo* in individuals with major depressive disorder (MDD) and healthy volunteers (HVs). We did not find relationships between KOR binding and diagnosis, depression severity, and both recent and early life stress in initial analyses within hypothesized *a priori* regions of interest (ROIs) selected based on rodent findings (amygdala, hippocampus, ventral striatum and raphe nuclei; Miller et al. 2018). In the current analyses, we examined regions identified from a secondary ICA analysis, where KOR binding in those regions was related to depression severity (Smart et al. 2021). Hypotheses tested were: (1) lower KOR binding in participants with MDD *vs.* HVs, and (2) lower KOR binding in participants with greater early life stress.

Methods: [^{11}C]GR103545 PET scans were obtained in 10 unmedicated, currently depressed individuals with MDD (32.6±6.5 years, 5 women) and 13 HVs (34.8±10 years, 6 women). The tracer total volume of distribution (V_T) was quantified from the [^{11}C]GR103545 PET scans and was normalized by the fraction of tracer not bound to plasma proteins in blood (free fraction: f_P), to yield an outcome measure (V_T/f_P) that was previously unanalyzed in this dataset. Considered bilateral ROIs were from our in-house atlas and were the closest regions to those reported with the strongest weighting in Smart et al. 2021: anterior cingulate (ACC), dorsolateral prefrontal (dlPFC), and insula. Linear mixed models were fit with ROI-level KOR binding (V_T/f_P) as outcome, participant as a random effect, and either diagnosis or recent life stress and ROI as fixed effects. Age and sex were tested as covariates and subsequently dropped from all models. Standardized betas (ß) are reported as effect sizes. Recent life stress was assessed with the Interview for Recent Life Events (IRLE). We handled zero-weighting by dichotomizing by the presence of at least one stressor of moderate impact in the prior six months ("recent major life stress").

Results: Across ACC, dlPFC, and insula, KOR binding was not different between MDD and HVs ($F=0.69$ $p=0.41$). However, when examining the full sample according to the presence of recent major life stress, individuals with recent major life stress had significantly higher KOR binding than those without recent major life stress (main effect of stress: $F=32.05$, $p<0.001$; interaction of stress-by-ROI: $F=11.03$, $p<0.001$; individual post-hocs: ACC: ß=2.09, 95% confidence interval (CI): [1.22, 2.95], $p<0.001$, dlPFC: ß=1.71, 95% CI: [0.84, 2.57], $p<0.001$, insula: ß=1.97, 95% CI: [1.11, 2.84], $p<0.001$). An analysis was also run examining all HVs vs. individuals with MDD with recent life major life stress. There were only 3 participants in the group of MDD without recent major life stress and so it was excluded. Individuals with MDD with recent life major life stress had higher KOR binding than HVs (main effect of group: $F=8.01$, $p=0.011$; interaction of group-by-ROI: $F=4.14$, $p=0.015$; individual post-hocs: ACC: ß=1.02, 95% confidence interval (CI): [0.32, 1.73], $p=0.006$, dlPFC: ß=0.60, 95% CI: [0.10, 1.31], $p=0.089$, insula: ß=1.04, 95% CI: [0.33, 1.74], $p=0.006$). Using a conservative Bonferroni correction for the six post-hoc comparisons ($p=0.05/6=0.008$), all effects remained significant after correction, except the dlPFC for HVs vs. individuals with MDD and recent major life stress. Effects were consistent but slightly weaker using the original outcome measure in Miller et al. 2018.

Conclusions: This is a re-analysis of human *in vivo* [^{11}C]GR103545 PET data using data-driven selection of brain regions. Contrary to our hypothesis, we did not find evidence for a diagnosis effect. However, when analyzed from the perspective of stress responsiveness, we saw an effect of higher KOR binding with recent major life stress and an effect specific to recently stressed individuals with MDD *vs.* HVs. *In vitro* and preclinical studies show that life stress elevates dynorphin, yielding compensatory KOR downregulation. Though these results require follow-up in larger samples, they suggest a possible model of blunted dynorphin/KOR response from major life stress exposure. Perhaps less dynorphin release over time from more life stress amplifies KOR binding. Further characterization has the potential to advance ongoing development of individualized antidepressant medications that target KOR.

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VTA Kappa opioid control of Stress-Induced Dopamine Dynamics in the dmPFC.

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The medial prefrontal cortex (mPFC) is critical for executive function, integrating sensory, emotional, and cognitive information to generate reasoned, behavioral responses to external stimuli. Within this region, the dorsal mPFC (dmPFC) plays a pivotal role in mediating goal-directed behaviors, decision-making, and aversive learning. Dopamine signaling within the dmPFC is essential for maintaining these processes, as it modulates neuronal activity and synaptic plasticity that underpin adaptation and cognitive flexibility. Acute aversive stressors both require their own behavioral responses and also can impede subsequent, optimal decision making in response to other stimuli. Disruptions in dmPFC dopamine function have been implicated in the development of stress-related neuropsychiatric disorders, including depression, anxiety, and addiction.

Kappa opioid receptors (KORs) are expressed on dopamine neurons in the ventral tegmental area (VTA), which provides the primary dopaminergic input to the dmPFC. In control conditions, KOR activation in the VTA inhibits dmPFC-projecting dopamine neurons by activating a G-protein-gated inwardly rectifying potassium (GIRK) current (Margolis et al. 2003, 2006). This is consistent with VTA KOR activation being aversive and the hypothesis that decreases in dopamine release are aversive. VTA KORs are thought to contribute to aversive and dysphoric states in both acute and chronic stress contexts through this mechanism.

Here we show that acute stress disrupts this canonical KOR function, leading to unexpected excitatory signaling. Using *ex vivo* electrophysiology, we found that a single foot shock stress session induced a switch in KOR signaling in a subset of VTA dopamine neurons, changing their KOR agonist response from inhibitory to excitatory. This phenomenon was absent in unstressed controls and was specific to dmPFC-projecting neurons. Complementary *in vivo* fiber photometry revealed that dopamine release in the dmPFC during foot shock stress was reduced in rats pretreated with intra-VTA microinjection of the KOR selective antagonist NorBNI compared to saline controls, indicating that VTA KOR activation actually *promotes* dopamine release in the dmPFC in response to acute aversive stimuli.

These results challenge the established understanding of KOR function as strictly inhibitory, revealing a stress-induced reconfiguration of KOR signaling in a mesocortical dopamine pathway.

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Serotonin and dopamine dynamics during KOR activation

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Stress activates the release of neuropeptide transmitters, including endogenous dynorphin (Dyn) opioids that activate $G\alpha_{i/o}$-coupled Kappa Opioid Receptors (KOR) to encode the dysphoric properties of stress. KOR activation leads to the recruitment of the three major mitogen-activated protein kinases (MAPK), and a unique role for p38α MAPK in regulating serotonin (5-HT) and dopamine (DA) tone has been established by p38α inhibitor and gene deletion studies. Further, p38α MAPK mediates dysphoria and promotes relapse of drug seeking during periods of drug abstinence. KOR activation of p38α in 5HT neurons has been shown to mediate stress-induced potentiation of cocaine preference and increased serotonin transporter (SERT) function in the DRN. This increase in SERT activity is hypothesized to cause a hyposerotonergic state in the nucleus accumbens (NAc). However, the hyposerotonergia remains to be shown. Additionally, KOR activation of p38α in the ventral tegmental area (VTA) DA neurons is also required for aversion in mice, and pharmacological inhibition or selective gene deletion of p38α in VTA DA neurons blocks the aversive response to stress. Together, the Dyn/KOR system remains a potent modulator of both the 5HT and DA systems that regulate the stress response. Characterization of the pharmacological and behavioral role of stress-induced activation using *in vivo* fiber photometry and the genetically-encoded sensor, $GRAB_{5HT,}$ and $GRAB_{DA}$ in combination with CRISPR gene deletion allows us to monitor real-time 5-HT DRN neurons and DA VTA neurons projecting to the NAc. Injection of cocaine produces a robust increase in endogenous 5-HT and DA compared to control. Injection with an unbiased KOR agonist that activates p38α, U50,488 (10 mg/kg) decreases 5-HT and DA tone, which can be blocked by pretreatment with naloxone (10 mg/kg) 15 minutes prior. Nalfurafine is a G-biased KOR agonist that does not efficiently activate p38α at low doses. Consistent with this, 5-HT and DA tone were not reduced in mice injected with nalfurafine (50 ug/kg). These studies will extend to using gene deletion methods and a repeated forced swim paradigm to reveal DRN to NAc 5-HT dynamics and VTA to NAc DA dynamics. Understanding 5HT and DA dynamics regulated by KOR/Dyn will provide critical insight into the mechanisms by which stress disrupts the 5HT/DA system to increase susceptibility to subsequent reward.

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Prefrontal cortical opioid receptor regulation of recurrent inhibitory microcircuits: from mice to man

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The prefrontal cortex exerts top-down control over motivated, goal-directed behavior through long-range inputs, local recurrent excitatory, and inhibitory microcircuits. Endogenous opioid neuropeptides and their receptors are expressed in the prefrontal cortex, but their principles underlying their anatomical organization and modulation of "hard-wired" synaptic circuits are just beginning to be uncovered. We and others have demonstrated that prefrontal cortical opioid receptors regulate excitatory and inhibitory synapses innervating the prefrontal cortex with specificity, but how endogenous opioid neuropeptide neurons communicate with opioid sensitive synapses was unclear. Further it is unclear whether functional regulation of inhibitory microcircuits is conserved in human and non-human primates (NHPs). Here, we will describe organizing principles of the endogenous dynorphin (Dyn) / kappa-opioid receptor (KOR) system and enkephalin (Enk) signaling through mu- (MOR) and delta-opioid receptors (DOR) from slice electrophysiology and anatomical studies performed in cortical slices from mice, NHPs, and human cortex. Our laboratory has demonstrated that in addition to directly inhibiting dopaminergic and recurrent local circuits and afferent excitatory synapses in a pathway specific manner, Dyn / KOR signaling also potently suppresses inhibitory microcircuits. Furthermore, Enk regulates inhibitory SST neurons through joint actions on MOR/DOR while inhibiting PV microcircuits solely through DOR. Through the NIH Comparative Brain Physiology Consortium and leveraging interneuron-specific enhancer driven optogenetic constructs our laboratory has been able to demonstrate that Dyn and Enk differentially regulate GABA release from distinct interneurons in NHPs. Moreover, using MOR promoter viruses we gained genetic access to MOR-positive neurons in NHPs and functionally demonstrated robust Enk inhibition of GABA release from MOR-positive interneurons. Further, we demonstrated that Dyn inhibits GABA release onto pyramidal neurons via KORs, while Enk inhibits GABA release through both MOR and DOR in human cortex. We also found the existence of functional Dynergic and Enkergic neuropeptidergic transmission that operates within prefrontal cortical circuits to inhibit glutamate and GABA release from opioid receptor positive neurons or hyperpolarize interneurons in mice. In collaboration with the NIMH Human Brain Collection Core, we have employed computational analyses (NeuronChat) that have revealed existence of intra-cortical Enkergic interactions within disinhibitory microcircuits similarly established in mice. Together, these findings demonstrate that endogenous opioid signaling shapes the activity of defined inhibitory microcircuits, a function that is conserved in primates. Future work is aimed at understanding how endogenous opioid systems regulate defined connectivity in primate cortex, specifically with novel tools implemented across species. These studies provide a framework for understanding opioid receptors in regulating prefrontal cortical circuits in health and neuropsychiatric disorder and elucidate multi-modal treatments consisting of existing and novel opioid therapeutics in tandem with cognitive therapy and/or neuromodulation (e.g. TMS) of cortical circuits.

Dynorphinergic extended amygdala circuitry regulates nociception and negative affect

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Chronic pain is a growing global public health concern, significantly reduces quality of life, and is highly co-morbid with negative affective conditions. The endogenous opioid system, including kappa opioid receptors (KORs) and their endogenous ligand dynorphin (DYN), is heavily implicated in pain chronification and negative affect. This is due, in part, to the concentrated expression of DYN and KOR in the extended amygdala, a cluster of regions including the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST). Here, we use electrophysiological, chemogenetic, and photometric approaches to characterize a role for dynorphinergic CeA-BNST circuitry in regulating nociception and negative affective-like behaviors. Our electrophysiological studies show that persistent inflammatory pain, induced by complete Freund's adjuvant (CFA), decreases excitability of prodynorphin (*Pdyn*) cells in the right CeA in both sexes, and further anatomical tracing shows that CeA^{Pdyn} cells send dense projections to the BNST. Consistent with these findings, using wireless fiber photometry, we find that terminal activity of CeA^{Pdyn}-BNST neurons is attenuated during thermal nociception, and this effect is exacerbated by CFA. Further, pain/stress-predictive stimuli appear to trigger anticipatory increases in CeA^{Pdyn}-BNST terminal activity prior to pain/stress-induced suppression of CeA^{Pdyn}-BNST terminal activity precipitated by foot shock. This anticipatory response persists in the absence of the shock in CFA-treated mice, suggesting that persistent activation of CeA^{Pdyn}-BNST in anticipation of a painful stimulus may reflect an anxiogenic state or a compensatory mechanism by which CeA^{Pdyn}-BNST neurons act to offset pro-nociception. Finally, to characterize the functional contributions of CeA^{Pdyn}-BNST neurons, we chemogenetically manipulated the circuit prior to testing anxiety-like behavior (light-dark box test) and mechanical nociception. Chemogenetic activation of the circuit produces anti-nociception in both females and males regardless of pain status but only decreases anxiety-like behavior in males not treated with CFA. Alternatively, chemogenetic inhibition of CeA^{Pdyn}-BNST neurons produces hyperalgesia in both sexes in the absence of CFA, attenuates hyperalgesia in males treated with CFA, and increases anxiety-like behavior in females. Collectively, these preliminary data indicate that CeA^{Pdyn}-BNST neurons regulate nociception and anxiety-like behavior in a sex-dependent manner. Ongoing studies are further investigating terminal activity of this circuit in response to innocuous and noxious stimulation and during the light-dark box test to further characterize its contributions to nociception and negative affect.

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Surgical incision engages endogenous kappa opioid receptor (KOR) activity in spinal KOR-expressing neurons to keep chronic postsurgical pain in remission

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Chronic postsurgical pain (CPSP) develops in millions of patients that undergo surgeries. Prolonged opioid therapy is contraindicated, and other therapeutic approaches lack sufficient analgesic efficacy. We employed a latent sensitization (LS) model of CPSP to test two hypotheses: 1) whether peripheral or central KORs suppress LS; 2) whether chemogenetic inhibition of spinal KOR expressing interneurons (KOR-INs) prevent LS. LS is a silent, long-lasting sensitization of nociceptive neurons that is tonically masked by compensatory activity of inhibitory G-protein coupled receptors (Taylor and Corder, 2014), including kappa opioid receptor (KOR) (Basu et al., 2021). To test Hypothesis 1 with a conditional deletion approach, we crossed *Pirt*^{Cre} mice with *Oprk1*$^{loxP/loxP}$ mice to create *Oprk1*$^{DRG-/-}$ conditional knockout (cKO) mice. We also crossed *Lbx1*^{Cre} mice with *Oprk1*$^{loxP/loxP}$ mice to create *Oprk1*$^{SC-/-}$ conditional knockout mice. We performed plantar incision in *Oprk1*$^{loxP/loxP}$ controls and cKO mice. 21-days later, we injected either long-acting (LY2456302, 10 µg, i.t.) or short-acting (BT-3761, 30 mg/kg, i.p.) KOR antagonists, and measured mechanical and heat hypersensitivity. The results indicate that both agents reinstated mechanical and heat hypersensitivity in controls for both *Oprk1*$^{DRG-/-}$ and *Oprk1*$^{SC-/-}$ mice. However, both long- and short-acting antagonists reinstated hypersensitivity in *Oprk1*$^{DRG-/-}$ but not *Oprk1*$^{SC-/-}$ cKO mice. To test Hypothesis 2 with a chemogenetics approach, *Oprk1*^{Cre} mice received intraparenchymal injections of AAV8-hSyn-hM4D$_i$, or AAV8-hSyn-mCherry (control) into lumbar enlargement of dorsal horn. Three weeks after AAV injection, clozapine N-oxide (3 mg/kg, i.p.) was injected 15 minutes before LY2456302 administration. Chemogenetic inhibition of KOR-INs prevented LY2456302-induced mechanical and heat reinstatement without changing the motor coordination in an accelerating rotarod test. For the first time, we report that surgical incision engages endogenous KOR activity in spinal KOR-expressing neurons to keep chronic postsurgical pain in remission. Current studies will optogenetically manipulate KOR-INs for a better understanding of endogenous opioid analgesia and neural circuits that underlie LS.

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Opioid modulation of contralateral effects of brain and spinal cord injury: a potential for pharmacological treatment.

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The crossed descending neural tracts set a basis for contralateral effects of traumatic brain injury (TBI). Previously, we demonstrated that the effects of unilateral brain lesions are also mediated by the neuroendocrine system through the humoral pathway. In rats with completely transected spinal cords, unilateral brain injury induced hindlimb postural asymmetry with contralateral flexion, a proxy for neurological deficit. Here, we hypothesize that this neurohormonal system consists of the left and right counterparts and examine if they differ in neural and molecular mechanisms. We report that the left and right-side hormonal signaling is differentially blocked by the selective opioid antagonists. The effects of left- and right-sided TBI are inhibited by delta- and kappa-opioid antagonists, respectively. The left and right neurohormonal signaling differ in targeting the afferent and efferent spinal mechanisms, further suggesting a lateralized organization of opioid-mediated neuromodulation. New findings also suggest that, following lateral spinal cord injury (SCI), asymmetric postural and motor deficits result from the interplay between opioid-mediated lumbar mechanisms and non-opioid pathways. Analysis of gene-gene co-expression patterns identified the left and right side-specific gene co-expression networks involving opioid genes that were coordinated across the hypothalamus and lumbar spinal cord through the humoral pathway. Notably, this coordination was ipsilateral and perturbed by TBI. The novel unusual opioid mechanism that mediates the contralateral effects of unilateral neurotrauma opens a window of opportunities for side-specific pharmacological treatment of hemiparesis and hemiplegia secondary to TBI and SCI.

Lukoyanov et al, Elife. 2021 10:e65247; Watanabe et al, eNeuro 0548-20.2021; Bakalkin, Cell Mol Life Sci 2022 79: 545; Watanabe et al., Function doi: 10.1093/function/zqae013.

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Peripheral kappa opioid receptor activation drives cold hypersensitivity in the oxaliplatin-dependent model of cold allodynia

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Noxious cold sensation is commonly associated with peripheral neuropathies; however, there has been limited progress in understanding the mechanism of cold pain. We previously found that peripherally expressed kappa opioid receptors (KOR) potentiate cold hypersensitivity, and others have shown that silent large-diameter neurons contribute to cold allodynia in neuropathic conditions such as in oxaliplatin-induced cold allodynia. Here we identify a role for kappa opioid receptors (KOR) in cold hypersensitivity associated with cold allodynia in an acute oxaliplatin mouse model. Wildtype mice (WT) injected with oxaliplatin (100 µg, 50 µL, i.pl.) in both paws show a significant increase in the paw-directed responses (nocifensive behaviour) 4 hours post-injection on the cold plate at 3ºC compared to saline-injected counterparts. To understand the role of KOR in this model of oxaliplatin-induced cold allodynia, we injected NorBNI (KOR antagonist) and repeated the cold plate behaviour at 4 hours post-oxaliplatin injection in WT mice. NorBNI injection attenuated oxaliplatin-induced nocifensive responses in both WT male and female mice at 3ºC. To further understand whether peripheral KOR plays a role in the oxaliplatin-induced cold sensitivity model, we used a conditional knockout mouse of KOR in the dorsal root ganglia using Pirt-cre$^{+/-}$ x Oprk$^{fl/fl}$. In the oxaliplatin-induced nocifensive model, Pirt-cre^{+} X Oprk$^{fl/fl}$ showed attenuated oxaliplatin-induced cold nocifensive responses compared to Pirt-cre^{-} X Oprk$^{fl/fl}$ on a cold plate at 3ºC in males. A similar effect was seen in Pirt-cre^{+} X Oprk$^{fl/fl}$ females compared to Pirt-cre^{-} X Oprk$^{fl/fl}$, but the nocifensive response was significantly higher in females versus male mice, suggesting a sexually dimorphic response of KOR-induced cold hypersensitivity in the oxaliplatin-induced cold allodynia model. Overall, our data suggests that peripheral KORs modulate cold hypersensitivity in the oxaliplatin-dependent model of cold allodynia.

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POSTER ABSTRACTS

Kappa opioid receptor (KOR) induced analgesia and aversion modulated by R7 Regulator of G protein signaling (RGS) Family.

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The Kappa opioid receptor (KOR) system is a neuromodulator system that represents a potential alternative to current analgesics, but aversive side effects of KOR activation have hindered its drug development. Deciphering the intracellular signaling events activated by KOR and its modulators that modify its therapeutic and aversive effects may help aid in the development of novel compounds. In this project we interrogate the involvement of G protein signaling by targeting their endogenous antagonist, Regulators of G protein signaling [RGS]. The R7 RGS family is highly expressed in the central nervous system (CNS), modulates $G\alpha_{i/o}$ proteins, and has been shown to regulate opioid receptors. To target the R7 family of RGS proteins, we used RGS7-family binding protein (R7BP) knockout (KO) mice as R7BP is a membrane anchor for all R7 family members. Using a Conditioned Place Aversion test, we found that R7BP KOs have enhanced KOR-induced aversion compared to WT littermates at 5 and 10 mg/kg of U50,488. This implicated the R7 family in modulating KOR-induced aversion. We further delineated which member of the R7 family is responsible for mediating this effect as well as modulating KOR induced analgesia. Using global KOs for two members of the R7 family that are widely expressed in the CNS, RGS6 and RGS7, we investigated their role on mediating KOR-induced aversion and analgesia. We found that RGS6 nor RGS7 alone modulate KOR-induced aversion at 2 and 5 mg/kg U50,488. This suggests that either a more spatially restricted R7 family member or RGS6 and RGS7 together modulate KOR-induced aversion. KOs of RGS6 but not RGS7 has been implicated in modulating KOR-induced analgesia in a thermal pain assay in both males and females. Using a traditional 55^{oC} hot plate assay, we found a significant increase in the peak analgesic response in response to 15 mg/kg U50,488 in RGS6 KOs compared to WT. Together, this data suggests that the R7 RGS family may modulate KOR in diverse ways to modulate behavioral output. Further research will provide valuable knowledge that will advance our understand of the contribution of intracellular signaling and regulators at opioid receptors towards specific behavioral outcomes.

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KOR in Paraventricular nucleus of the thalamus (PVT)

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The PVT is the most dorsal nucleus of the thalamic midline nuclei and is involved in stress responses, fear, anxiety, arousal, and reward. In the KOR-tdTomato mouse line, the PVT expressed a moderately high level of KOR. In situ hybridization showed that in the PVT, KOR mRNA co-localized substantially with vGluT2 mRNA (but not vGluT1 mRNA), indicating presence of KOR in Glu neurons. As the KOR is also involved in stress responses, anxiety, and reward, we characterized KOR in the PVT with regard to the brain regions KOR-expressing neurons projected to and the role of KOR in the PVT in KOR-related behaviors. By use of Cre-dependent anterograde tracer AAV-FLEx^{loxP}-mGFP-2A-synaptophysin-mRuby and KOR-iCre mice, we found that KOR-expressing neurons projected to many brain regions, with the NAc, CeA, BLA, zona incerta and stria terminalis among the major projection targets. We conditionally knock-downed KOR (KOR cKD) in the PVT by stereotaxic injection of AAV-eGFP-Cre into anterior PVT (aPVT) or posterior (pPVT) of KOR-floxed mice. The degree of KOR cKD was determined by quantitative [^{3}H]U69,593 receptor autoradiography. Data on effects of KOR cKO on open arm entry in the elevated plus maze test, immobility in the forced swim test, and U50,488H-induced anti-scratching and conditioned place aversion will be presented and discussed. .

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None.

Title: Characterizing the role of Mu opioid receptors in metabolism

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Fifty percent of individuals with some form of diabetes will develop a painful and chronic comorbidity called peripheral neuropathy. Medications that act on the mu opioid receptor (MOPR) like tramadol, fentanyl or oxycodone are prescribed to treat the painful elements of this disease. Importantly, decades of research suggests that MOPR stimulation may additionally have direct effects on pancreatic function, however, concrete understanding of that relationship remains poorly understood. Here, we seek to comprehensively determine how endogenous MOPRs regulate endocrine pancreas function by using a combination of genetic and pharmacological interventions during in vivo metabolic and behavioral tests. To test if loss of MOPRs influence overt ingestive behaviors, we measured 24-hour ad libitum food consumption using Feeding Experimental Devices (FEDs) in wildtype or *OPRM1* knockout (MOPR-deficient) mice. We found that *Oprm1* KO males were heavier and exhibited increased food intake, feeding frequency, and increased bout sizes relative to wildtype controls. In contrast, female Oprm1 KO mice did not differ from wildtype control mice. To test whether constitutive knockout of MOPRs impact glucose metabolism, we conducted glucose and insulin tolerance tests (GTT, ITT) in Oprm1 KO and wildtype mice. We found that at young adult ages (10-12 weeks) both male and female OPRM1 KO mice displayed enhanced glucose tolerance. However, this phenotype was absent at later ages (25-27 weeks). Finally, to test whether the effects observed in MOPR constitutive knockout mice were driven by developmental versus dynamic changes in metabolic regulation, we used a pharmacological approach to selectively antagonize and agonize MOPRs (CTAP, DAMGO, respectively) in wildtype mice, and then tested them on GTT. Our results suggest that acute blockade and agonism of MOPRs in both male and female mice significantly enhance GTT. Future directions will include a later time point to compare during old age (50-52 weeks).

Ligand Recognition and G Protein Signaling Diversification in the Kappa Opioid Receptor

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The kappa-opioid receptor (KOR) shows promise as a pain therapeutic target, providing analgesic effects without causing respiratory depression and addiction. However, undesirable effects such as dysphoria and hallucination with KOR-targeted ligands remain a concern. Canonically, KOR signals are transduced through the activation of heterotrimeric G proteins, including seven Gi/o proteins (Gi1, Gi2, Gi3, GoA, GoB, Gz, Gg), alongside various Gβ and Gγ subunits. The multiple potential KOR-G protein combinations raise questions regarding selectivity and functionality. In this study, we determined atomic structures of KOR in complexes with multiple G protein heterotrimers and ligands. These structures provide valuable insights into the key determinants responsible for opioid recognition and G protein subtype selectivity. Furthermore, our analysis demonstrated that the seven Gi/o protein subtypes exhibit differences in the allosteric enhancement of agonist binding, GDP/GTP turnover rates, and the kinetics of KOR signaling responses. These findings lay the foundation for designing Gi/o protein subtype selectively targeted drugs to improve therapeutic effects.

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Reproducible interrogation of opioid withdrawal using novel machine-learning enabled approaches in mice

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Modeling opioid withdrawal in rodents has presented several challenges due to the wide variability of drugs, routes of exposure, and behavioral methods. To achieve consensus among different lab groups, using objective measures of withdrawal behaviors allows for rigor and reproducibility. To model opioid exposure and withdrawal, we utilized both a chronic exposure paradigm to fentanyl, using osmotic minipumps, and an acute exposure paradigm, using subcutaneous injections of fentanyl. We validated the delivery of fentanyl using the osmotic minipumps by pharmacokinetic analysis at different time-points using liquid chromatography/ mass spectrometry. To assess withdrawal, we first used the anxiety-like behavior assays to determine if we could infer differences via the Open Field Test during exposure, and the Elevated Plus Maze (EPM) test during withdrawal. Contrary to our hypothesis, we found that mice undergoing withdrawal exhibited decreased anxiety-like behaviors in the EPM. Seeing as that contradicts with human data during withdrawal, we concluded that these standard approaches do not capture the withdrawal experience in rodents appropriately. We sought to make use of the advances in machine-learning based algorithms for behavioral analysis to better score the somatic signs of withdrawal in mice which offers both automation and scaling compared to subjective scoring by experimenters of each video. We used the pose- estimation toolkit Deeplabcut (DLC) and the supervised machine learning behavioral classification tool, Simple Behavioral Analysis (SimBA) to label body-parts and behaviors respectively to quantify the total time and frequency of grooming, rearing, lifting, jumping, and climbing after precipitated and spontaneous withdrawal from fentanyl. We observed differences between acute and chronic fentanyl exposure. We also found that the opioid receptor antagonist, naloxone, causes changes in the duration and frequency of the behaviors regardless of prior fentanyl exposure. This work is of interest to the Kappa Therapeutics Community given the established role of the dynorphin/KOR system in mediating negative affective states, and likely the somatic symptoms of withdrawal presented here. Future work will aim to draw a direct correlation between the activity of the kappa opioid receptor system and the duration and frequency of the behaviors presented here.

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Title: Second generation SAR studies on Akuammicine guided by cryoEM structure of an AKC complex in the κOR.

The κ-Opioid Receptor (κOR) is a promising target for treating different neurological conditions, including pain and addiction. There exist a multitude of agonists discovered which are highly potent, yet few have passed clinical trials due to their sedative, dysphoric, and/or hallucinogenic properties. Thus, novel κOR ligands are essential for developing effective κOR agonists. Akuammicine (AKC), an alkaloid found in the seeds of the akuamma plant (*Picralima nitida*), is an agonist of the κOR that is structurally distinct from existing classes of κOR ligands. Recently, structure-activity relationship (SAR) studies on AKC scaffold identified that 10-(3-Furanyl)-AKC, 10-Bromo-AKC, and 10-Iodo-AKC have 1300-, 300- and 200-fold increase compared to AKC, suggesting that modification at the C10 position produce additional ligand-receptor interactions within the κOR binding site. Animal studies with 10- Bromo- and 10-Iodo-AKC showed inhibition of compound 48/80-induced scratching with ED_{50} value of 3.0 and 1.3 mg/kg respectively. However, both exhibit conditioned place aversion (5 mg/kg for both) and impaired rotarod performance at effective anti-scratch doses (10 mg/kg for 10-Bromo-AKC and 5 mg/kg for 10-Iodo-AKC). Herein we present our on-going efforts to employ a recent cryoEM structure of an AKC in complex with the κOR to guide the synthesis of additional C10-modified AKC derivatives to explore and exploit these interactions.

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Efforts to Define the Mechanism of Action of Ibogaine

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Ibogaine is a psychoactive indole alkaloid, found in the iboga shrub (*Tabernanthe iboga*) which is used as a traditional medicine and in spiritual rituals in Central Africa. Anecdotal evidence also suggests that ibogaine may be effective in the treatment of several substance use disorders, including opioid use disorder (OUD). Although several receptors and transporters have been proposed as ibogaine's target, including the α3β4 nicotinic acetylcholine receptor, serotonin transporter (SERT), dopamine transporter (DAT), and κ opioid receptor, ibogaine has low affinity for each of these potential targets. However, it is possible that a metabolite of ibogaine may bind with higher affinity and contribute to its psychoactive and anti-addictive properties. To test this hypothesis, we have synthesized a collection of fourteen metabolites of ibogaine and related natural products. His collection is being evaluated for activity against previously proposed targets including the opioid receptors, SERT, DAT, and the α3β4 nAChRs as well as an unbiased screen against the GPCRome through the Psychoactive Drug Screening Program.

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The role nociceptin opioid peptide in dorsal raphe nucleus on pain and motivation

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In 2021, it was estimated that 51.6 million people in the United States experienced chronic pain. Even though emotional regulation plays a vital role in pain, research in this area is limited. The dorsal raphe nucleus has been previously studied for its role in emotional regulation. Recent work in our lab has shown that the opioid peptide enkephalin strongly regulates appetitive and aversive behaviors, including those that impact nociceptive behaviors. In addition to enkephalin, the opioid peptides dynorphin and nociceptin are also highly expressed in the dorsal raphe, but preliminary in situ hybridization experiments suggest that these three peptides are largely separate populations. While some research has shown that dynorphin regulates aversive processing, the role of nociceptin has not been characterized. To address this, we have used a CRISPR-Cas9 approach to selectively knockdown nociceptin opioid peptide in dorsal raphe nucleus of Pnoc-cre mice and measured changes in a variety of appetitive, aversive, and nociceptive behaviors. Results so far suggest that nociceptin may play an opposite role relative to enkephalin. Specifically, CRISPR knockdown of nociceptin blunts mechanical allodynia induced by inflammatory pain, and enhances food intake in both ad libitim and food deprived conditions. Future work will continue to characterize this subpopulation, as well as assess how dynamic nociceptin activity changes via fiber photometry recordings of a nociceptin biosensor.

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Study of conformational dynamics of k-Opioid Receptor to probe ligand efficacy using single-molecule FRET

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Opioid drugs are essential for pain management, but κ-opioid receptor (KOR)-targeting compounds offer distinct advantages over μ-opioid receptor (μOR) agonists by providing analgesia with reduced risk of addiction and overdose. Understanding the molecular basis of KOR ligand efficacy is critical for developing safer and more selective therapeutics. Here, we employ single-molecule fluorescence resonance energy transfer (smFRET) to investigate the conformational dynamics of transmembrane helix 6 (TM6) in KOR upon binding to ligands as a measure to probe the receptor activation dynamics. Our results reveal that agonists and antagonists differentially modulate TM6 motions, influencing the formation of the KOR-G protein complex, hence tuning the G-protein signaling pathways. Unlike the μ-opioid receptor, which fully shifts to the activated G-protein bound state in the presence of a full agonist and absence of GDP, the κ-opioid receptor exhibits a dynamic equilibrium between the active and inactive state at the same experimental condition. Additionally, we identified a distinct inactive resting state that could significantly influence the dynamic transition between the active and inactive state in the presence of the ligand, hence modulating the allosteric coupling. These findings provide a single-molecule perspective on KOR activation, offering new insights into the structural dynamics of ligand-dependent signaling and a quantitative approach to assessing the efficacy of specific drugs.

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JWT-101 acts as a long-lasting KOR antagonist

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Kappa opioid receptor (KOR) ligands have been explored for anti-anxiolytic, anti-depressive, pain, and substance use disorder therapeutics. These therapeutic effects are partly attributed to biased signaling through the cJun N-terminal Kinase (JNK) pathway, which involves complex molecular interactions and downstream effects. JWT-101, a novel KOR ligand, has demonstrated long-lasting therapeutic effects; however, its underlying mechanism remains poorly understood. I assessed KOR agonist-induced analgesia by measuring the latency of tail withdrawal from 52.5°C water after treatment with U50,488, a KOR agonist. Pretreatment with JWT-101 (15 mg/kg) 24 hours before U50,488 injection effectively blocked KOR-induced analgesia in wild-type male mice. This effect was itself blocked by the short-acting, KOR-selective antagonist Aticaprant (5 mg/kg), suggesting that JWT-101's action is specifically mediated through KOR. Further investigation using in-vivo fiber photometry and ex-vivo slice imaging with the novel peroxide sensor oROS-Gr revealed that JWT-101 (15 mg/kg for fiber photometry and 10 uM for slice imaging) significantly increased ROS production in KOR-expressing cells of the prefrontal cortex that could be blocked with KOR antagonism. These findings indicate that JWT-101 activates the KOR/JNK signaling pathway, leading to increased ROS levels. The enhanced ROS production and subsequent receptor inactivation suggest a mechanism where JWT-101's therapeutic effects may be attributed to its ability to modulate KOR signaling through oxidative stress pathways. Understanding this mechanism could provide valuable insights into the development of more targeted KOR-based therapies and advance our knowledge of how JWT-101 exerts its long-lasting effects.

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Structural Basis of Inverse Agonism via Kappa Opioid Receptor:G protein Complexes

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Opioid receptors, belonging to the G protein-coupled receptor (GPCR) superfamily, are important targets for managing pain and addressing various neuropsychiatric disorders. Despite the therapeutic value of opioids, their addiction potential and resulting abuse has led to a public health crisis which targeting kappa opioid receptors (kOR) offers the potential to mitigate. Kappa opioid receptors are a promising target for selective antagonists which can provide analgesics, withdrawal treatments, as well as anxiety management without the negative effects of traditional opioid based treatments. Utilizing kOR's therapeutic potential, hinges on a comprehensive understanding of opioid receptor activation and inactivation which remains incomplete by the canonical ternary complex model. The canonical model asserts that ligands acting as agonists or antagonists either promote or inhibit the formation of receptor:G protein complexes. By this model, inhibitors bind G protein-free receptors to prevent G protein coupling. However, the canonical model is incomplete, as it fails to account for the fact that many GPCRs are constitutively active and can associate with G proteins in the absence of agonists. Alternative models have proposed pathways involving G protein binding to various receptor states in the absence of agonists yet, the signaling and properties of these non-canonical receptor:G protein complexes remain largely unexplored. We provide the first insights into the pharmacology and structural features of these complexes. Our data from biochemical, pharmacological and single particle cryoEM experiments reveal the first evidence of non-canonical inverse agonist-receptor states, challenging the canonical GPCR activation model. By capturing these novel states, we can expand upon the conformational landscape that receptors can access beyond those in the canonical model to further our understanding of fundamental aspects of signaling inhibition. Our findings shed light on mechanisms of opioid receptor signaling dynamics and provide additional receptor states for targeting and inhibition of KOR as well as other GPCRs. Understanding the regulation of opioid receptors and signaling dynamics could significantly contribute to the framework for a structure-guided approach to KOR drug design which has far-reaching impacts in improving the treatment of substance abuse and neuropsychiatric disorders.

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Title:

NAc-VTA dynorphin modulation of dopamine dynamics in associative learning

Authors:

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These authors contributed equally: Michael R. Bruchas, Ana João Rodrigues

Abstract:

Dopaminergic neurons in the ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc) are essential for mediating both rewarding and aversive responses. Various neuropeptides, including the endogenous opioid dynorphin, intricately regulate dopaminergic activity. It is believed that the primary source of dynorphin in the VTA is from nucleus accumbens (NAc) D1-MSNs and that dynorphin acts through kappa opioid receptors (KORs) as a negative feedback mechanism to regulate dopamine release. The NAc is divided into core and shell, with the shell further subdivided into dorsal and ventral regions, each exhibiting distinct anatomical and functional characteristics. We recently showed evidence for a functional dynorphin gradient across the dorso-ventral axis of the NAc shell, promoting divergent motivated behaviors. However, how dynorphin regulates dopamine signaling during associative learning and whether dopamine dynamics differ between NAc shell subregions remains unknown.

In this study, we utilized the newly developed fluorescent dynorphin sensor kLight1.3, along with multiple genetic manipulations, to characterize dynorphin signaling between the NAc and VTA during associative learning. We observed dynamic dynorphin release in the VTA after cue presentation, which increased over several days of the Pavlovian conditioning task. High-frequency-like and prolonged optogenetic stimulation of NAc neurons induced dynorphin release in the VTA, confirming the specificity of NAc-originated release. We also assessed whether optogenetic stimulation of dynorphinergic neurons in the NAc shell shapes dopamine release during conditioning. We observed an unexpected increase in dopamine release upon stimulation in the NAc during cue-reward presentations, suggesting a more complex role of dynorphin in modulating VTA activity. To further examine its role, future studies will employ various strategies to downregulate dynorphin signaling.

These findings highlight that dynorphin/KOR signaling between the NAc and VTA shapes dopamine dynamics and plays a key role in associative learning.

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

Kappa opioid receptor availability and interoceptive awareness: Investigating links across disordered eating behaviors

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Background: Interoception, the ability to perceive internal bodily states, relies on the thalamus, insular cortex, amygdala, anterior cingulate cortex (ACC), and frontal brain regions. Disruptions in interoception are implicated in disordered eating behaviors (DEB). The kappa opioid receptor (KOR) plays a role in stress, emotion regulation, and reward processing; however, its relationship with interoception remains underexplored. Using [^{11}C]EKAP PET imaging, this study examines relationships between KOR availability, interoceptive awareness (IA), and DEB. First, we hypothesized that lower KOR availability, reflecting increased receptor activity due to dynorphin release triggered by stress, will be associated with reduced IA (aspects of emotional awareness; attention regulation; body trust). Second, we hypothesized that relationships between KOR availability and IA will be stronger in individuals with DEB, as greater emotional and stress-related demands may amplify KOR's impact on interoceptive processing. **Methods:** 26 individuals (69% female, M_{age} = 31; diagnoses of borderline personality disorder, post-traumatic stress disorder, or healthy controls) participated in an [^{11}C]EKAP PET scan and MRI (Outcome measure = volume of distribution [V_T]). Regions of interest included amygdala, thalamus, ACC, insula, and frontal regions, averaged to create a KOR circuit value. IA was assessed with the Multidimensional Assessment of Interoceptive Awareness (MAIA-2). The high DEB group included those above the clinical cut-off on the Eating Disorders Examination Questionnaire (n=6). **Results:** In the full sample, KOR availability was positively correlated with Attention Regulation (r(21)=.603, p=.004) and Trust (r(22)=.499, p=.018), but not Emotional Awareness (p=.328). The high DEB group exhibited lower Trust scores (t(18)=-3.471, p=.003) and lower KOR availability (t(15)=-2.260, p=.039) than the low DEB group. Within-group correlations showed a significant positive correlation between Trust and KOR availability in the low DEB group (r(11)=.724, p=.012) only. **Discussion:** Lower KOR availability was observed among those with more interoceptive deficits and with DEB, suggesting a potential neurobiological mechanism underlying DEB. This aligns with previous work linking the kappa opioid system to maladaptive reward processing and stress-related eating behaviors. KOR was correlated with IA (attention regulation; body trust) in the full sample, but not in the DEB group. KOR antagonists could be a potential treatment for improving IA, supporting personalized therapeutic strategies. Future studies should explore KOR, IA, and DEB in larger clinical samples.

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

Role of central amygdala dynorphin neurons in inflammatory pain and negative affective behaviors

Léa J. Becker, Gustavo Borges, Jessica Cucinello-Ragland, Yolanda Campos-Jurado,
Jose Moron-Concepcion, Jordan G McCall

Pain is a risk factor for the development of negative affective states. The extended amygdala circuit, comprising the central amygdala (CeA), the nucleus accumbens, and the bed nucleus of the stria terminalis (BNST), is central to the pathophysiology of pain and affective disorders comorbidity. Kappa opioid receptors (KORs) and their endogenous ligand, dynorphin (dyn), are expressed throughout these regions. Here, we show that persistent inflammatory pain, induced by complete Freund's adjuvant (CFA) intra-plantar injection, decreases excitability of prodynorphin (Pdyn) cells in the right CeA in both sexes. Interestingly, in males inflammatory pain seems to concomitantly increase left CeA^{pdyn} cells excitability. Using in vivo chemogenetics, we show that mimicking this left right imbalance in CeA^{pdyn} cells excitability is sufficient to induce pain and negative affective behaviors. Finally, we show using anatomical tracing in these neurons, that CeA^{pdyn} cells send dense projections to the BNST. We determined the nature of this input using acute slice electrophysiology and show that CeA^{pdyn}→ BNST is a monosynaptic GABAergic exhibiting short-term synaptic depression. We will further investigate the effect of inflammatory pain on this CeA^{pdyn}→ BNST circuit.

Support: Work supported by R01NS135401 (JGM) & R01NS117899 (JGM).

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

Novel Synthesis of Kappa Opioid Receptor Agonists

Alexis Knoll[a], Nokomis Ramos-Gonzalez[a], Rohini Ople[a,] Balazs R. Varga[a], Kevin Appourchaux[a], Haoran Zhu[a], Tao Che[a], Susruta Majumdar[a]

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Opioids are primarily used in the treatment of moderate to severe pain. Clinically used opioid analgesics such as fentanyl and oxycodone target the mu opioid receptor (MOR). The use of opioids in medical settings is slowly decreasing because of the negative side effects such as respiratory depression, and abuse potential. Targeting the kappa opioid receptor (KOR) represents a viable option as they lack the MOR mediated adverse effects although dysphoria rather than euphoria seen with MOR activation has limited clinical usage.

The goal of our current project is to separate kappa mediated analgesia from dysphoria. Towards this, we utilized the Ugi multicomponent reaction to synthesize KOR modulators that are structurally distinct from existing KOR chemotypes. The Ugi multicomponent reaction is a one-step, overnight reaction that involves an amine, isocyanide, ketone, and carboxylic acid combined in solution that results in our novel ligands with minimal purification. Compounds were characterized in Bioluminescence resonance energy transfer (BRET) based assays for G-protein activation and β-arrestin2 recruitment. These compounds were found to be highly KOR selective and served as full agonists. Using the lead compound, AKN036, a Cryo-EM structure was solved of KOR in complex with Gαi1β1γ2 further stabilized by ScFv16 which revealed how the ligand was bound to the receptor and identified the molecular determinants of selectivity.

Structure based design was next pursued to rationally design G-protein biased ligands with high efficacy and potency similar to U50,488H.

In summary, using the Ugi multicomponent reaction and structure-based design we developed new chemical entities targeting the KOR with differential activity.

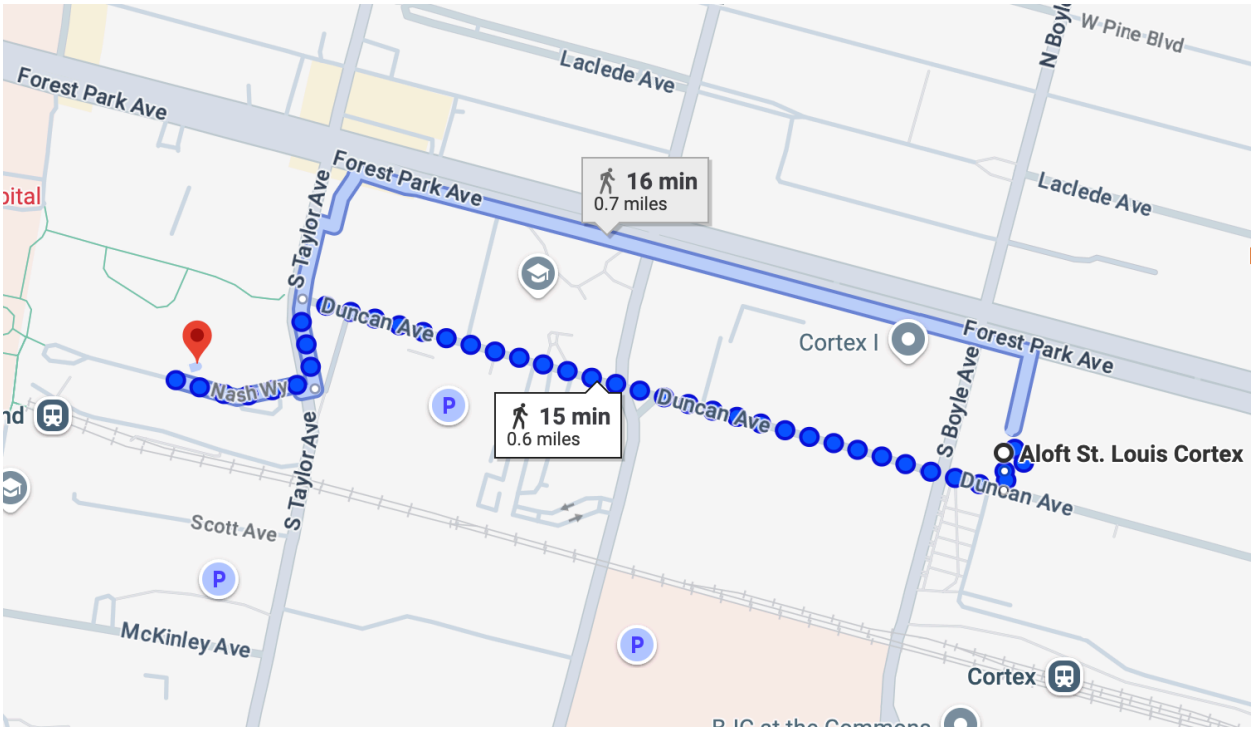
Support: This work is supported by NIH/NIDA grants (R01DA057790, R01DA059978, R61NS136307, R01DA036246).

Presentation Preference: Poster

Important Information

* Getting to the Conference Venue (UHSP, Academic and Research Building, 2 Pharmacy Place, 63110) from the Aloft Hotel (4245 Duncan Ave, 63110)

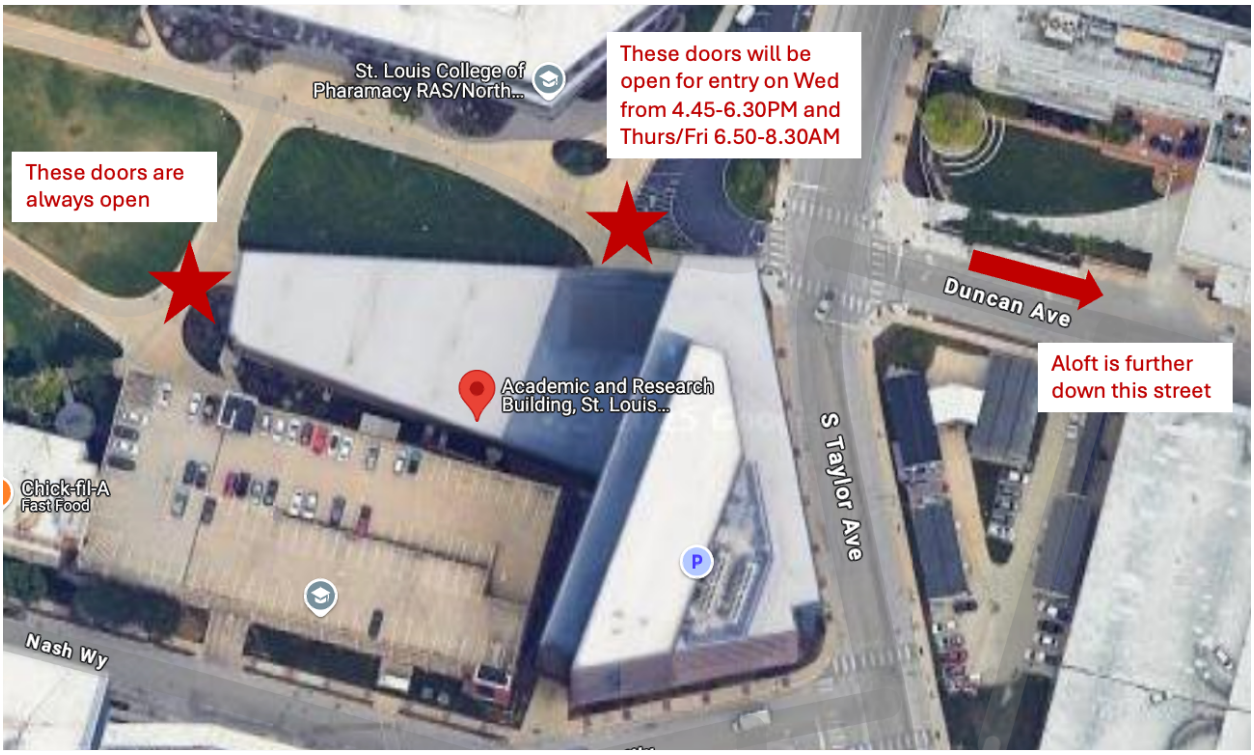
From the Aloft hotel head west on Duncan towards Taylor Avenue (WashU's Med campus and UHSP), which will take you past WashU's Neurosciences Research Building. When you cross Taylor you will see signs to enter the building. You can also take the metro from the Cortex stop opposite the hotel to Central West End stop, trains run every 20 mins.



*Getting in and out of the building

ARB has two sets of doors (big red stars in the satellite image below). The ones on Duncan and Taylor are usually locked to the public but will be open for the reception on Wed and on Thurs and Fri between 7-8.30 AM. You can always leave through these doors

but in order to re-enter you need to use the second set of doors located on the west side by the quad (towards starbucks/Chick-fil-A) and then walk through the building towards the stairwell and up to the second floor.



Local Recommendations for Dining and Nightlife

***Short walk from the Conference Venue**

Havana's Cuisine Restaurant
(https://www.havanascuisine.com/)
Cuban – 12 S. Euclid Ave – 6 minutes

Brasserie by Niche
(https://brasseriebyniche.com/)
French - 4580 Laclede Ave – 7 minutes

Saigon Café
(https://www.saigoncafestl.com/)
Vietnamese – 10 N. Euclid Ave – 7 minutes

West End Wok
(https://www.westendwokstl.com/)
Chinese– 4577 Laclede Ave – 7 minutes

Vicia
(https://www.viciarestaurant.com/)
Modern American, Seasonal – 4260 Forest Park Ave – 11 minutes

Yellowbelly
(https://www.yellowbellystl.com/)
Seafood & Cocktails – 4659 Lindell Blvd – 11 minutes

Bootleggin' Tavern
(https://www.bootlegginbbq.com/)
Large beer selection & quality bar food - 4501 Chouteau Ave – 12 minutes

Lazy Tiger
(https://www.lazytigerstl.com/)
Asian-inspired Bar & Small Plates – 210 N. Euclid – 12 minutes

El Burro Loco
(https://www.elburrolocostl.com/)
Mexican - 313 N Euclid Ave - 14 minutes

Edera
(https://www.ederastl.com/)
Italian – 48 Maryland Plaza – 16 minutes

Maryland House
(https://www.themarylandhouse.com/)
Cocktails & Appetizers - 44 Maryland Plaza Rear – 16 minutes

Scarlett's Wine Bar
(https://www.scarlettscwe.com/)
Wine & Pizza – 4253 Laclede Ave - 16 minute

Platypus
(https://www.drinkplatypus.com/)
Neighborhood bar – 4501 Manchester Ave – 18 minutes

Urban Chestnut
(https://www.urbanchestnut.com/)
Brewery & German-inspired Food – 4465 Manchester Ave – 18 minutes

Session Taco
(https://www.sessiontacostl.com/)
Mexican – 398 N. Euclid – 19 minutes

Up-Down
(https://www.updownstl.com/)

Arcade Bar – 405 N Euclid Ave – 20 minutes

***Longer Walk or Car from the Conference Venue**

Good Company
(https://www.goodcompanystl.com/)
Southern & Comfort Food – 4317 Manchester Ave – 22 minutes – 1.0 mile

Gramophone
(https://www.gramophonestl.com/)
Bar & Music Venue – 4243 Manchester Ave – 23 minutes – 1 mile

Retreat Gastropub
(https://www.retreatgastropub.com/)
Gastropub - 6 N Sarah St – 22 minutes – 1 mile

Sultan
(https://www.sultanrestaurantstl.com/)
Mediterranean & Middle Eastern – 4200 Manchester Ave – 22 minutes – 1 mile

Grace Meat + Three
(https://www.gracestl.com/)
Southern Comfort Food – 4270 Manchester Ave– 22 minutes – 1 mile

Sanctuaria
(https://www.sanctuaristl.com/)
Latin Fusion – 4198 Manchester Ave – 24 minutes – 1.1 miles

Bowood by Niche
(https://www.bowoodbyniche.com/)
Seasonal & Farm-to-Table – 4605 Olive St – 24 minutes – 1.1 miles

Chao Baan
(https://www.chaobaanstl.com/)
Thai & Asian Fusion – 4087 Choteau Ave #5 – 26 minutes – 1.2 miles

Rockwell Beer Company
(https://www.rockwellbeerco.com/)

Craft Brewery & Taproom – 1320 S. Vandeventer – 29 minutes – 1.3 miles

***By Car from the conference venue**

Modern Brewery
(https://www.modernbrewery.com/)
Craft Brewery – 5200 Oakland –25 minutes – 1.1 miles

Union Loafers
(https://unionloafers.com/)
Artisan Bread & Sandwiches – 1629 Tower Grove Ave – 33 minutes – 1.5 miles

Indo
(https://www.indostl.com/)
Southeast Asian & Asian Fusion – 1641D Tower Grove – 1.5 miles

Sado
(https://www.sadostl.com/)
Sushi & Japanese – 5201 Shaw Ave – 1.9 miles

2nd Shift Brewery
(https://www.2ndshiftbrewing.com/)
Craft Brewery – 1601 Sublette Ave #2 – 2.0 miles

Anthonino's Taverna
(https://www.anthoninostaverna.com/)
Italian & Mediterranean – 2225 Macklind Ave – 2.2 miles

ITAP (https://www.itapstl.com/)
Huge beer selection, BYO food – 6217 Delmar Blvd – 3.2 miles

Heavy Riff Brewing
(https://www.heavyriffbrewing.com/)
Craft Brewery & Taproom – 6413 Clayton Ave – 3.2 miles

Plantar's House
(https://www.plantars-house.com/)
Cocktails – 1000 Mississippi Ave – 3.3 miles

Blueberry Hill
(https://www.blueberryhill.com/)
Chuck Berry's bar – 6504 Delmar Blvd – 3.4 miles

Asador del Sur
(https://www.asadordelsur.com/)

South American fusion – 7322 Manchester Rd – 4 miles

Ballpark Village
(https://www.stlballparkvillage.com/)
Sports Bar & Entertainment – 601 Clark Ave – 4.2 miles

Taco Buddha
(https://www.tacobuddhastl.com/)
Mexican & Street Food – 7405 Pershing Ave – 4.3 miles

4 Hands Brewery
(https://www.4handsbrewery.com/)
Craft Brewery – 1220 S. 8th St – 4.6 miles

Molly's in Soulard
(https://www.mollysinstl.com/)
Irish Pub & American – 816 Geyer Ave – 5.3 miles

Cate Zone
(https://www.catezonestl.com/)
Chinese – 8148 Olive Blvd – 6.4 miles

Perennial Artisan Ale
(https://www.perennialbeer.com/)
Craft Brewery – 8125 Michigan Ave – 10.9 miles

Il Bel Lago
(https://www.ilbellago.com/)
Italian – 11631 Olive Blvd – 11.9 miles

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Chair of the Meeting: Ream Al-Hasani
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