KAPPA THERAPEUTICS CONFERENCE

March 29-31, Bethesda, MD

March 2023

Program Book

KappaCon2023 - Bethesda, MD

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Program Committee

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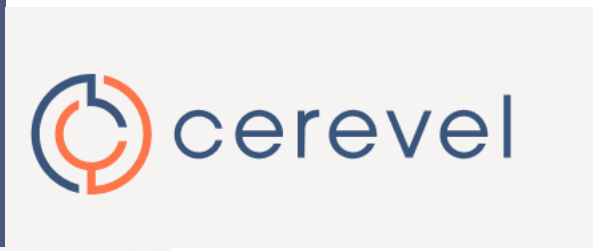
for additional information, go to: https://kappatherapeutics.org/

Sponsors

The Kappa Therapeutics Conference was sponsored by the National Institute on Mental Health. The program content is the sole responsibility of the speakers and does not necessarily reflect the views of NIH / NIMH.

We also wish to thank Neumora, Cerevel and Sosei Heptares for their generous support that helped pay for the Hyatt receptions, Conference Coffee breaks, Trainee Mixer, and the Shippenberg Young Investigator Awards.



General Information

The 7th Conference on the Therapeutic Potential of Kappa Opioids in Pain and Addiction.

Conference Venue

Natcher Conference Center (Building 45)
NIH Campus, Bethesda, MD
Conference Rooms E1 / E2

Internet Access in Meeting Rooms

WiFi: NIH Guest Wireless No password is required

Zoom Access

https://us06web.zoom.us/j/82480666205

Mute your microphone & use the chat feature to ask questions.

Badges

Every registered participant will receive a name badge that must be worn to gain access to scientific sessions and meals/coffee breaks onsite.

Registration Desk

The personnel at the registration desk will assist in all conference needs. The registration desk will be open:

Wednesday, March 29,	4 pm	-	6 pm (Bethesda Hyatt)
Thursday, March 30,	7 am	-	8 am (outside the lecture Room)
Friday, March 31,	7 am	-	8 am (outside the lecture Room)

Bethesda, MD Dining Guide

- https://www.bethesda.org/dining-guide

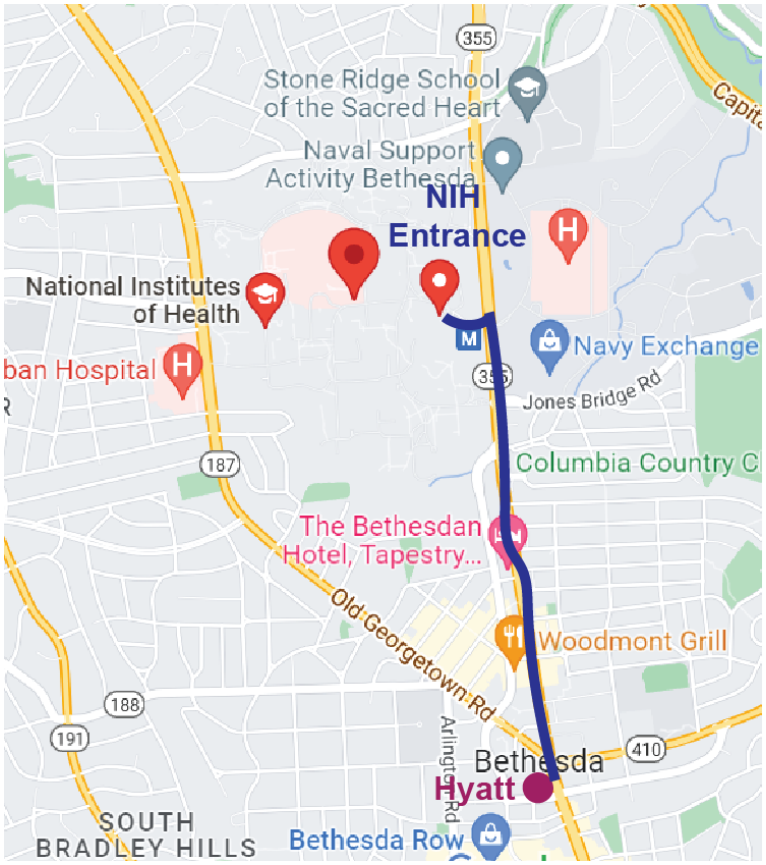
Getting from the Hyatt to the NIH Entrance (see map below)

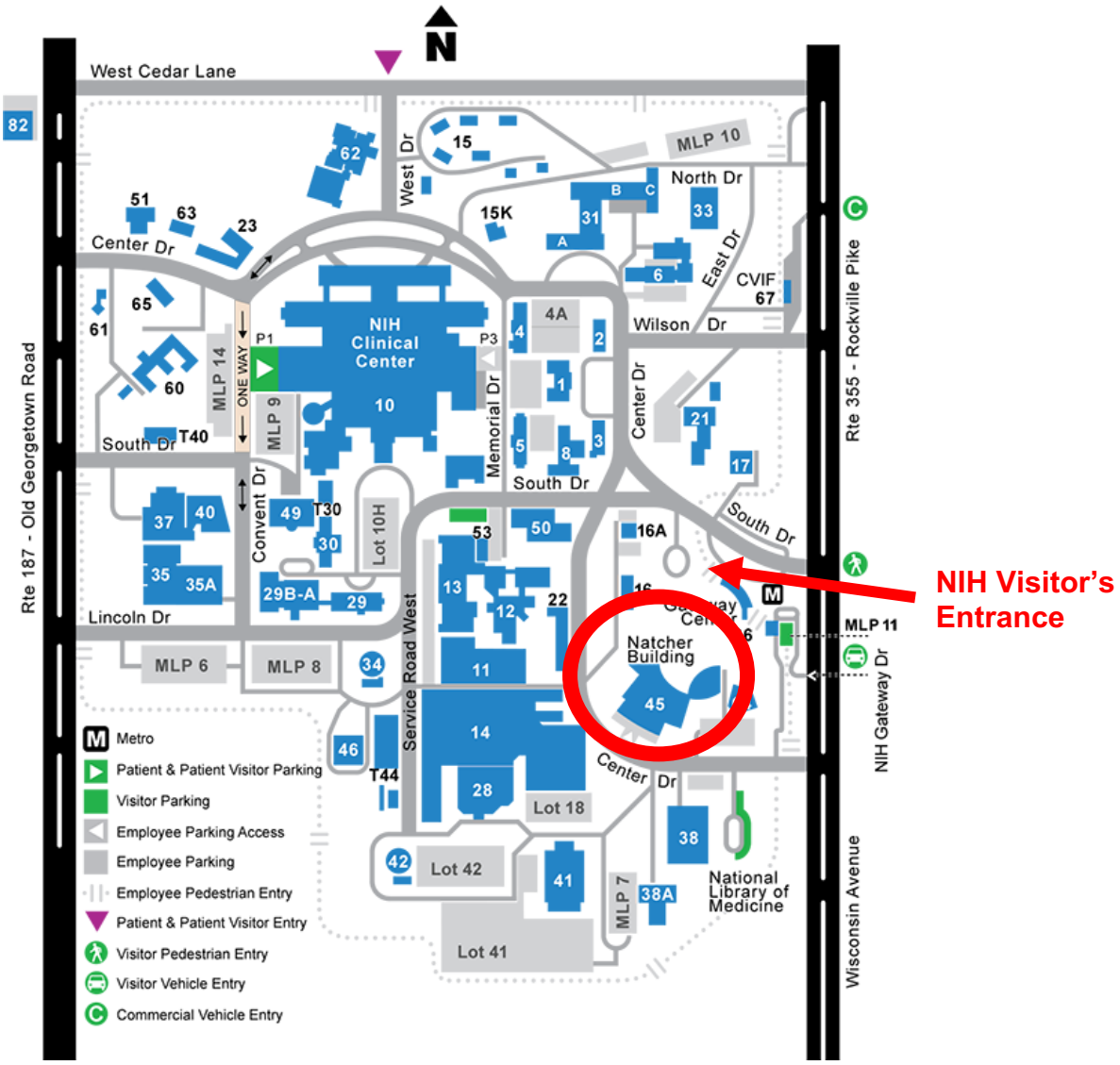
- Metro
  - Bethesda Metro Stop to Medical Center Metro Stop
- 15-20 minute walk north on Wisconsin Ave
- NIH Campus Map
  - https://ors.od.nih.gov/maps/pages/nih-visitor-map.aspx

At the NIH visitor's center please mention you are attending the "Kappa-opioid therapeutic potentials" conference in the Natcher Conference Center (Building 45).

- Visitors will need a driver's license or passport to enter campus
- If security requests a point of contact please tell them to notify:
  - Juan Enriquez-Traba: 301-792-0695
  - Hugo Tejeda: 443-538-9318

Map of Downtown Bethesda





Getting to hotels and NIH from Ronald Reagan International Airport (DCA) using the metro.

"Take the yellow metro line from Ronald Reagan Airport (DCA) to Gallery Place – Chinatown. Switch to the red metro line headed towards Shady Grove. Depart the red metro line at Bethesda for hotels and reception or Metro Center for NIH."

Getting to hotels and NIH from Dulles International Airport (IAD) using the metro.

"Take the silver metro line from Dulles Airport (IAD) to Metro Center. Switch to the red metro line headed towards Shady Grove. Depart the red metro line at Bethesda for hotels and reception or Metro Center for NIH."

https://www.nih.gov/about-nih/visitor-information/getting-nih
https://www.wmata.com/schedules/maps/

Kappa Therapeutics Conference Code of Professional Conduct

Professional Societies (ACNP, WCBR, & SfN) have become increasingly proactive about promoting professional behavior by all participants at our meetings. We are working together to make our meetings inclusive, safe, positive and a diverse experience for everyone.

All participants are expected to treat others with appropriate respect and civility at all times. We strive to sustain an environment in which a free exchange of ideas and opinions can occur. Discrimination and harassment in any form will not be tolerated. If you witness or experience an interaction that makes you uncomfortable during the sessions or associated social events, please intervene immediately or report the incident to a member of the Program Committee (whichever you feel is appropriate).

Incidents of unprofessional conduct will be documented as completely as possible. Documented incidents will be reviewed by the Program Committee, and if the majority concurs, the alleged offender will be informed, and an incident report will be forwarded to their supervisor (e.g. Dean or Department Chair) for appropriate action.

This is a proactive policy statement. We have not been informed of any previous incidents at Kappa Therapeutics Conferences, but by explicitly stating our expectations, we will hopefully reinforce everyone's positive experience.

Instructions for Presenters

Posters

Poster boards are 4 feet x 6 feet. Pushpins will be provided. Posters may be hung before the first coffee break on Thursday, March 30 (preferably during registration) and taken down at the close of Friday's session (before 6:30 pm).

Your poster number is listed in the Program

Oral presentations

We will have a computer with Microsoft Office software. All talks **must** be loaded onto the conference computer the morning of the talk (i.e. during breakfast or the morning coffee break) at the latest. Talks can be emailed or brought to our A/V specialists (to be announced) on a USB stick for uploading at the registration desk.

7th Conference on the "Therapeutic Potential of Kappa Opioids"

March 29-31, 2023
NIH Campus, Bethesda, MD
Natcher Conference Center (Building 45)
Conference Rooms E1 / E2 (next to main auditorium)

Wednesday, March 29th

4 - 6 PM Registration (Bethesda Hyatt Rooftop Lounge)

4 - 6 PM Opening Reception (Bethesda Hyatt Rooftop Lounge)

Thursday, March 30th

7 - 8 AM Coffee, Registration, and Poster Setup (atrium)

8:00 AM Welcome: Charles Chavkin, Hugo Tejeda, & George Koob

8:15 – 9:55
Oral Session 1: (Monitoring Dyn / KOR signaling; Chair: Niko Massaly)

8:15 AM
Chunyang Dong, Raajaram Gowrishankar, Yihan Jin, Jenny He, Huikun Wang, Nilüfer Sayar Atasoy, Rodolfo Floresgarcia, Karan Mahe, Achla Gupta, Jennifer Whistler, Ivone Gomes, Hugo Tejeda, Atasoy Deniz, Lakshmi Devi, Michael Bruchas, Matthew Banghart, & Lin Tian. **Shedding Light on Neuropeptide Dynamics: Genetically Encoded Biosensors to Illuminate Cellular and Systemic Actions of Opioids.** University of California, Davis, CA, USA.

8:35 AM
Michael Rappleye, Carlie Neiswanger, Selena S. Schattauer, Kandace Kimball, Adam Gordon-Fennell, Catalina A. Zamorano, Daniel C. Castro, Avi K. Matarasso, Carrie Stine, Sarah J. Wait, Justin Daho Lee, Jamison C. Siebart, Azra Suko, Netta Smith, Jeanot Muster, Kenneth A. Matreyek, Douglas M. Fowler, Garret D. Stuber, Michael R. Bruchas, Charles Chavkin, & Andre Berndt. **Enlighten the neurophysiology of opioid signaling with genetically encoded sensors.** University of Washington, Seattle WA, USA.

8:55 AM
Huikun Wang, Rodolfo Flores-Garcia, Hector Yarur-Castillo, Aaron Limoges, Hector Bravo-Rivera, Sanne M. Casello, Niharika Loomba, Juan Enriquez-Traba, Miguel Arenivar, Queenie Wang, Grace Or, Chunyang Dong, Lin Tian, & Hugo A. Tejeda. **Prefrontal cortical dynorphin peptidergic transmission constrains threat-driven behavioral and network states**. NIMH, Bethesda, MD, USA.

9:15 AM
Antony D. Abraham, Sanne M. Casello, Selena S. Schattauer, Brenden A. Wong, Grace O. Mizuno, Karan Mahe, Lin Tian, Benjamin B. Land & Charles Chavkin. **Detection of endogenous dynorphin release *in vivo* in the mouse prefrontal cortex with the fluorescent sensor kLight1.2a** Research Triangle Institute, Research Triangle Park, NC, USA.

9:35 AM
R. Gowrishankar, A. E. Elerding, S.E. Shirley, J. Van Tilburg, K. Abrera, D.J. Marcus, K. Motovilov, S.C. Piantadosi, A.A. Gordon-Fennell, C.Z. Zhou, C. Dong, L. Tian, G.D.& M.R. Bruchas **Dynorphin-Kappa Opioid receptor control of amygdalo-striatal circuits for reward-seeking** University of Washington, Seattle, WA, USA.

9:55 AM
Sineadh M. Conway, Chao-Cheng Kuo, Woodrow Gardiner, Rui-Ni Wu, Loc V. Thang, Graydon B. Gereau, John R. Cirrito, Carla M. Yuede, Jordan G. McCall, Ream Al-Hasani. **An electrochemical approach for rapid, sensitive, and selective detection of dynorphin.** Washington University School of Medicine, St. Louis, MO, USA.

10:15 AM Discussion

10:25 AM Coffee Break (with Posters in atrium)

10:45 AM – 12:00 PM
Oral Session 2: (Novel views on the Dyn / KOR system, Chair: Irwin Lucki)

10:45 AM
Marwa Mikati, Shenjian A, Sarah Rosen, Ayah Hamdan, Justin Woods, Robyn Klein, & Ream Al-Hasani **Investigating the role of the Kappa Opioid Receptor in the immunomodulatory effects of fentanyl exposure and withdrawal** Washington University School of Medicine in St. Louis, MO, USA.

11:05 AM
John Grothusen & Renyu Liu **Salvinorin A, a natural Kappa opioid receptor agonist, for acute stroke treatment** University of Pennsylvania, Philadelphia, PA, USA

11:25 AM
Abigail G. Schindler, Bryan Schuessler, Ari Asarch, Janet Lee, Britahny Baskin, Mayumi Yagi, & Mackenzie Patarino, **Mapping time-dependent dynorphin/kappa opioid receptor activation to Polytrauma Clinical Triad-related outcomes following repetitive blast trauma** University of Washington and VA Puget Sound, Seattle, WA, USA

11:45 AM
Travis D. Goode, Delara Chizari, Nina Sachdev, Antoine Besnard, Michael D. Kritzer, Devesh Pathak, Evan Z. Macosko, & Amar Sahay **Hippocampus-Dependent Calibration of Context-Evoked Feeding by a Prodynorphin-Expressing Lateral Septum to Lateral Hypothalamus Circuit** Mass. Gen. Hosp., Boston, MA, USA

12:05 AM Discussion

12:15 PM Nora Volkow, NIDA Director

12:30 PM -1:30 PM Buffet Lunch (rooms F1 / F2, G1 / G2)

1:30 PM – 2:40 PM
Oral Session 3: (Dyn / KOR and addiction, Chair: Bill Carlezon)

1:30 PM
Matthew B. Pomrenze, Daniel F. Cardozo Pinto, Peter A. Neumann, Pierre Llorach, Jason M. Tucciarone, Neir Eshel, Boris D. Heifets, Robert C. Malenka **Modulation of 5-HT release by**

dynorphin mediates social deficits during opioid withdrawal Stanford University School of Medicine, Stanford, CA, USA

1:50 PM
Ruby A. Holland, Kelly M. Smith, Michael C. Chiang, Jeffrey Okoro, Eileen K. Nguyen, Isabel H. Bleimeister, Samantha A. Sherman, Ava V. Zoltanski, Sarah E. Ross, **Kappa-opioid receptor-expressing neurons in the ventral tegmental area reverse opioid withdrawal symptoms** University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

2:10 PM
Brendan M. Walker, and Gengze Wei **Inducible *Oprk1* knockdown in the basolateral amygdala rescues dysphoria cue-induced escalation of alcohol self-administration.** University of South Florida, Tampa, FL, USA

2:30 PM Discussion

2:40 PM Coffee Break (with Posters in atrium)

3:00 PM
Oral Session 4: (Molecular mechanisms, Chair: Lee Yuan Liu-Chen)

3:00 PM
Jianming Han, Jingying Zhang, Antonina Nazarova, Sarah M. Bernhard, Brian E. Krumm, Lei Zhao, Jordy Homing Lam, Vipin Rangari, Susruta Majumdar, David E. Nichols, Vsevolod Katritch, Peng Yuan, Jonathan F. Fay, Tao Che, **Ligand and G Protein Selectivity in Kappa Opioid Receptor Revealed by Structural Pharmacology** Washington University in St. Louis, St. Louis, MO, USA

3:20 PM
Carlie Neiswanger, Selena S. Schattauer, Kandace Kimball, Micaela V. Ruiz, Justin Lee, Andre Berndt, Charles Chavkin **Kappa Receptor Partial Agonists Inactivate KOR through a JNK/ROS Mechanism** University of Washington, Seattle, WA, USA

3:40 PM
Anushka Dikshit, Sayantani Basak, Laetitia Chatelain, Steve Zhou, Ching-Wei Chang, Connie Zhang, John Pulliam. **Spatial mapping of pain-associated G-protein coupled receptors and biomarker localization in mouse brain using RNAscopeTM HiPlex v2 and RNA-protein Co-detection assay.** Advanced Cell Diagnostics, a Bio-Techne brand, Newark, CA, USA

4:00 PM
Vladana Vukojević, Sho Oasa, Aleksandar J. Krmpot, Stanko N. Nikolić, Lars Terenius **Ethanol effect on nalfurafine (NFF) binding to kappa-opioid receptor (KOP). Live cell study using Fluorescence Lifetime Imaging Microscopy (FLIM)** Karolinska Institutet, Stockholm, Sweden

4:20 PM Discussion

4:30 PM – 5:30 PM Poster session (atrium)

6:00 PM – 9:00 PM Student / Postdoc Mixer
(Rock Bottom Brewery, 7900 Norfolk Ave, Bethesda, MD)

Dinner (no host, maps to local restaurants provided)

Friday, March 31st

7 - 8 AM Coffee & Registration

8:00 – 9:55 AM
Oral Session 5: (Pain, Chair: Catherine Cahill)

8:00 AM
Olayinka Idowu, Hye Jean Yoon, Rossana Sandoval, Catherine Cahill, Marco Pignatelli, Jose Moron-Concepcion, & Nicolas Massaly **Dissecting the accumbal dynorphinergic outputs underlying affective pain** Washington University in St. Louis and University of California, Los Angeles, CA, USA

8:20 AM
Morón-Concepcion J.A, Lorente, J.D., Campos-Jurado, Y., Ibrahim, K.M., Massaly, N., & Hipólito, L. **Dissecting the role of a central amygdala to nucleus accumbens dynophinergic projection in pain-induced negative affect** Washington University in St. Louis, St. Louis, MO, USA

8:40 AM
Manish K. Madasu, Loc V. Thang, Sriya Chebrolu, Priyanka Chilukuri, Fiona Bree, Tayler D. Sheahan, Richard A. Houghten, Jay P. McLaughlin, Jordan G. McCall, & Ream Al-Hasani **Kappa opioid receptor potentiated cold hypersensitivity is transient receptor potential ankyrin 1-dependant in male mice but not in female mice** Washington University School of Medicine, St. Louis, MO, USA

9:00 AM
Ellen S. Staedtler, Michael J. Iadarola, Matthew R. Sapio, Diana King, Dragan Maric, André Ghetti, & Andrew J. Mannes **Opioid receptor expression in human dorsal root ganglion: a perspective on peripheral opioid analgesia and alternative targets.** National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

9:20 AM
Mariana Spetea, Aina-Leonor Olivé-Marti, Alexandra Peer, Edin Muratspahić, Helmut Schmidhammer, & Christian W. Gruber **Comparison of antinociceptive effects of structurally diverse, selective kappa-opioid receptor agonists in chronic inflammatory pain in mice** University of Innsbruck, Innsbruck, Austria

9:40 AM
Paramita Basu & Bradley K. Taylor. **Spinal kappa opioid receptor maintains latent postsurgical pain sensitization in a state of remission through tonic inhibition of a sex-specific, MEK→ ERK pronociceptive signaling pathway** University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

10:00 AM Discussion

10:10 AM Coffee Break (with posters in atrium)

Oral Session 6: (Drug development, Ream Al-Hasani Chair)

10:30 AM
Jane V. Aldrich, Tanvir Khaliq, Shainnel Eans, Ariana C. Brice-Tutt, & Jay P. McLaughlin. **Pharmacokinetics of macrocyclic tetrapeptide kappa opioid peptide antagonists and their potential for drug development.** University of Florida, Gainesville, FL, USA

10:50 AM
Simone M. Creed, Anna M. Gutridge, Jillian L. Kyzer, J. Brent Friesen, Guido F. Pauli, Cody J. Wenthur, Richard M. van Rijn, Andrew P. Riley. **Functionalization of Akuammicine – A Naturally Occurring Kappa Opioid Receptor Agonist** University of Illinois, Chicago, IL, USA

11:10 AM
Christian W. Gruber, Edin Muratspahić, Kristine Deibler, Jianming Han, Nataša Tomašević, Kirtikumar B. Jadhav, Aina-Leonor Olivé-Marti, Roland Hellinger, Johannes Koehbach, Jonathan F. Fay, Timothy Craven, Balazs R. Varga, Gaurav Bhardwaj, Kevin Appourchaux, Susruta Majumdar, Markus Muttenthaler, Parisa Hosseinzadeh, David J. Craik, Mariana Spetea, Tao Che, David Baker **Design of nature-inspired macrocyclic peptide ligands for the κ-opioid receptor** Medical University of Vienna, Vienna, Austria

11:30 AM
Landsberg N, Guillot A, Riera-Calabuig, Cervera-Sospedra M, Polache A, Morón JA, Melero A, & Hipólito L. **Development and characterization of a new nose-to-brain liposomal Pharmaceutical formulation targeting the kappa opioid receptor.** University of Valencia, Spain

11:50 Discussion

12:00 PM -1:30 PM Buffet Lunch (rooms F1 / F2, G1 / G2)

Oral Session 7: (Dyn / KOR and reward function, Chair: Elyssa Margolis)

1:30 PM
Zahra Z. Farahbakhsh, Keaton Song, Hannah E. Branthwaite, Kirsty R. Erickson, Snigdha Mukerjee, Suzanne O. Nolan, & Cody A. Siciliano **Systemic kappa opioid receptor antagonism accelerates reinforcement learning via augmentation of novelty processing in male mice** Vanderbilt University, Nashville, TN, USA

1:50 PM
Wallace, C.W., Holleran, K.M., Centanni, S.W. & Jones, S.R. **Kappa opioid receptors modulate real-time reward-related dopamine signaling in a sex-dependent manner** Wake Forest University School of Medicine, Winston-Salem, NC, USA

2:10 PM
Valentina Martinez Damonte, & Julie A. Kauer. **Kappa opioid control of a GABAergic stress-sensitive circuit involved in reinstatement.** Stanford University, Palo Alto, CA, USA

2:30 PM
Aida Mohammadkhani, Min Qiao, & Stephanie L. Borgland. **Effects of chronic morphine on LH orexin and dynorphin modulation of VTA dopamine neurons** University of Calgary, Calgary, Canada

2:50 PM
Hector Bravo-Rivera, Aaron Limoges, Christina T LaGamma, & Hugo Tejeda. **Dyn in vmPFC facilitates optimal approach/avoidance conflict resolution.** NIMH, Bethesda, MD, USA

3:10 PM Discussion

Coffee Break 3:20 PM (with posters in atrium)

3:30 PM – 4:30 PM HOT Topics (Chair: Hugo Tejeda)

3:30 AM
Hector E. Yarur, Sanne M. Casello, Valerie Tsai, Juan Enriquez-Traba, Rufina Kore, Huikun Wang, Miguel Arenivar, & Hugo A. Tejeda. **Dynorphin / kappa-opioid receptor regulation of excitation-inhibition balance toggles afferent control of prefrontal cortical circuits in a pathway-specific manner** National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA

3:40 PM
Bernard N. Johnson, Kiran Solingapuram Sai, Susan Nader, Songye Li, Yiyun Huang & Michael A. Nader. **Kappa Opioid Receptor Availability, Social Rank, and Cocaine Self-Administration in Socially Housed Female and Male Monkeys** Wake Forest University School of Medicine, Winston-Salem, NC, USA

3:50 PM
EJ Kuijer, SJ Bailey, DJ Heal, S Smith, S Wonnacott, & CP Bailey **Electrophysiological analysis of kappa opioid receptor activation in mouse paraventricular thalamus** University of Bath, Bath, UK

4:00 PM
Sofia Shirley, Hugo Tejeda, & Mario Penzo. **Characterization of Endogenous Opioid Systems within the Paraventricular Nucleus of the Thalamus** Johns Hopkins University and NIMH, Bethesda, MD, USA

4:10 – 5:00 PM Where do we go from here? A kappaphile community discourse on how to advance the field and increase translational impact (Moderator: Charles Chavkin)

Wayne Drevets (JNJ/Janssen) **An update on the ongoing Aticaprant Clinical Trial**

Brandi Quintanilla (NIMH) **KOR plasma levels are associated with sex and diagnosis of major depressive disorder but not response to ketamine.**

Bill Carlezon (McLean Hospital) **Kappa receptors and the damaging effects of stress in the periphery**

Lee-Yuan Liu-Chen (Temple) **Kappa Opioid Receptor and Endogenous Ligand Visualized Together in a New Mouse Line: (Pdyn-iCre x Ai6) x KOR-tdTomato**

Charles Chavkin (Univ Washington) **What makes norBNI a long-lasting antagonist?**

5:00 – 6:00 PM Poster Session (atrium)

6:30-8:30 PM Closing Reception (wine & cheese)
(Bethesda Hyatt Rooftop)

7:30 PM Presentation of the *2023 Young Investigator Awards* (Hugo Tejeda)

Saturday, April 1st. Checkout & Departure

Poster Presentations

(Atrium; 4ft high x 6ft wide, pins provided)

1. Andrea Bedini, Elisabetta Cuna, Federica Santino, Luca Gentilucci, Santi Spampinato. **RDM1127 is a novel, kappa opioid receptor (KOR)-selective ligand that displays a pharmacological profile suggestive of negative allosteric modulation.** University of Bologna, Bologna, Italy

2. Dominika J. Burek, Mykel A. Robble, Andrew G. Hall, Elisa M. Taylor-Yeremeeva, Eric J. Nestler, William A. Carlezon, Jr. **Cell-type-specific regulation of Fosb gene expression in nucleus accumbens moderates effects of chronic stress on sleep and diurnal rhythms** Harvard Medical School and McLean Hospital, Belmont, Massachusetts

3. Chongguang Chen, Kathryn Bland, Peng Huang, Conrad K. Ho, and Lee-Yuan Liu-Chen **Kappa Opioid Receptor and Endogenous Ligand Visualized Together in a New Mouse Line: (Pdyn-iCre x Ai6) x KOR-tdTomato** Temple University, Philadelphia, PA

4. Benjamin C. Coleman, Kevin M. Manz, Brad A. Grueter **Kappa opioid receptor modulation of nucleus accumbens microcircuits** Vanderbilt University, Nashville, TN

5. Monica Dawes, Katherine Holleran, Sara Jones **Methamphetamine and fentanyl co- self-administration modifies fentanyl taking and exacerbates mesolimbic dopamine deficits** Wake Forest School of Medicine, Winston-Salem, NC

6. Hastings, Lyndsay; Marchette, Renata; Frye, Emma; Carlson, Erika; Vendruscolo, Leandro; Koob, George F **Extended kappa-opioid receptor antagonism reduces opioid self-administration in dependent mice** National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism Intramural Research Programs, Baltimore, MD

7. Peng Huang, Conrad K. Ho, Kathryn Bland, and Lee-Yuan Liu-Chen **β-Arrestin 2 (arrb2) deletion has end-point dependent and sex-specific effects on the kappa opioid receptor (KOR)-mediated behaviors in mice** Temple University, Philadelphia, PA

8. Michael J. Iadarola, Ellen S. Staedtler, Matthew R. Sapio, Diana M. King, Dragan Maric, André Ghetti, Kenneth A. Jacobson, Andrew J. Mannes **Trans-species Transcriptomics of Receptors in Dorsal Root Ganglia to Identify Potential Analgesic Targets.** NIH Clinical Center, Bethesda, MD

9. Khairunisa Mohamad Ibrahim, Nicolas Massaly, Hye-Jean Yoon, Rossana Sandoval, Sidney Williams, Hannah Frye, William Post, Waylin Yu, Olayinka Idowu, Azra Zec, Sulan Pathiranage, Thomas L. Kash, Jose A. Morón **Dorsal Hippocampus to Nucleus Accumbens Projections Drive Reinforcement Via Activation of Accumbal Dynorphin Neurons** Washington University in St. Louis, St. Louis, MO

10. EJ Kuijer, SJ Bailey, DJ Heal, S Smith, S Wonnacott, CP Bailey **Nalfurafine is aversive in doses that produce equi-effective antinociception to U50,488** University of Bath, Bath, UK

11. EJ Kuijer, SJ Bailey, DJ Heal, S Smith, S Wonnacott, CP Bailey **A single injection of kappa opioid receptor agonist inhibits contextual heroin cues by counter-conditioning, not by enhancing extinction** University of Bath, Bath, UK

12. C. Lebonville, H. Haun, W. Griffin, J. Rinker, P. Mulholland, and H. Becker **Dissecting selective responses of dynorphin-expressing neurons during voluntary alcohol consumption in the central amygdala** Medical University of South Carolina & VAMC, Charleston, SC

13. Aaron Limoges, Huikun Wang, Rodolfo Flores-Garcia, Hugo Tejeda **Dynorphin signaling in the ventromedial prefrontal cortex regulates state transitions during acute threat exposure.** National Institute of Mental Health, NIH, Bethesda MD

14. Jacob K. Meariman, Juan Gao Donald E. Mercante, Daniel R. Kapusta, **Difelikefalin, a peripherally restricted KOR (kappa opioid receptor) agonist, produces diuresis through a central KOR pathway** Louisiana State University, New Orleans, LA

15. Galen Missig, Sokhom Pin, Srinivas Chakilam, Sridhar Duvvuri, Philip Iredale Georgette L. Suidan **CVL-354, a novel, brain penetrant and selective kappa opioid receptor antagonist** Cerevel Therapeutics, Cambridge, MA

16. Siavash Shahbazi Nia, Guangchen Ji, Samuel Obeng, Ashrafur Rahman, Christopher R. McCurdy, Lance McMahon, Thomas J. Abbruscato, Paul C. Trippier, Volker Neugebauer, Nadezhda A. German **Development of novel diketopiperazine and dipeptide analogs as selective KOR ligands as potential pain therapy** Texas Tech University Health Sciences Center, Amarillo, Texas

17. Aaron J. Norris, Aaron L. Cone, Kenny K. Wu, and Alexxai V. Kravitz **Kappa Opioid Receptor Activation Increases Energy Expenditure, Body Temperature, and Feeding Through Central Regulation of Brown Adipose Tissue** Washington University in St. Louis, St. Louis, MO

18. Breanne E. Pirino, Brody A. Carpenter, Pelagia G. Candelas, Annie Hawks, Genevieve R. Curtis, Andrew T. Gargiulo, Anushree N. Karkhanis, & Jessica R. Barson **Effects of the kappa-opioid receptor in the nucleus accumbens shell on ethanol drinking: Influence of sex, subregion targeted, and prior ethanol intake** Drexel University College of Medicine, Philadelphia, PA

19. Sho Oasa, Erdinc Sezgin, Yuelong Ma, David A. Horne, Mihajlo Radmilović, Tijana Jovanović-Talisman, Rémi Martin-Fardon, Vladana Vukojević, Lars Terenius **Live-cell time-resolved fluorescence microscopy/spectroscopy assess ethanol and kappa-opioid receptor (KOP) antagonists effect on KOP and lipid dynamics** Karolinska Institutet, Stockholm, Sweden

20. Georgette L. Suidan, Megan Neal, Gillian Driscoll, Philip Iredale, Sridhar Duvvuri, Srinivas Chakilam, Giri Gokulrangan, Scott Carrier, Elena Chartoff **A novel, short-acting kappa opioid receptor antagonist blocks the analgesic effects of U50,488 and attenuates symptoms of spontaneous oxycodone withdrawal in rats.** Cerevel Therapeutics and McLean Hospital, Harvard Medical School, Boston, MA

21. Gavin J. Vaughan, Laura Navarro Gomez, Chelsea M. McNamara, Jessica R. Barson, Anushree N. **Karkhanis Kappa opioid receptor control over monoaminergic transmission is differentially modulated by ethanol consumption along the NAc shell rostral-caudal axis** Binghamton University, Binghamton, NY

22. Alyssa West, Katherine Holleran, Sara Jones **Kappa opioid receptors reduce serotonin uptake and escitalopram efficiency in the substantia nigra pars reticulata** Wake Forest University School of Medicine, Winston-Salem, USA

23. Isabel H. Bleimeister, Sarah E. Ross. **Mapping kappa and mu opioid receptor expression in the amygdala.** University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

24. Sanne M. Casello, Huikun Wang, Hector E. Castillo, Niharika Loomba, Queenie Wang, Hugo A.

Tejeda. **Unique Morphological and Electrophysiological Characteristics Define Dynorphin Neurons in the Medial Prefrontal Cortex.** National Institute of Mental Health, NIH, Bethesda MD

Double-sided 4' x 6' corkboards and push-pins will be provided. Please put up your poster on Thursday before the first coffee break and take it down at the end of the poster session <6:30PM on Friday.

ABSTRACTS for ORAL PRESENTATIONS

Shedding Light on Neuropeptide Dynamics: Genetically Encoded Biosensors to Illuminate Cellular and Systemic Actions of Opioids.

Chunyang Dong[1, †], Raajaram Gowrishankar[3, †], Yihan Jin[1], Jenny He[2], Huikun Wang[5], Nilüfer Sayar Atasoy[4], Rodolfo Floresgarcia[5], Karan Mahe[6], Achla Gupta[7], Jennifer Whistler[8], Ivone Gomes[6], Hugo Tajeda[5], Atasoy Deniz[4], Lakshmi Devi[6], Michael Bruchas[3], Matthew Banghart[2], Lin Tian[1].

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Neuropeptides are the largest and most diverse class of signaling molecules in central and peripheral systems. Research on neuropeptide systems has been difficult because of the need for experimental tools that allow for the precise dissection of their complex and diverse signaling in a circuit-specific manner. The opioid system is the most functionally complex and clinically relevant family, modulating pain, reward, and aversive behaviors. A comprehensive understanding of the spatiotemporal release of endogenous opioids and their actions on each receptor is fundamental to revealing neural circuits mediating these behaviors. However, it has been challenging to achieve. Toward this end, we developed a class of genetically encoded fluorescent biosensors κLight, δLight, and μLight to rapidly probe ligand binding-induced conformational changes of kappa, delta, and mu receptors, respectively. With the biosensors, we determined the electrical parameters that trigger endogenous opioid release in acute brain slices. Furthermore, we characterized circuit-specific endogenous opioid release upon optogenetic stimulations and revealed fast, subregional differences in opioid release in response to fearful and rewarding conditions in vivo. These novel biosensors will enable a fundamental understanding of the cellular and systemic actions of opioid signaling, with potential implications for combatting opioid abuse and overdose.

Enlighten the neurophysiology of opioid signaling with genetically encoded sensors.

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Conflicts of interest: None

Genetically encoded sensors, combined with fluorescent microscopy, have revolutionized the real-time monitoring of dynamic physiological processes. However, the number of high-performing sensors that meet experimental needs, such as ligand sensitivity and specificity, is still limited. At the same time, there are hundreds of biological compounds for which no sensors exist. These include a range of endogenous opioids and second messengers involved in opioid signaling. The Berndt lab is at the center of sensor development by combining state-of-the-art approaches such as high-throughput screening, ensemble machine learning, and predictive protein engineering. These efforts aim to address the intrinsic complexity of protein sensors which effectively hinders the fast deployment of new tools or optimization of existing sensors. Utilizing these approaches, we engineered new sensors for reactive oxygen species (ROS), calcium, dopamine, and opioids with significantly increased dynamic range, kinetics, sensitivity, and red-shifted excitation/emission spectra. For example, ROS dynamics are inherently coupled to the activation of kappa and mu-opioid receptors. We used our genetically encoded ROS sensor oROS to detect increased ROS signaling in response to receptor activation in MOR-positive VTA neurons in acute slices of mouse brains upon morphine stimulation. Specifically, male mice between 5 and 7 weeks of age were given bilateral injections of AAV1-DIO-oROS-Gr-mCherry into the VTA. Brains were sliced 2-4 weeks after the viral injection into 2-4 200 um sections. Imaging was conducted using 2-photon excitation at 920nm. Slices were imaged for 5-10 minutes to acquire a baseline and then washed with either 10 uM morphine or 10 uM morphine + 10 uM naloxone for 30 minutes, followed by 250 uM H_2O_2 as a positive control. Monitoring these processes could reveal critical insights into the signaling of reward circuits and reveal the neurophysiology of opioid signaling with increasing precision. Our sensors have been optimized and validated in various relevant preparations, such as acute brain slices, in vivo 2-photon microscopy, and fiber photometry. Our future efforts aim to build custom-designed tools that meet the specific requirements of neuroscience research, such as high ligand selectivity, affinity, large response amplitudes, low toxicity, and fast kinetics.

Funding: R01GM139850, R21 DA051193

Prefrontal cortical dynorphin peptidergic transmission constrains threat-driven behavioral and network states

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Prefrontal cortical (PFC) circuits provide top-down control of threat reactivity. This includes ventromedial PFC (vmPFC) circuitry, which plays a role in suppressing fear-related behavioral states. Dynorphin (Dyn) has been implicated in mediating negative affect and mal-adaptive behaviors induced by severe threats and is expressed in limbic circuits, including the vmPFC. However, there is a critical knowledge gap in our understanding of how vmPFC Dyn-expressing neurons and Dyn transmission detect threats and regulate expression of defensive behaviors. Here, we utilized *in-vivo* fiber photometry and single cell imaging to demonstrate that vmPFC Dyn-expressing neurons are recruited during processing of threats and during retrieval of threat-associated memories. By expressing a novel, fluorescence-based kappa opioid receptor (KOR) sensor kLight in the vmPFC, we monitored the *in-vivo* dynamics of Dyn / KOR transmission during fear conditioning. Similar to the activity of Dyn neurons, the KOR sensor signal is increased by threats and the cues that predict them, while responses to on-going threats are modulated in a dynamic manner. Furthermore, we demonstrated that manipulating Dyn / KOR signaling by knocking-down Dyn expression via viral-mediated expression of prodynorphin (PDyn) small hairpin RNA (shRNA) modifies defensive behaviors in response to threats. PDyn-shRNA mice exhibit enhanced freezing to threat associated cues compared to control group during Pavlovian threat conditioning, without impacting expression of long-term memories or their extinction. Finally, we demonstrated that vmPFC Dyn-mediated signaling is required to promote a switch of vmPFC network activity to a fear-related state. In conclusion, this study demonstrates that Dyn cells are broadly activated by threats and release Dyn locally in the vmPFC to limit passive defensive behaviors. We further reveal a previously unknown role of vmPFC Dyn neurons and Dyn neuropeptidergic transmission in promoting active defensive behaviors in response to threats via state-driven changes in vmPFC networks.

Support.
This work was supported by the National Institute of Mental Health Intramural Research Program, a Brain and Behavior Research Foundation NARSAD Young Investigator Award to HAT, and a NIH Center for Compulsive Behaviors Fellowship to HEY and RFG.

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

Detection of endogenous dynorphin release *in vivo* in the mouse prefrontal cortex with the fluorescent sensor kLight1.2a

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Kappa opioid receptor (KOR) antagonists show promise for decreasing stress-induced cognitive dysfunction in preclinical studies with rodents and non-human primates. However, effective translation of these therapies into clinical populations requires a better understanding of the behavioral and neural systems regulated by the dynorphin/KOR system. Recent technological advancements allow for high-resolution measurements of dynorphin release and kappa opioid receptor activation in the brain. Here, we describe studies using a fluorescent sensor and bulk fluorescent imaging in awake-behaving mice to examine dynorphin release *in vivo* in the cortex. We found that morphine withdrawal disrupted working memory and elicited dynorphin release in the mouse prefrontal cortex (PFC) as detected by the fluorescent KOR ligand sensor kLight1.2a and confirmed with immunohistochemistry (IHC). kLight1.2a fluorescence in the PFC was also increased by systemically administering the KOR agonist U50,488. Optogenetic stimulation of prodynorphin^{Cre} PFC neurons was sufficient to activate PFC KORs and generate behavioral disruptions. In summary, we will describe how targeting cortical dynorphin/KOR activity could improve treatments for substance use disorders and other psychiatric disorders.

Support: P30-DA048736

The authors have no financial conflicts of interest

Title:
Dynorphin-Kappa Opioid receptor control of amygdalo-striatal circuits for reward-seeking

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Abstract:
Targeting the endogenous opioid dynorphin (dyn), via the kappa-opioid receptor (KOR) has shown promise in the treatment of multiple neuropsychiatric disorders including substance use disorder (SUD); yet how dyn-KOR signaling impacts natural reward-seeking, its locus of action, and the critical period for intervention are unknown. The dorsomedial striatum (DMS), where dyn is abundant, is critical for reward-seeking behavior. Furthermore, enhanced dyn-KOR signaling and aberrant striatal activity are associated with the transition of recreational to persistent drug-seeking. Yet, how dyn-KOR signaling refines natural reward-seeking behavior is unknown. Here, using *in vivo* fiber photometry of a novel dyn biosensor to assay DMS dyn dynamics when mice perform nosepokes (seeking) to obtain sucrose (reward), we observe that DMS dyn tone increases as animals learn reward-seeking behavior, and is released specifically during reward retrieval and consumption upon learning. In support, stimulating DMS dyn release using optogenetics specifically during reward delivery enhances seeking, without being inherently reinforcing. Conversely, we find that conditional deletion of dyn from the DMS decreases the learning, flexibility and extinction of reward-seeking behavior, without affecting the innate preference for sucrose. To determine the locus of action of dyn-KOR signaling, we measured the activity of BLA terminals in the DMS (BLA-DMS), as BLA terminals preferentially activate DMS dyn neurons, and a majority of BLA-DMS neurons express KOR. Using *in vivo* fiber photometry, we show that BLA-DMS terminal activity is increased during seeking, and inhibited during reward. To determine if this is purely a function of BLA soma activity, we used *in vivo* two-photon imaging of DMS-projecting BLA neurons during head-fixed reward-seeking behavior. We observe that distinct BLA-DMS ensembles are active during seeking or reward, not inhibited. Furthermore, using optogenetic manipulation of BLA-DMS terminals, we show that photoactivation during reward (when BLA-DMS terminals are inhibited) disrupts seeking and photoinhibition enhances seeking. This suggests that BLA-DMS inhibition during reward is essential for seeking, and occurs locally at BLA-DMS terminals. In accordance, we find that DMS dyn or BLA KOR deletion, or dyn-KOR antagonism via the short-acting, reversible KOR antagonist aticaprant, significantly diminishes BLA-DMS activity and the learning, and maintenance of reward-seeking. Altogether, we reveal that retrograde dyn transmission from the DMS onto KOR at BLA terminals during reward shapes seeking, thereby enabling dynamic changes in BLA-DMS activity and flexibility of reward-seeking behavior.

Funding:
R37DA033396 (MRB), P30DA048736 (NAPE Center)

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

An electrochemical approach for rapid, sensitive, and selective detection of dynorphin

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The endogenous opioid peptide systems are critical for analgesia, reward processing, and affect, but research on their release dynamics and function has been challenging. Here, we have developed microimmunoelectrodes (MIEs) for the electrochemical detection of opioid peptides using square-wave voltammetry. Briefly, a voltage is applied to the electrode to cause oxidation of the tyrosine residue on the opioid peptide of interest, which is detected as current. To provide selectivity to these voltammetric measurements, the carbon fiber surface of the MIE is coated with an antiserum selective to the opioid peptide of interest. To test the sensitivity of the MIEs, electrodes are immersed in solutions containing different concentrations of opioid peptides, and peak oxidative current is measured. We show that dynorphin antiserum-coated electrodes are sensitive to increasing concentrations of dynorphin in the attomolar range. To confirm selectivity, we also measured the oxidative current from exposure to tyrosine and other opioid peptides in solution. Our data show that dynorphin antiserum-coated MIEs are sensitive and selective for dynorphin with little to no oxidative current observed in met-enkephalin and tyrosine solutions. Additionally, we demonstrate the utility of these MIEs in an *in vitro* brain slice preparation using bath application of dynorphin as well as optogenetic activation of dynorphin release. Future work aims to use MIEs *in vivo* for real-time, rapid detection of endogenous opioid peptide release in awake, behaving animals.

Funding Sources: NIDA R00 DA038725 (RA), NIDA R21 DA048650 (RA), NINDS R01 NS123070 (RA), NIDA F32 DA053093 (SC), NINDS R01 NS117899 (JGM), NARSAD Young Investigator Grant from the Behavior Research Foundation, grant no. 28243 (RA)

The authors have no COIs

Investigating the role of the Kappa Opioid Receptor in the immunomodulatory effects of fentanyl exposure and withdrawal

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Opioid withdrawal is associated with distressing physiological symptoms that contribute to continued use. Importantly, epidemiological studies have shown that opioid exposure and withdrawal increase the susceptibility to opportunistic infections. Opioids directly impact the immune system likely through opioid receptors on immune cells. It has been shown that morphine inhibits the activity of innate immune cells such as macrophages and affects adaptive immunity by inhibiting antigen presentation and T cell proliferation and activity, suggesting that opioids perform complex immunomodulatory effects. Recent literature has demonstrated that immune cells regulate anxiety-like and depressive behaviors, which are also features of opioid withdrawal in humans. Interestingly, studies have shown that the expression of somatic withdrawal symptoms relies on an intact immune system in rodents yet whether this effect is mediated by endogenous opioid signaling remains unknown. Here, we investigate the immunomodulatory consequences of fentanyl treatment and withdrawal in mice. We demonstrate decreased cortical CD4+ and CD8+ T cells following chronic fentanyl treatment. We also show decreased expression of MHCII in myeloid cells in the blood both after chronic fentanyl treatment and during abstinence, suggesting potentially impaired antigen presentation capabilities. We hypothesized that the lasting immunomodulatory effects of fentanyl withdrawal may be mediated by the Kappa Opioid Receptor (KOR). Therefore, in KOR knockout animals, we showed rescue of both T cell and B cell changes that we observed in wildtype animals, suggesting that KOR is necessary for some of the alterations in the immune system. We also investigated the role of T cells during withdrawal behaviors and found that T cells may be necessary for the manifestation of the somatic signs of withdrawal in mice. In the future, we will investigate whether the KOR knockout animals show decreased somatic signs of withdrawal and whether withdrawal behaviors are mediated by KOR activity on immune cells. Our studies demonstrate complex interactions between the opioid and immune systems, offering insight into the utility of these interactions in designing better treatments.

Support: Brain, Immunology, and Glia inter-lab collaboration grant at Washington University School of Medicine, St. Louis, MO, USA (M.O.M). NIH-R00DA038725 National Institute on Drug Abuse (R.A)

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

Salvinorin A, a natural Kappa opioid receptor agonist, for acute stroke treatment

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Stroke is a sudden loss of blood flow to a region of the brain from a clot or bleeding and is a leading cause of death and disability. At the present time the only treatments are to remove the blockage with tPA or with surgical thrombectomy, both of which can only be performed in a hospital setting and are extremely time sensitive. Many compounds have been tested in attempts to protect the brain during the acute phase of stroke, but so far none have proved to be effective clinically. A major reason for this is that these potential neuroprotective agents are based on single mechanisms and stroke is a complex injury requiring a multiple mechanism approach for neuroprotection. Kappa opioid receptor (KOR) agonists have shown unique promise as potential pleiotropic neuroprotective agents. Salvinorin A (SA) is the most potent KOR agonist from a natural source and is structurally unrelated to any other known opioid agonists. SA easily crosses the blood-brain barrier and rapidly enters the brain within seconds. SA selectively dilates brain vessels and preserves cerebral vascular autoregulation in piglet models of hypoxia and ischemia,(1, 2) improves neurological outcome in rodent middle cerebral artery occlusion models,(3) and improves outcome in a rodent subarachnoid hemorrhage model.(4) The most clinically relevant results were recently published showing significant improvement in both short and long term outcomes following intranasal administration of SA to monkeys who were subjected to permanent occlusion of a branch of the middle cerebral artery using autologous blood clots.(5) (**Figure** 1) The brain protective effects of SA in multiple preclinical stroke models offer solid evidence to develop SA as a potential medication for stroke rescue purposes.

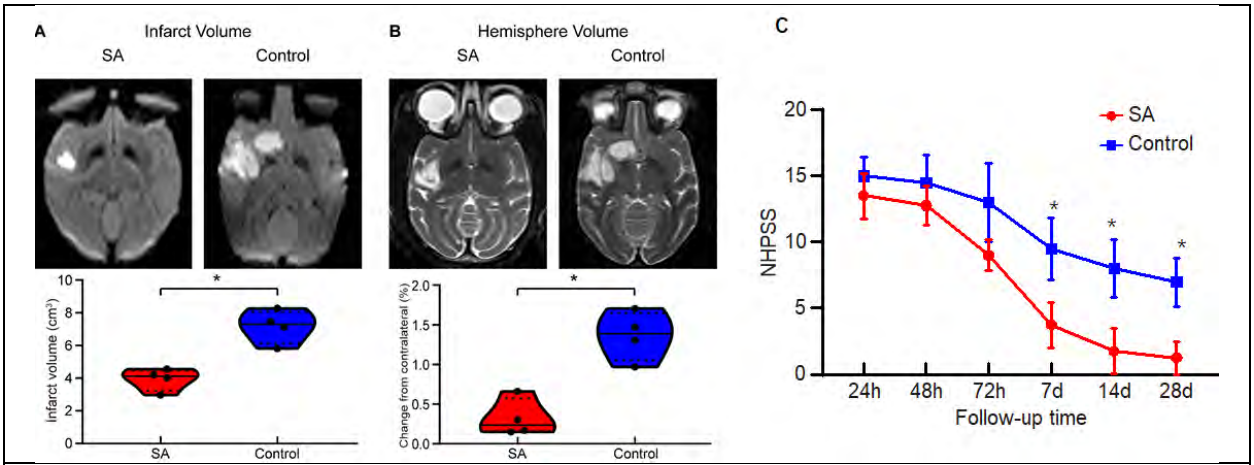

Figure 1. Left: MRI of monkeys 24h post stroke. The infarct size diminished with SA. Right: Neurological outcome improved almost back to normal with SA treatment. (5) NHPSS: Non-human Primate Stroke Scale

Funding: This project was funded by the University of Pennsylvania.

Conflict of interest statement: Dr. Renyu Liu is the founder of the Neurokappa Therapeutics.

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Mapping time-dependent dynorphin/kappa opioid receptor activation to Polytrauma Clinical Triad-related outcomes following repetitive blast trauma

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Adverse pathophysiological and behavioral outcomes related to mild traumatic brain injury (mTBI), posttraumatic stress disorder (PTSD), and chronic pain (e.g., Polytrauma Clinical Triad) are common following blast exposure and contribute to decreased quality of life, but underlying mechanisms and prophylactic/treatment options remain limited. The dynorphin/kappa opioid receptor (KOR) system helps regulate behavioral and inflammatory responses to stress and injury and we recently reported that KOR antagonism at the time of blast exposure is sufficient to prevent or reduce adverse immunological and behavioral outcomes in male mice at the one month time point. Critically, whether KOR antagonists can also reverse or decrease adverse outcomes when administered chronically following trauma exposure remains unknown and has important implications for subsequent clinical trial development. Likewise, while our recent report used relatively basic behavioral tests (place conditioning, elevated zero maze, acoustic startle), little is known regarding whether blast-induced KOR activation also mediates more complex behavioral dysfunction in relation to higher order cognitive processes and executive functioning. Here we report unpublished results using our well established pneumatic shock tube in male and female adult mice with KOR antagonism either 1) during blast exposure, 2) 24 hours after blast exposure, or 3) chronically six-months post-exposure at the time of behavioral testing. Preliminary results demonstrate a dissociation across time points and behavioral tests, where KOR antagonism during or acutely following blast is able to decrease acute anxiety and locomotor deficits but only KOR antagonism during or chronically after blast is sufficient to change behavioral outcomes related to aversion and executive function. Our findings demonstrate a previously unreported role for the dynorphin/KOR system as a mediator of behavioral dysfunction within specific time windows following repetitive blast exposure and highlight this system as a potential prophylactic/therapeutic treatment target.

Funding:
I01 RX003087/RX/RRD VA/United States
I01BX002311/U.S. Department of Veterans Affairs
IK2BX003258/U.S. Department of Veterans Affairs
T32DA007278/Office of Extramural Research, National Institutes of Health

Disclosure:
None.

Hippocampus-Dependent Calibration of Context-Evoked Feeding by a Prodynorphin-Expressing Lateral Septum to Lateral Hypothalamus Circuit

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Defining novel brain circuits of food-seeking may guide future therapies for eating disorders. Context-appropriate initiation or termination of motivated behaviors, including feeding, is thought to depend on the successful recognition, linkage, and relay of internal states (e.g., hunger vs. satiation), external cues (e.g., novelty vs. familiarity), and outcomes (e.g., food consumption) to and from the brain's hippocampus (HPC) to goal-relevant subcortical structures. Upstream to hypothalamic regions known to be central to consummatory behaviors, the long-range inhibitory (GABAergic) network of the lateral septum (LS) is well-positioned to integrate environmental and contextual cues from its dense HPC afferents for modulation of situation-specific feeding. However, the cells and efferent circuits by which the LS may calibrate context-dependent food-seeking remain unresolved. Leveraging single-cell transcriptomics (DROP-seq) and multiplex fluorescent *in situ* hybridization (FISH), we have discovered a previously uncharacterized subpopulation of inhibitory LS cells, defined in both male and female mice by the expression of the neuropeptide, prodynorphin (Pdyn). LS(Pdyn) neurons are genetically distinct and topographically restricted within the LS, having little to no overlap with other neuropeptidergic cell populations of the LS, such as neurotensin or proenkephalin. Monosynaptic neural tracing of LS(Pdyn) neurons revealed dense input from CA3/2 of the dorsal HPC, and robust innervation of the lateral hypothalamus (LH) by LS(Pdyn) cells (while avoiding other hypothalamic subregions). Accordingly, we tested whether LS(Pdyn) neurons critically regulate context-dependent motivation and food-seeking behaviors. Activity-related (c-fos) experiments showed that LS(Pdyn) neurons exhibit low levels of activation in fasted animals, while optogenetic interrogation of LS(Pdyn) cells was found to bidirectionally modulate spontaneous food consumption in a familiar place. Moreover, optogenetic inhibition of LS(Pdyn) cells (or of dorsal HPC terminals in the dorsal LS, where its Pdyn-expressing cells are located) eliminated the context-specificity of food-seeking in a model of context-driven nonhomeostatic overconsumption. Conversely, optogenetic excitation of LS(Pdyn) cells attenuated food consumption in fasted animals and induced real-time place avoidance. Furthermore, preliminary experiments indicate that conditional genetic deletion of Pdyn in LS(Pdyn) neurons augments spontaneous feeding of palatable food. In total, we have identified a novel subpopulation of LS neurons (Pdyn), whose connectivity may bridge contextual signals of the dorsal HPC to gate motivation- and feeding-regulating cells of the LH—thereby, LS(Pdyn) circuit dysfunction may contribute to disordered eating. An understanding of Pdyn signaling in these circuits may edify biological actions of kappa-opioid receptor (KOR) modulators in regulation of appetitive behaviors.

Funding: BBRF YI GRANT (TDG); R01MH111729 (AS); R01AG076612 (AS); SIMONS FOUNDATION GRANT (AS); MGH ECOR SCHOLARS PROGRAM GRANT (AS)

COI Disclosures: None

Modulation of 5-HT release by dynorphin mediates social deficits during opioid withdrawal

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Lethal overdoses from opioids have increased dramatically over the past decade, critically contributing to the "opioid crisis". Despite the economic and societal costs of opioid use disorder (OUD), existing treatments are often inadequate in helping users maintain abstinence over the long-term. During the abstinent weeks, months, and years following opioid use (i.e. protracted withdrawal or abstinence), the risk of relapse and fatal overdose is increased by an array of associated emotional symptoms, such as social avoidance and opioid cravings. We designed a procedure in which mice are administered escalating doses of morphine in conditioned place preference (CPP) chambers and tested for social behaviors and place preference after three weeks of abstinence. We observed robust sociability deficits during protracted withdrawal that correlated with long-term morphine place preference. The sociability deficits required activation of kappa opioid receptors (KORs) in the nucleus accumbens (NAc) medial shell. Blockade of transmitter release from dynorphin (*Pdyn*) expressing dorsal raphe neurons (DR^{Pdyn}), but not from NAc^{Pdyn} neurons, with tetanus toxin prevented these deficits in prosocial behaviors as well as morphine preference during withdrawal. Conversely, optogenetic activation of DR^{Pdyn} neurons or their inputs in NAc medial shell reproduced NAc KOR-dependent decreases in sociability. Deletion of KORs from serotonin (5-HT) neurons, but not NAc neurons or dopamine neurons, prevented both sociability deficits and morphine preference. Finally, fiber photometry recordings with the genetically encoded $GRAB_{5\text{-}HT}$ sensor revealed that during withdrawal, KORs reduce the 5-HT release into the NAc that normally occurs during social interactions. These results define a neuromodulatory mechanism that is engaged during protracted opioid withdrawal to induce maladaptive deficits in prosocial behaviors, which contribute to relapse in humans with OUD.

Support: K99DA056573-01 to M.B.P. and P50DA042012 to R.C.M.

Conflict of interest: R.C.M. is on the scientific advisory boards of MapLight Therapeutics, Bright Minds, MindMed, Cyclerion, AZTherapies, and Aelis Farma.

Kappa-opioid receptor-expressing neurons in the ventral tegmental area reverse opioid withdrawal symptoms

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Opioids, while effective in the treatment of pain, cause troubling side effects such as dependence and withdrawal. The kappa-opioid receptor (KOR) and its endogenous ligand dynorphin are recruited in aversive states and play an opposing role to the mu-opioid receptor in reward circuits emanating from the ventral tegmental area (VTA). However, our understanding of the functional role of KOR-expressing VTA (VTA^{KOR}) neurons in the aversive, negative affective, and dysautonomic dimensions of opioid withdrawal remain unclear. Here we show that VTA^{KOR} neurons are anatomically and neurochemically diverse, predominantly dopaminergic, and send dense projections throughout the CNS down to the sacral spinal cord. We find that chronic opioids diminish the neuronal activity of VTA^{KOR} neurons and their disinhibition by mu-opioid agonists. Chemogenetic activation of VTA^{KOR} neurons in opioid withdrawn mice reduces jumping, body weight loss, diarrhea, and micturition. Finally, we explore the specific role of VTA^{KOR} neurons projecting to the periaqueductal gray (PAG) in withdrawal symptoms. Taken together, these findings support a model in which chronic opioids recruit KOR/dynorphin circuits in the midbrain to diminish mesolimbic dopamine circuitry, leading to dependence and withdrawal. Furthermore, we highlight the importance of a central mechanism contributing to opioid withdrawal-induced gastrointestinal distress.

Support
Work supported by NINDS R01NS096705 and F31NS116981.

Conflict of Interest
The authors declare no conflict of interest.

Inducible *Oprk1* knockdown in the basolateral amygdala rescues dysphoria cue-induced escalation of alcohol self-administration.

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Alcohol use disorder (AUD) is a major problem worldwide and is highly comorbid with post-traumatic stress disorder (PTSD). Dysregulation of *Oprk1* gene expression (encoding the kappa opioid receptor; KOR) can contribute to AUD-related behaviors by increasing dysphoria, which has implications for trauma-related psychopathology, and can lead to excessive self-administration of alcohol to alleviate dysphoric states. To evaluate this 'self-medication' concept relevant to both AUD and AUD / PTSD comorbidity, we leveraged Cre-Lox technology with a goal of inducibly excising the *Oprk1* gene in the basolateral amygdala (BLA) to rescue maladaptive excessive alcohol consumption. Male and female transgenic floxed *Oprk1* mice were first phenotyped in our previously-developed dysphoria cue-induced alcohol escalation model. Initial results showed that female, compared to male, floxed *Oprk1* mice showed a shift to the left in the KOR agonist-associated cue-induced alcohol escalation dose-response curve. Next, using a KOR agonist dose that showed efficacy in both males and females, AAV2-Cre-GFP (or control) infusions in the BLA occurred to inducibly excise *Oprk1* after KOR agonist / cue conditioning occurred and alcohol consumption was assessed during limited access sessions. The results showed that three weeks following viral infusions in the BLA, male and female mice receiving the control viral construct showed significant dysphoria cue-induced escalation of alcohol self-administration, whereas the active AAV2-Cre-GFP viral construct ameliorated escalated alcohol consumption in male and female mice when exposed to dysphoric cues alone. These results have considerable implications for public health surrounding trauma / AUD comorbidity and support the continued focus on the dynorphin / KOR system as a therapeutic target for the treatment of certain neuropsychiatric conditions.

Support:
This research was supported by R01AA020394 from NIAAA (to BMW).

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

Ligand and G Protein Selectivity in Kappa Opioid Receptor Revealed by Structural Pharmacology

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The kappa opioid receptor (KOR) represents a highly desirable therapeutic target for treating not only pain but also addiction and affective disorders. The development of KOR analgesics, however, has been hindered by the hallucinogenic side effects mediated by KOR signaling. The initiation of KOR signaling requires the Gi/o family proteins. However, how hallucinogens exert their actions via KOR and how KOR determines the G protein subtype selectivity are not well understood. Here we determined the active-state structures of KOR in complex with G protein heterotrimers by cryo-electron microscopy (Cryo-EM). Comparisons of these structures reveal molecular determinants critical for KOR-G protein interactions as well as key elements governing Gi/o family subtype selectivity and KOR ligand selectivity. These results provide novel insights into the actions of opioids and G protein-coupling specificity at KOR and establish a foundation to explore the therapeutic potential of pathway-selective agonists of KOR.

Support:
The work is supported by NIH grants R35GM143061 (T.C.) and R01NS099341 (P.Y.).

Conflict of interest:
The authors declare no competing financial interests.

Kappa Receptor Partial Agonists Inactivate KOR through a JNK/ROS Mechanism

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Kappa opioid receptor (KOR) antagonists have potential therapeutic utility in the treatment of stress disorders, substance use disorders, and cognitive disruption resulting from excess dynorphin tone. Two forms of receptor-selective KOR antagonists have previously been distinguished: short-acting competitive antagonists (e.g. Aticaprant & NMRA-140) and long-acting noncompetitive antagonists (e.g. norBNI & JDTic) that activate cJun kinase (JNK) to irreversibly inactivate KOR by blocking Gαi•GDP dissociation. In this study, we are testing whether a 3rd form of KOR functional antagonist might have potential therapeutic utility. In contrast to the effects of repeated treatment with highly efficacious KOR agonists (e.g. U50,488), which result in readily-reversible β-arrestin-dependent receptor desensitization, we hypothesize that repeated exposure to a partial or G-biased agonist that activates JNK without activating β-arrestin might also cause long-lasting KOR inactivation. Nalfurafine and nalmefene are two medications of this type that are already approved for human use, and we are asking if they can cause JNK-dependent KOR inactivation with repeated treatment. We find that nalfurafine (1 mg/kg) and nalmefene (10 mg/kg) injected daily for 1 week, block U50-induced tail-flick analgesia for up to 21 days in male C57BL6 mice, like a single high dose of norBNI. MJ33, which inhibits pJNK-activated PRDX6 and blocks reactive oxygen species (ROS) generation, prevents this effect when given 1 hour prior to each daily dose of nalmefene or nalfurafine, suggesting that the long-acting antagonist action is ROS-dependent. Similar results can be seen with both compounds given in the same regimen in their ability to block KOR activation-induced prolactin increase but do not block the increase in total urine output. To confirm the generation of ROS via JNK activation, in vitro testing in HEK293 cells showed that both nalmefene and nalfurafine increase pJNK and stimulate ROS production by KOR activation. Ex vivo 2-photon imaging using a novel ROS sensor (oROS-Gr) expressed in the ventral tegmental area (VTA) shows ROS generation by acute treatment of these drugs as assessed by fluorescence increase. The same results were found when using oROS-Gr in the VTA with in vivo fiber photometry and the same dose of nalmefene and a 10-fold lower dose of nalfurafine as used in our daily dosing experiments. Short-acting opioid antagonist naloxone blocked these effects in both slice and fiber photometry to confirm ROS generation was due to KOR activation. These results suggest that chronic nalmefene or nalfurafine treatment may functionally antagonize KOR. Further, their well-tolerated actions in humans suggest that KOR inhibition by this mechanism may be a viable path for the treatment of substance use disorder.

Supported by R01 GM139850 and R21 DA051193.

The authors declare that they have no financial conflicts of interest.

Spatial mapping of pain-associated G-protein coupled receptors and biomarker localization in mouse brain using RNAscope™ HiPlex v2 and RNA-protein Co-detection assay.

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The mammalian brain is highly complex, comprised of distinct cell populations that contribute to the function of each distinct neuroanatomical area. Spatially mapped gene expression and cellular resolution is critical for better understanding the phenotypes of various central nervous system (CNS) disorders such as Alzheimer's disease, schizophrenia, autism and epilepsy, with poorly defined etiologies. The G-protein coupled receptors (GPCR) are an important component of pain modulation. They are widely distributed in the peripheral and CNS and are one of the most important therapeutic targets in pain medicine. However, detection of GPCRs can be challenging due to difficulties in obtaining suitable antigen accessibility and their low expression levels. In this study we demonstrate detection of GPCRs and Neuropeptide Y (NYP), known to be involved in pain perception and transmission in the brain, using the RNAscope HiPlex v2 and integrated codetection workflow assays.

The RNAscope HiPlex v2 assay can detect 48 targets in fixed and fresh frozen samples and up to 12 targets in formalin fixed paraffin embedded (FFPE) tissues. We have leveraged this technology to investigate spatial expression profile of 12 GPCR targets implicated in the pain modulation in the mouse brain. Spatial expression profile of 12 GPCR family receptors included opioid receptors, dopamine receptors and GABAergic receptors along with a neuronal marker. The copy numbers of opioid receptors in different regions of the brain were quantified using HALO Image analysis platform.

The spatial expression profiles of these markers showed high concordance to the Allen Brain Atlas resources for validating spatial gene expression (ABA Mouse Atlas ISH database). The copy number analysis indicated differential expression of *Oprm1*, *Oprd1* and *Oprk1* transcripts between cortex and nucleus accumbens, implying region-specific functions. Simultaneous RNA and protein co-detection indicated colocalization of NPY and opioid receptors in the astrocytes and microglia of the nucleus accumbens.

In summary, using the highly sensitive HiPlex v2 assay and the RNA-protein Codetection assay, we established a spatial gene expression map for visualizing GPCR family receptors within the normal mouse brain. The findings revealed cell type specific gene expression in different regions of the brain to establish functional significance of different cell types in the pain pathway. The study provides deeper insights into understanding the spatial crosstalk as well as functional significance of different cell populations within the various brain regions thereby leading to broader understanding of disease pathology.

COI: The authors are employees of Advanced Cell Diagnostics

Ethanol effect on nalfurafine (NFF) binding to kappa-opioid receptor (KOP). Live cell study using Fluorescence Lifetime Imaging Microscopy (FLIM)

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Nalfurafine (NFF), a selective kappa-opioid receptor (KOP) agonist approved for the treatment of chronic pruritus in Japan, was recently shown to reduce excessive alcohol drinking in mice *via* a KOP-mediated mechanism (Zhou Y, Kreek MJ. Brain Res. 2019 1724:146410). To examine whether acute exposure to ethanol (EtOH) affects NFF binding to KOP, we used live PC12 cells stably transformed to express KOP genetically fused at the C-terminal end with the enhanced Green Fluorescent Protein (KOP-eGFP) and massively parallel Fluorescence Correlation Spectroscopy integrated with Fluorescence Lifetime Imaging Microscopy (mpFCS/FLIM) to quantitatively characterize EtOH effects on NFF interactions with KOP. Dose-response analysis under normal physiology conditions showed that NFF concentration that gives half-maximal change in eGFP fluorescence lifetime is $EC_{50,NFF}^{Ctrl}$ (0.15 ± 0.05) nM and the factor of binding cooperativity α_{NFF}^{Ctrl} -1. Pretreatment of KOP-eGFP expressing PC12 cells for 30 min with 100 nM NFF followed by 1 hour incubation with 40 mM ethanol in the presence of NFF, did not alter NFF binding to KOP, yielding $EC_{50,NFF}^{EtOH}$ (0.17 ± 0.02) nM and α_{NFF}^{EtOH} -1. Interestingly, 40 mM EtOH treatment increased the EC_{50} values for all KOP antagonists tested: naltrexone (NTX), nor-BNI, LY-2444296 and BTRX335140. Of note, for JDTic, which was also tested, changes in eGFP lifetime were not observed in the absence of EtOH. We note also that the factor of binding cooperativity, α, is negative for NFF and all antagonists tested except for nor-BNI, for which it was positive but < 1. Our results suggest that the observed reduction in NFF-mediated alcohol intake is not due to EtOH-induced effects of NFF binding to KOP.

Support: Research reported in this publication was supported by the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number R01AA028549. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Support by the Swedish Research Council (2022-03402) is gratefully acknowledged.

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

Dissecting the accumbal dynorphinergic outputs underlying affective pain

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Pain represents a growing epidemic in the U.S., afflicting more than 30% of the population. Despite the availability of effective treatments for acute nociceptive pain conditions, negative affective states induced by persistent or chronic pain remain under- or untreated. The nucleus accumbens (NAc) is a critical component of the mesolimbic system and is involved in integrating both reinforcing and aversive properties of external stimuli. Activation of kappa opioid receptors (KORs) through exogenous or endogenous agonist, dynorphin, produces dysphoric effects and impairs active coping strategies in preclinical models of pain. Using chemogenetic approaches and microPET imaging, we recently demonstrated that 1) dynorphin-containing (Dyn+) neurons in the NAc are necessary to drive pain-induced negative affect and 2) inflammatory pain increases overall central KORs occupancy. However, the nature of the downstream structures through which Dyn+ neurons mediate behavioral adaptations to pain remain to be determined. Indeed, NAc Dyn+ neurons project to many structures involved in motivation including the Ventral Pallidum, the Ventral Tegmental Area (VTA) and the Lateral Hypothalamus (LH). Recent evidence has uncovered that Dyn+ neurons projecting from the Nac to the LH ($Dyn^{NAc \rightarrow LH}$) are necessary to drive stress-induced anhedonia. In this line of thoughts, using both males and females adult mice we employed a combination of ex vivo physiology, imaging and behavioral pharmacology and determined that pain 1) increases the excitability of $Dyn^{NAc \rightarrow LH}$ projections, 2) increases KOR function in the LH and 3) engages LH KOR signaling to decrease reward-driven motivation (n=8, $p<0.0001$). Our results participate in further understanding the allostatic changes in Dyn+ NAc synaptic efferents in pain and their impact on negative affective states.

Support: McDonnell Foundation Small Grant (NM), R21DA055047 (NM)

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

Dissecting the role of a central amygdala to nucleus accumbens dynophinergic projection in pain-induced negative affect

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Opioids, like other drugs of abuse, activate the structures within the mesolimbic reward pathway via three types of opioid receptors: mu, delta, and kappa. Activation of the mu opioid receptor (MOR) is largely responsible for encoding dopamine (DA)-dependent reward and reinforcement, for both opioids and other reinforcers. In contrast to MOR activation, kappa opioid receptor (KOR) activation in this pathway blocks the rewarding effects of MOR agonists, leading to diminished DA transmission in the nucleus accumbens (NAc). These alterations in DA transmission are likely to underlie the dysphoria and other negative side effects elicited by KOR activation. The KOR system has a role in motivation, and pain-induced adaptations in the KOR system could explain the associated negative affect, which is known to promote reductions in motivation observed during pain states. Our data reveal specific recruitment of the KOR system in discrete brain areas to drive persistent pain-induced negative affect. Similar findings have reported a role for KOR in the tonic aversive component of pain. In addition, we have reported that blockade of KOR or silencing the activity of neurons containing the endogenous KOR ligand, dynorphin (dyn) within the NAc shell with a prevent pain-induced decrased in motivation for goal directed behaviors. However, accumbal dyn/KOR containing neurons receive multiple projections/inputs from different brain areas. Therefore, dissection of specific inputs modulating NAc in response to pain are potential targets to prevent pain induced negative affect. The Central Amygdala (CeA) is a key brain area involved in emotion, aversion and other behaviors that are also reported in pain patients. In addition, the CeA is an area rich in dyn. Therefore, we decided to uncover a novel dynophinergic CeA-Nac input and dissect its role in pain-induced negative affect.

First, we show that opto-stimulation of this input induces real time place aversion and alters local field potentials in a sex- and time-dependent manner supporting the role for this novel dynophinergic input in pain-induced negative affect. In addition to the impact of pain on motivated behaviors, negative affective states promoted by pain may reflect the emergence of anxiety like behaviors. In order to assess whether pain-induced anxiety-like behaviors may be prevented by the local blockade of KOR, we used a well-validated model of anxiety-like behavior, the light/dark box (LDB). The LDB test is commonly used rodent test of unconditioned anxiety-like behavior that is based on an approach/avoidance conflict between the drive to explore novel areas and an aversion to brightly lit, open spaces. Using this model, we have data showing that inflammatory pain produces an avoidance of the time spent in the light compartment. In addition, our data indicate that this effect is prevented following the intracranial administration of the KOR antagonist, norBNI, in the NAc

In conclusion, we demonstrate that inflammatory pain promotes the recruitment of dynorphin projections from CeA to NAc in a sex- and time-dependent manner to drive the emergence of negative affective states. Our findings shed light on a novel pathway governing pain-induced negative affect and further extend the role of the CeA on pain associated behavior.

Funding:

NIH DA045463, DA042499 and DA041781 grants to JAM. MICINN PID2019-109823RB-100 and DGPNSD 2019I038 grants to LH. JD Lorente received funding from the University of Valencia and The Company of Biologists.

Kappa opioid receptor potentiated cold hypersensitivity is transient receptor potential ankyrin 1-dependant in male mice but not in female mice

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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. Here we investigate the potential mechanism through which KOR can drive cold hypersensitivity. Using Fluorescent in-situ hybridization (FISH), we found that KOR colocalized with transient receptor potential ankyrin 1 (TRPA1) transcripts in dorsal root ganglia (DRG) neurons of the wild-type (WT) mice. To investigate a potential mechanism through which KOR and TRPA1 interact, we use calcium (Ca^{2+}) imaging to show that simultaneous application of TRPA1 agonist, mustard oil (MO), and KOR agonist (U50) potentiates Ca^{2+} release compared to MO alone, suggesting that activation of KOR potentiates MO-induced Ca^{2+} release. To further understand the downstream signaling pathway underlying the interaction between the KOR and TRPA1, we used pertussis toxin (peTX) known to impair G-protein receptor signaling, and phospholipase-C (PLC) inhibitor (PLCi) (U73122) known to inhibit early KOR activated transmembrane signaling. PeTX application decreased KOR potentiated MO-induced Ca^{2+} release, suggesting the pathway is G-protein signaling dependent. Furthermore, using PLCi, we report no change in KOR-mediated MO-induced Ca^{2+} release, suggesting KOR-potentiated MO-induced Ca^{2+} release independent of the PLC-pathway.

To understand whether KOR-mediated cold hypersensitivity is TRPA1-dependant, we used $TRPA1^{-/-}$ mice. We found that KOR activation did not increase noxious cold hypersensitivity in $TRPA1^{-/-}$ male mice, suggesting KOR potentiated cold hypersensitivity is TRPA1-dependant. Moreover, we do not see potentiated Ca^{2+} release following activation of TRPA1 and KOR simultaneously, suggesting that TRPA1 likely mediates peripheral KOR-induced noxious cold hypersensitivity in males. Conversely, KOR activation in the $TRPA1^{-/-}$ female mice potentiated noxious cold hypersensitivity, and interestingly, we do not see potentiated Ca^{2+} release following activation of TRPA1 and KOR simultaneously, suggesting that in females peripheral KOR-induced noxious cold hypersensitivity is likely through TRPM8 channels. Our work suggests that in males, KOR drives cold hypersensitivity through TRPA1, is G-protein dependent and PLC-pathway independent. In females, however, KOR-induced hypersensitivity may be through both TRPA1 and TRPM8 but is also G-protein dependent and PLC-pathway independent.

Support: R01 NS123070 (NINDS) R.A

Conflict of interest statement: None

Opioid receptor expression in human dorsal root ganglion: a perspective on peripheral opioid analgesia and alternative targets.

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The present study examines the neuronal co-expression matrix for algesic ion channels and analgesic GPCRs in the human dorsal root ganglion. The idea of analgesics based on opioid receptors located in the peripheral nervous system has been examined for more than 50 years. Most of the foundational neurobiology was conducted in animal models. While this information formed the basis for drug development, little verification of the rodent data was obtained for the human. Using multiplex *in situ* hybridization in human DRGs provided by tissue donors, we characterized the molecular expression profiles regarding the canonical opioid-receptors, the nociception receptor, and transcripts for the algesic ion channels Nav1.8, Nav1.9, TRPV1, TRPA1, TRPM8, and P2RX3. We were able to identify three different nociceptive populations, which all expressed at least one opioid or opioid-like receptor and combined represent 75% of DRG neurons. The first population consists of small-diameter neurons that express the μ-opioid receptor, partially in combination with the δ-opioid receptor and/or the nociceptin-receptor. The second population expresses the same opioid receptors, but consists of medium/large diameter neurons. A third population of small-diameter neurons only expresses the δ-opioid receptor and therefore escapes attenuation of its input to the central nervous system by clinically used μ-receptor agonists. The κ-opioid receptor, which has been reported to be significantly expressed in mouse and rat DRG neurons, was largely absent from human DRG neurons, but ubiquitously expressed in satellite glial cells. Notably, only the μ-opioid receptor showed moderate neuronal expression levels, a fact that confirms its central role in analgesia lasting for thousands of years. Algesic ion channels were broadly expressed in DRG neurons. TRPV1, Nav1.8, Nav1.9 and, to a lesser extend P2RX3, are essential ion channels represented in all nociceptive neurons. They do show differential expression patterns within, and more importantly, across different nociceptive populations, which makes them attractive alternative targets for pain medications. For example, Nav1.9, a threshold channel responsible for increasing the excitability of DRG neurons, is preferentially expressed in the nociceptive population that does not express the μ-opioid receptor. Further detailed molecular phenotyping of DRG neurons is likely to reveal more possible targets for future drug development.

Conflict of interest:
None of the authors declares a conflict of interest.

Comparison of antinociceptive effects of structurally diverse, selective kappa-opioid receptor agonists in chronic inflammatory pain in mice

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Adequate treatment of chronic pain remains an unmet clinical need at the beginning of the third millennium. Chronic pain is still poorly managed because of the lack of efficacious therapies, high side effect burden or abuse liability of the present pharmacotherapies. Drugs targeting the mu-opioid receptor (MOR) are very effective analgesics. With continued use, opioid safety is dramatically reduced because of the side effects of physical dependence and addiction, promoting development of opioid use disorders and overdose deaths.
The kappa-opioid receptor (KOR) has a central role in modulating neurotransmission in central and peripheral neuronal circuits that subserve pain and other behavioral responses. Among alternative treatment strategies, the KOR is viewed as a promising avenue for pain therapeutics without the deleterious adverse effects of the MOR. In this study, we report and compare the antinociceptive effects of structurally distinct and selective KOR agonists (peptides and small molecules) in a mouse model of chronic inflammatory pain. The investigated KOR agonists include the prototypical ligand U50,488, the clinically used antipruritic drugs nalfurafine and difelikephaline, the two diphenethylamines HS665 and HS666 and the peptide-small molecule conjugate DNCP-β-NalA(1). Chronic inflammatory pain was induced in mice by injection of Complete Freund's Adjuvant (CFA) to the dorsal side of the right hindpaw. Nociceptive behavior was assessed 72 hours post-CFA by measuring paw withdrawal latencies to thermal stimulation using the Hargreaves test. All KOR agonists produced significant, dose-dependent antinociceptive effects after subcutaneous administration in mice with CFA-induced inflammatory hyperalgesia. However, characteristic differences were observed in the time-course of the antinociceptive effects of the investigated KOR agonists. We also show that the attenuation in pain-related behavior was antagonized by the selective KOR antagonist nor-binaltorphimine, demonstrating a KOR-dependent mechanism of action. In the rotarod test, no sedation or motor incoordination was caused by HS665, HS666 or DNCP-β-NalA(1), whereas locomotor dysfunction was produced by U50,488. These results indicate that selective activation of the KOR effectively attenuates thermal sensitivity in mice with chronic inflammatory pain with a favorable safety profile.

Support:
Supported by the Austrian Science Fund (FWF: I4697 and P32109).

Conflict of interest:
Patent applications EP22191168 and EP22193238 have been filed on peptide-small molecule conjugates targeting the kappa-opioid receptor. The authors declare no other financial interests.

Spinal kappa opioid receptor maintains latent postsurgical pain sensitization in a state of remission through tonic inhibition of a sex-specific, MEK→ ERK pronociceptive signaling pathway

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Acute surgical pain is frequently followed by chronic postsurgical pain (CPSP) (Kehlet et al., 2006). Prolonged opioid therapy is contraindicated, and other therapeutic approaches lack sufficient analgesic efficacy. We reported that tissue injury sensitizes nociceptive neurons in the dorsal horn of the spinal cord *via* activation of N-methyl-D-aspartate (NMDA) receptor and its downstream signaling elements adenylyl cyclase isoform 1 (AC1), cyclic adenosine monophosphate (cAMP), and the cAMP receptors protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac) (Corder et al., 2013; Basu et al, 2021). We reported sex differences in which NMDAR→AC1→cAMP→Epac signaling pathways in dorsal horn of spinal cord maintain latent sensitization (LS) in both male and female mice; however only males recruit a parallel PKA-dependent mechanism (Basu et al., 2021). The downstream signaling pathways beyond Epac include Ras-associated protein-1 (Rap1)→mitogen-activated protein kinase (MEK) →extracellular signal-regulated kinases (ERK). In our first studies in a mouse model of postsurgical pain, we used a behavioral pharmacology approach to test the hypothesis that Rap1, MEK and/or ERK drive acute pain as well as the latent surgical pain that is maintained in remission by kappa opioid receptors (KOR). After plantar incision in male and female C57BL/6 mice, we waited either 2 days for peak of hyperalgesia (acute phase) or 21 days for behavioral signs of hyperalgesia to resolve (latent phase). Next, we intrathecally administered pharmacological inhibitors of Rap1 (GGTI, 10µg / 5µL, i.t.,), MEK (PD98059, 10µg / 5µL, i.t.,) or ERK (SCH772984, 10µg / 5µL, i.t.,), or vehicle controls (10% dimethyl sulfoxide in corn oil). We measured mechanical and heat sensitivity at the plantar hindpaw at 60-, 120-, and 180-minutes post-injections. Adverse pharmacological effects (ataxia, sedation) were assessed with an accelerating rotarod test. Rap1, ERK, and MEK inhibitors did not change heat sensitivity or disrupt motor coordination in naïve mice of either sex. GGTI but neither PD98059 nor SCH772984 prevented acute phase of incision-induced mechanical and heat hypersensitivity in females. All the drugs prevented hypersensitivity in males. Similarly, GGTI prevented KOR antagonist LY2456302-evoked reinstatement of mechanical and heat hypersensitivity in both male and female mice, while PD98059 and SCH772984 did so only in male mice. We conclude that Rap1→MEK→ERK signaling components maintain the acute and postoperative LS masked by sustained KOR signaling in a sex-dependent manner. The male-specific recruitment of a MEK→ERK signaling pathways add to our understanding of sexual dimorphism in chronic pain. In our second study, we conditionally deleted KORs from the dorsal root ganglion (DRG). In $Pirt^{cre}$ x $Oprk1^{lox/lox}$ mice, Oprk1 mRNA expression was eliminated in DRG but did not change in the dorsal horn of ($Oprk1^{lox/lox}$ WT: 17.7±1.4% of Oprk1-mRNA positive cells; cKO: 16.4±1% of Oprk1-mRNA positive cells) mice. Conditional deletion did not change long-acting (LY2456302, 10µg, i.t.) or short-acting (BT-3761, 30mg/kg, i.p.) KOR antagonist induced reinstatement of mechanical and heat hypersensitivity. Together, the data confirm that endogenous KOR inhibits pronociceptive signaling in spinal cord but not DRG neurons suppress LS. Our findings support the development of Rap1, MEK, and ERK inhibitors towards a new pharmacotherapy for CPSP. Current studies will investigate the underlying mechanism of sexual differences in MEK→ERK signaling pathways of the LS. We will test whether testosterone is responsible for activating MEK→ERK signaling components of the latent postsurgical pain pathway.

Support: Work supported by NIH R01DA037621, R01NS045954 and R01NS062306 (to BKT).

Conflict of interest: Authors declare no potential conflict of interest.

Pharmacokinetics of macrocyclic tetrapeptide kappa opioid peptide antagonists and their potential for drug development

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Kappa opioid receptor (KOPr) antagonists have potential application in the treatment of substance abuse by preventing stress-induced reinstatement of drug seeking behavior. The macrocyclic tetrapeptide (MTP) KOPr antagonist [D-Trp]CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-D-Trp]) prevents stress-induced reinstatement of cocaine-seeking behavior, and we have shown that related MTPs also prevent stress-induced reinstatement of morphine-seeking behavior in conditioned place preference (CPP) assays after oral administration. In order to guide the optimization and identification of MTP analogs for potential development we evaluated the pharmacokinetics of three additional MTPs with KOPr antagonist activity compared to [D-Trp]CJ-15,208. *cyclo*[Pro-Sar-Phe-D-Phe] and another MTP with decreased lipophilicity exhibited increased free plasma fractions, but also lower oral bioavailability. The brain concentrations following oral administration varied substantially for the MTPs, with one of the MTP analogs exhibiting much higher brain concentrations than [D-Trp]CJ-15,208 and the other MTPs. The results for the pharmacokinetic evaluations will be compared to the KOPr antagonist activity observed for these MTPs. Based on these results we have identified a second generation MTP that is a promising lead compound for potential further development of treatments for substance abuse.

Support:
Work supported by R01 DA023924 (to JVA and JPM) and the Office of the Assistant Secretary of Defense for Health Affairs awards W81XWH-15-1-0452 (to JVA) and W81XWH- 15-1-0464 (to JP.M). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

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.

Functionalization of Akuammicine – A Naturally Occurring Kappa Opioid Receptor Agonist

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The akuamma plant (*Picralima nitida*) has been used for centuries in West African traditional medicine as a remedy for multiple ailments, including pain and fever. Akuammicine, an indole alkaloid isolated from the seeds of *P. nitida*, is a kappa opioid receptor (κOR) agonist with submicromolar affinity (K_i = 89 nM) and potency (EC_{50} = 240 nM). Notably, akuammicine is structurally distinct from traditional κOR agonists like U50,488 and salvinorin A, suggesting it may possess unique pharmacology or signalling properties. Leveraging akuammicine isolated from commercially available akuamma seeds, we used organic synthesis for late-stage functionalization of the molecule to study its structure-activity relationships (SAR) at the κOR. We have discovered analogues which exhibit improved affinity (K_i = 0.087-0.36 nM) and potency (EC_{50} = 1.4-4.6 nM) while maintaining selectivity for the κOR over the other opioid receptors and >45 other CNS receptors. Interestingly, in contrast to U50,488, in a condition place aversion assay, akuammicine and its derivatives do not appear aversive. Ongoing work is focused on further characterizing the behavioural effects of this novel class of potent κOR agonists. Future work will involve completing the synthetic SAR campaign of the akuammicine scaffold and evaluating the functional activity of akuammicine and current derivatives in animal models of pain and itch.

Support:
This research is supported by funding from the National Institute of General Medicine (R35GM147005).

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

Design of nature-inspired macrocyclic peptide ligands for the κ-opioid receptor

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The development of peptide therapeutics targeting G protein-coupled receptors (GPCRs) has been limited by poor pharmacokinetics (metabolic instability, short half-life, and rapid clearance) and lack of oral bioactivity (low gastrointestinal stability and membrane permeability). To tackle these challenges, we explore genomes/transcriptomes to identify evolutionary-related human neuropeptides in animals and plants and utilize pharmacology-guided screening to discover novel GPCR ligands.

Using state-of-the-art medicinal chemistry and computational tools such as molecular grafting, macrocyclization, cysteine stapling and *de novo* design, we have been developing novel ligands for the κ-opioid receptor that exhibit enhanced stability, improved receptor subtype selectivity and functional bias. As proof-of-concept, two lead candidates derived from different approaches, exhibited potent *in vivo* activity in mouse models of chronic inflammatory pain and chronic visceral hypersensitivity.

Specifically, the computational *de novo* platform overcomes extensive lead optimization encountered in ultra-large library docking and virtual small molecule screening campaigns and offers innovation for GPCR ligand discovery. This may aid the development of next-generation therapeutics for chronic pain.

DEVELOPMENT AND CHARACTERIZATION OF A NEW NOSE-TO-BRAIN LIPOSOMAL PHARMACEUTICAL FORMULATION TARGETING THE KAPPA OPIOID RECEPTOR

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The Dynorphin/Kappa Opioid Receptor (Dyn/KOR) system have shown promising data to treat several neuropsychiatric disorders including mood disorders, drug addiction and post-traumatic stress disorder. However, systemic administration of KOR ligands exhibits reduced efficacy and is prone to increase the severity of unwanted side effects. Furthermore, poor solubility of KOR ligands can also limit their bioavailability by oral route of administration, thus impacting their translational validity. Indeed, several compounds, including KOR ligands, to treat neuropsychiatric disorders have failed in clinical trials due to lack of efficacy and safety issues. Thus, the development of adequate pharmaceutical dosage forms is warranted to improve the accessibility of these drugs to their target to produce the desirable pharmacological effect without jeopardizing their safety. Nose-to-Brain delivery of drugs to treat central nervous system (CNS) disorders could be an option to reach the CNS avoiding collateral effects. Our objective here is to design, develop and assess the efficacy of an intranasal formulation that allows a gradual release and delivery to the CNS of norbinaltorphimine (norBNI), a KOR-antagonist. Liposomes were prepared by the Thin Film Method technique and characterized by Dynamic Light Scattering. These liposomes were included in an *in situ*-forming gel that was developed to guarantee the desired properties including temperature-controlled gel formation and muco-adhesion. Next, Franz Cells were used for the drug release assay. Finally, we designed and implemented *in vivo* studies to test the efficacy of these liposomes and to ensure norBNI distribution to the brain. Rats received 4 nasal administrations of 34µg/kg/kg/24h of norBNI. In order to assess the efficacy of the *in situ*-forming gel containing the liposomes loaded with norBNI, we used microdialysis since it's known that KOR agonists suppress dopamine release in the Nucleus accubens (NAc). At the end of this experiment, brains were collected and processed to measure the presence of norBNI in different brain areas. The liposomes obtained were homogeneous with a 2.62 Z-potential and 109 nm size and were adequately incorporated into the in situ-forming gel. Drug-release studies showed that liposomes achieve a controlled and gradual release of norBNI from the pharmaceutical dosage form, establishing equilibrium at 48 h with a maximum release of 60%. Finally, *in vivo* data demonstrated that norBNI administered intranasally in our formulation was able to successfully reach the brain to produce its pharmacological effects at 100-fold lower doses than those used in systemic administration. The microdialysis data revealed that the intranasal pretreatment for 4 days with 34 µg/kg, blocked the U50,488-induced decrease on dopamine release in the NAc. In conclusion, our intranasal pharmaceutical formulation containing norBNI-loaded liposomes reached key structures of the CNS providing the expected pharmacological effect.

Acknowledgements: This work was supported by Ministerio de Ciencia e Innovación PID2019-109823RB-100 (LH), PID2020-114530GA-100 (AM). Consellería de Innovación, Universidades, Ciencia y Sociedad Digital, Generalitat Valenciana CIAICO/2021/268 (LH)

The authors declare no conflicts of interest or competing financial interests.

Systemic kappa opioid receptor antagonism accelerates reinforcement learning via augmentation of novelty processing in male mice

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Selective inhibition of kappa opioid receptors (KORs) is highly anticipated as a pharmacotherapeutic intervention for substance use disorders and depression. The accepted explanation for KOR antagonist-induced amelioration of aberrant behaviors posits that KORs globally function as a negative valence system; antagonism thereby blunts the behavioral influence of negative internal states such as anhedonia and negative affect. While effects of systemic KOR manipulations have been widely reproduced, explicit evaluation of negative valence as an explanatory construct is lacking. Here, we tested a series of falsifiable hypotheses generated *a priori* based on the negative valence model by pairing reinforcement learning tasks with systemic pharmacological KOR blockade in male C57BL/6J mice. The negative valence model failed to predict multiple experimental outcomes: KOR blockade accelerated contingency learning during both positive and negative reinforcement without altering innate responses to appetitive or aversive stimuli. We next proposed novelty processing, which influences learning independent of valence, as an alternative explanatory construct. Hypotheses based on novelty processing predicted subsequent observations: KOR blockade increased exploration of a novel, but not habituated, environment and augmented the reinforcing efficacy of novel visual stimuli in a sensory reinforcement task. Together, these results revise and extend long-standing theories of KOR system function.

Support: This work was supported by NIH grants R00 DA04510 (NIDA) and U01 AA029971 (NIAAA), Alkermes Pathways Research Award, the Brain Research Foundation, Whitehall Foundation, and the Stanley Cohen Innovation Fund. Z.Z.F. is supported by an institutional training grant (T32 MH064913).

Conflict of Interest Statement: The authors have no conflicts to report.

Kappa opioid receptors modulate real-time reward-related dopamine signaling in a sex-dependent manner

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The dynorphin/κ-opioid receptor (KOR) system provides heterosynaptic regulation of dopamine axon terminals in the nucleus accumbens (NAc), which serves to dampen dopamine under stressful conditions. Using microdialysis and *ex vivo* voltammetry, our laboratory has consistently shown that KOR activation reduces basal dopamine levels and inhibits electrically-stimulated dopamine release. However, little is known about how KORs modulate real-time dopamine kinetics in awake and freely behaving animals. We sought to determine the impact of KOR activation on spontaneous and cued reward-related dopamine signaling events using fiber photometry with dLight. Male and female C57BL6/J mice underwent viral infusion (dLight1.2) and fiber optic cannula implantation in the NAc, and after four weeks of incubation, they were trained to lever press for sucrose pellets. After acquisition of the task, mice performed two sessions weekly to examine the effects of KOR ligands. Saline injections were counterbalanced with the KOR agonist U50,488H (U50; 1 or 5 mg/kg) or U50 (5 mg/kg) preceded by the KOR antagonist CERC-501 (3 or 10 mg/kg, 30 min). Behaviorally, we found that 5 mg/kg U50 increased the latency to the first lever press in males only. Further, females pressed the lever more and ate more sucrose than males, but there were no effects of KOR ligands on total lever presses or pellets consumed. Neurochemically, U50 (5 mg/kg) did not change the amplitude but reduced the width of spontaneous dopamine signals, potentially indicating augmented uptake rates, which are known to occur with KOR activation from prior voltammetry studies. Simple linear regression revealed a significant, dose-dependent reduction in the slope of the correlation between amplitude and width of the signal after U50, i.e., dopamine signals returned to baseline faster. This effect was stronger in males, but CERC dose-dependently reversed this effect in both sexes. For time-locked behavioral events, there were two phases of dopamine elevations, with a pre-lever press “ramping” of dopamine levels followed by a rapid “spike” that coincided with the lever press. U50 (5 mg/kg) dampened the dopamine “ramp” that preceded lever pressing, perhaps suggesting that KORs dampen reward anticipation and/or hamper dopamine responses to internal initiation of a planned motor sequence (deciding to press the lever). CERC-501 blocked suppression of the ramp by U50. Thus, KOR influences on real-time reward-related and spontaneous dopamine signaling are more uptake-focused than in *ex vivo* slice work, and dLight will allow us to assess the impact of KORs on dopamine signaling in a more holistic manner than previously possible.

Support: The National Institute on Alcohol Abuse and Alcoholism - U01 AA014091 & T32 AA007565

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

Kappa opioid control of a GABAergic stress-sensitive circuit involved in reinstatement.

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Plasticity at GABAergic synapses controlling dopamine neuron excitability in the ventral tegmental area (VTA) is a target of drugs of abuse and acute stress. We have previously shown that a single acute exposure to cold-water swim stress induces reinstatement of cocaine seeking via a long-lasting kappa opioid receptor (kOR) signaling. We also found that this stressor blocks nitric oxide-induced potentiation of inhibitory postsynaptic currents (LTP_{GABA}) onto VTA dopamine cells. Our work indicates that kOR activation upon stress contributes to reinstatement of drug-seeking by removing a normal brake (LTP_{GABA}) on VTA dopamine neuron activity.

Here we began to identify the kOR and dynorphin circuit elements responsible for the removal of this brake.

First, using selective activation with optogenetics, we identified that the nucleus accumbens (NAc) is one of the GABAergic inputs to VTA dopamine neurons that exhibit LTP_{GABA} and lose it upon acute stress. We are currently assessing if another important GABAergic afferent, the lateral hypothalamus, also exhibits these properties.

Since stress-induced block of LTP_{GABA} relies on activation of kOR, deleting kORs from the relevant cell type should prevent this block. Using a conditional knock-out approach, we found that kORs in dopamine cells are not required for stress-induced loss of LTP_{GABA}. Conversely, this suggests that the relevant kORs are instead located on presynaptic terminals. We found that GABAergic afferents from nucleus accumbens undergo LTP_{GABA}, and furthermore selectively deleting kORs from these terminals prevents stress-induced block of LTP_{GABA}.

It has become clear that VTA dopamine neurons are highly heterogeneous and participate in circuits that are differentially modulated. Using retrobeads, we started dissecting the participation of dopamine neurons with different projection targets in subcircuits sensitive to stress. We have found that NAc- projecting dopamine neurons receive synapses displaying LTP_{GABA} and have begun assessing whether PFC-projecting dopamine neurons share this feature.

To identify the dynorphin sources relevant for stress-induced block of LTP_{GABA}, we have begun to drive individual sets of dynorphin afferents to the VTA to induce dynorphin release in brain slices and test for the presence of LTP_{GABA}.

In summary, our work contributes to defining the circuit involved in stress-induced reinstatement and highlights the importance of inhibitory inputs for controlling dopamine neuron excitability in the context of addiction.

Funding sources:
-HHS | NIH | National Institute on Drug Abuse (NIDA) [011289] (JAK)
-Stanford Dean’s Award (VMD)

No conflicts of interest

Effects of chronic morphine on LH orexin and dynorphin modulation of VTA dopamine neurons

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The misuse of opioids such as heroin, morphine, and other prescription pain relievers has risen rapidly and continues to be a major health issue worldwide. Addiction is associated with neural circuit dysfunction characterized by serial changes in synaptic transmission in the ventral tegmental area (VTA), a region supporting the incentive value of drug related stimuli. Orexins (ox; also known as hypocretin) and dynorphin (dyn) are co-expressed lateral hypothalamic (LH) neuropeptides that project to VTA. These peptides have opposing effects on the firing activity of VTA dopamine (DA) neurons via orexin 1 (ox1) or kappa opioid (KOR) receptors, respectively. Therefore, it is unclear how the co-released peptides contribute to the activity of dopamine neurons in physiological and pathological states. This study sought to determine the effects of optically driven LHox/dyn release on VTA DA neuronal activity, and how chronic morphine alters the selective contributions of LHox/dyn to the firing of VTA dopamine neurons that project to either the basolateral amygdala (DA-BLA) or the lateral shell of the nucleus accumbens (DA-AcbSh). We expressed channel rhodopsin2 selectively in LHox/dyn neurons of orexin-cre mice and photostimulated terminals in the VTA while recording VTA neuronal firing using patch clamp electrophysiology. VTA dopamine neurons were labeled with biocytin during recordings and posthoc imaged for tyrosine hydroxylase expression. We first showed a diverse response of LHox/dyn photostimulation on DA neuronal firing rate. In the presence of synaptic transmission blockers, 30-Hz optical stimulation increased firing in 60% of DA-lAcbSh neurons and decreased firing in 72% of DA-BLA neurons. SB334687, an ox1 receptor inhibitor or NorBNI, a KOR inhibitor reversed the potentiation or inhibition of firing, respectively. In mice chronically exposed to morphine, 30-Hz stimulation increased firing in 62% of DA-BLA neurons. This raises two possible hypotheses; chronic opioid exposure upregulates dynorphin degrading enzymes, leading to dynorphin inactivation, or chronic opioid exposure desensitizes KORs, resulting in reduced effect of dynorphin on DA-BLA neuronal firing. Current ongoing experiments address these hypotheses. Taken together, LHox/dyn corelease may tune the output of the VTA by simultaneously inhibiting and activating different VTA projection neurons and this tuning shifts with morphine dependence.

Keywords: dynorphin, kappa opioid receptor, morphine, ventral tegmental area, dopamine

Support:

This work was supported by an NSERC Discovery grant (DG-343012/DAS-04060 to SLB) and a Canada Research Chair (950-232211). AM was supported by a Mathison Centre for Research and Education Postdoctoral Fellowship.

Conflict of interest:

The authors do not have any conflict of interests to disclose.

Dyn in vmPFC facilitates optimal approach/avoidance conflict resolution.

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Survival relies on the appropriate selection of active or passive defensive behaviors to minimize aversive and maximize appetitive outcomes, especially when motivations to approach rewards and avoid threats conflict. The prefrontal cortex (PFC) provides top-down control of threat reactivity. Specifically, the ventromedial PFC (vmPFC) plays a role in fear suppression. Furthermore, dynorphin (Dyn) is expressed in vmPFC, highlighting Dyn as a potential driver of the adaptive selection of defensive behaviors in conflict. We assessed the role of vmPFC-Dyn in conflict resolution using an approach/avoidance task where mice have the choice of attaining rewards during the presentation of a light cue vs. avoiding a tone-signaled footshock by mounting a platform opposite the reward port. We then generate conflict by co-presenting reward- and footshock-predictive cues. Early in training, control mice prioritize avoidance, and over days, they optimize their strategy by timing approach and avoidance behaviors to maximize reward and minimize footshocks. In contrast, vmPFC-Dyn knockdown mice (using PDyn-shRNA) failed to avoid footshocks early in training and also failed to optimize their approach/avoidance strategy over time. These observations corroborate the hypothesis that vmPFC-Dyn promotes active defensive behaviors over passive ones. We then used fiber photometry to record activity patterns of vmPFC-Dyn neurons during conflict resolution. vmPFC-Dyn neuronal activity increased in response to threats and during situations where mice suppressed avoidance to retrieve rewards. This increase was blunted when mice exhibited passive defensive behaviors. These findings suggest that vmPFC-Dyn regulates adaptive conflict resolution by suppressing passive and promoting active defensive behaviors, which is relevant to psychiatric disorders characterized by maladaptive threat responses.

This work was supported by the National Institute of Mental Health Intramural Research Program, the NIH Center for Compulsive Behavior Post-doctoral Fellowship (HB), NARSAD Young Investigator Award (HT), and NIH Medical Research Scholars Program (CTL).

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Dynorphin / kappa-opioid receptor regulation of excitation-inhibition balance toggles afferent control of prefrontal cortical circuits in a pathway-specific manner

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The medial prefrontal cortex (mPFC) controls emotional behaviors and cognition via connections with limbic excitatory afferents that engage various intra-mPFC inhibitory motifs The mPFC dynorphin (Dyn) / kappa-opioid receptor (KOR) system regulates affect and cognition and is implicated in neuropsychiatric disorders. However, it's unclear how neuropeptides in the mPFC, including the Dyn / KOR system, control excitatory and inhibitory circuit motifs integral in information processing. Here, we provide a circuit-based framework wherein selective KOR expression in mPFC afferents or within mPFC feedforward and feedback inhibitory circuits gates how distinct limbic afferent inputs control mPFC neurons. Dyn/KOR signaling directly decreases the ability of KOR-expressing afferent inputs to drive mPFC cell activity. Dyn/KOR signaling also suppresses afferent-driven recruitment of inhibitory sub-networks via several mechanisms, disinhibiting KOR-negative excitatory afferent control of mPFC ensembles. Thus, the Dyn/KOR system toggles which afferent input controls mPFC circuits, providing mechanistic insights into the role of neuropeptides in shaping mPFC function. These studies provide novel therapeutic approaches to target neuropsychiatric disorders characterized by dysregulation in prefrontal cortical Dyn / KOR systems and integration of long-range afferents with local inhibitory microcircuits.

Support:

Work supported by the NIMH Intramural Research Program (ZIA MH002970-04), a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation (HAT), and a NIH Center for Compulsive Behaviors Fellowship (HEY).

Conflict of interest:

The authors declare no conflict of interest.

Kappa Opioid Receptor Availability, Social Rank, and Cocaine Self-Administration in Socially Housed Female and Male Monkeys

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Cocaine use disorder (CUD) persists as a worldwide public health problem for which there is no FDA-approved pharmacotherapy. In the US, cocaine use is increasing, alongside cocaine overdose deaths, currently the second leading cause of overdose deaths associated with illicit drug use. While dopamine has been implicated in positive reinforcement, the kappa opioid receptor (KOR) system and its endogenous ligand, dynorphin, are implicated in the neurobiological regulation of aversive states, stress and substance abuse. Both chronic stress and cocaine exposure increase dynorphin/KOR system function and sensitivity to stress-induced relapse. A recent positron emission tomography (PET) study with a novel KOR agonist tracer [^{11}C]EKAP in humans reported an inverse correlation between social status and KOR availability in “anti-reward”/stress brain regions (Matuskey et al., 2019); females having higher availability than males. Differences in KOR availability have also been shown to correlate with cocaine choice in men with CUD (Martinez et al., 2019). We recently extended the investigation of the dynorphin/KOR system in socially housed cocaine-naïve male and female monkeys. PET imaging with [^{11}C]EKAP found significant interactions between sex and social rank in KOR availability/binding potential (BP), the overall lowest KOR availability across all brain regions was observed in dominant females and subordinate males (Johnson et al., 2023); the two most vulnerable phenotypes to cocaine reinforcement, based on earlier research. The present study extended the investigation of the dynorphin/KOR system using a homologous nonhuman primate model of CUD involving cocaine self-administration (SA), primate social behavior, and PET imaging (*N*=8/sex). Utilizing a longitudinal, within-subject design, in drug-naïve female and male monkeys, the first aim was to investigate the relationship between baseline KOR availability and vulnerability to cocaine reinforcement. In socially housed males, monkeys were first trained under a concurrent schedule of food reinforcement; then low doses of cocaine were substituted for one of the food options. Dominant males, those with the highest KOR availability, did not choose any cocaine dose over food, while 75% of the subordinates did. Using slightly different conditions, no differences in cocaine acquisition were observed between dominant and subordinate females. The second aim of this ongoing study is to investigate how those cocaine-naïve baseline KOR measures change following chronic cocaine SA and to assess the neural plasticity of KOR system following protracted time-off from cocaine. In these PET studies, the primary dependent variable was BP, which is an in vivo measure of the ratio of receptor density to receptor affinity. Following total cocaine intakes of ~100 mg/kg, monkeys were rescanned with [^{11}C]EKAP. In males, chronic cocaine SA produced robust increases in KOR BP, with [^{11}C]EKAP binding showing ~13.0% increase across all regions of interest (ROI), no rank differences observed. In males, baseline BP appeared to influence the magnitude of change in BP following chronic cocaine SA, correlating positively in 10/15 ROI. Preliminary data in females indicate chronic cocaine SA abolished the baseline differences between dominant and subordinate monkeys. In preliminary findings it appears that rate of recovery during time-off from cocaine varies across monkeys and, in some, has not returned to baseline after ~100 days. These findings implicate the KOR system in the neurobiology associated with the vulnerability and the long-term consequences of the reinforcing effects of cocaine and sex differences. The authors declare no conflict of interest. Supported by DA017763-15, DA053776-2

Electrophysiological analysis of kappa opioid receptor activation in mouse paraventricular thalamus

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Introduction

Kappa opioid receptors (KOPrs) encode the dysphoric component of stress and are postulated to be involved in drug addiction. The paraventricular thalamus (PVT) is thought to integrate reward-related and stress-related information. Evidence for the involvement of KOPrs in PVT in drug-seeking behaviour has emerged [1] although their precise role is unclear. Here, we investigate KOPr agonist-induced currents in PVT neurons: their distribution across the PVT, between ages and sexes, and co-expression with other GPCRs. KOPr effects on excitatory postsynaptic currents (EPSCs) were also determined.

Methods

Brain slices were prepared from 15 male and 20 female C57BL/6J mice either 4- or 8-weeks-old. Whole-cell voltage-clamp recordings were performed on single PVT neurons [2] perfused with: KOPr agonist spiradoline (30 μM) or dynorphin-A (1-13) (10 μM); KOPr antagonist norBNI (1 μM); mu opioid receptor (MOPr) agonist met-enkephalin (10 μM); MOPr antagonist CTAP [D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2] (1 μM). Recordings to measure excitatory post-synaptic potentials (EPSCs) were performed in the presence of $GABA_A$ antagonist picrotoxin (100 μM). Differences between sexes and ages were determined by unpaired t-tests. Anatomical locations were compared using one-way ANOVA. Co-expression of KOPr and MOPr was analysed using Chi-square test. EPSC effects were determined by paired t-test. Data shown as mean±SEM. Significance level: $p<.05$ (two-tailed).

Results

In 50 recordings designed to study postsynaptic KOPrs, 37 neurons responded to KOPr activation by inducing a GIRK-mediated current of 13.5±1.7pA. KOPr currents differed across the anterior-posterior axis with smaller currents in the medial PVT compared to more anterior regions (One-way ANOVA (location) $F(2,26)=3.7$, $p=0.039$) (n=4-13 per group). However, there were no differences between sexes ($p=0.67$) (n=13-16 per group), or ages ($p=0.63$) (n=13-16 per group). Interestingly, in 42 neurons tested for both KOPr and MOPr, 96% of KOPr+ neurons also expressed MOPr ($\chi2=45.1$, $df=3$, $p<0.0001$) and the KOPr response was smaller than the MOPr response (one-sample t-test against 100%: $t=9.2$, $df=27$, $p<0.0001$) (n=29).
In 11 recordings designed to study KOPrs on glutamatergic nerve terminals, KOPr activation with spiradoline did not affect EPSC frequency ($p=0.98$). However, MOPr activation did decrease EPSC frequency ($p<0.05$) (n=11).

Conclusion

Despite the opposing functions in emotional valence where KOPr mediates aversion whilst MOPr mediates reward, PVT neurons unexpectedly co-express both receptor subtypes. Ongoing work will seek to clarify the role of KOPr in the PVT, a promising structure in maladaptive circuits.

Funding and disclosure of conflict of interest

EJK's research project is 50/50 funded by University of Bath and DevelRx Ltd.
No conflict of interest to declare.

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Characterization of Endogenous Opioid Systems within the Paraventricular Nucleus of the Thalamus

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The paraventricular nucleus of the thalamus (PVT) is a stress-sensitive region of the brain that regulates emotional and motivational processes. Recent studies demonstrate that the PVT is a heterogeneous structure composed of molecularly distinct neuronal types that are distributed along its antero-posterior axis. These molecularly defined PVT cell types are embedded within divergent projections to various limbic regions, such as the nucleus accumbens (NAc), central nucleus of the amygdala (CeA), and medial prefrontal cortex (mPFC). Moreover, the heterogeneous composition of the PVT is functionally relevant because different subregions and cell types of the PVT have been linked to diverse behavioral outcomes. The kappa opioid receptor (KOR) is expressed throughout the PVT and has been shown to play important roles in mediating anxiety-like behavior. Additionally, the mu opioid receptor (MOR) is also expressed throughout the PVT and is established to promote euphoric emotional states. These two receptors are involved in modulating affective states, however it is currently unclear how endogenous opioids are integrated into heterogeneous PVT circuits. Here I used whole cell patch clamp electrophysiology to investigate the responsivity of PVT neurons to kappa (KOR) and mu opioid receptor (MOR) activation along the antero-posterior axis of the PVT. I found that KOR and MOR activation elicits inhibitory currents in PVT cells, which provides a mechanism by which KOR and MOR regulate PVT neural activity. Further, somatodendritic KOR and MOR inhibit PVT neurons projecting to the NAc and mPFC. On-going work is aimed at identifying if somatodendritic KOR and MOR differ based on molecular identity, electrophysiological properties, and/or projections of PVT cells. This work will provide a framework for our lab to further dissect the role of PVT opioid systems in mediating stress-related behaviors and affect. Understanding these PVT opioid systems will inform us of the mechanisms underlying emotional behavior and can provide insight into how these systems are disrupted in anxiety and psychological disorders.

Support:
This work was supported by the NIMH Intramural Research Program (ZIA MH002970-04), a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation

Conflict of interests:
The authors have no conflicts of interest to declare

ABSTRACTS for POSTER PRESENTATIONS

RDM1127 is a novel, kappa opioid receptor (KOR)-selective ligand that displays a pharmacological profile suggestive of negative allosteric modulation.

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Kappa opioid receptor (KOR)/dynorphin system contributes to modulate multiple physiological functions, including stress, mood, and emotional behavior, and has been implicated in the development or symptomatology of a variety of neuropsychiatric conditions as depressive disorders, anxiety, and substance abuse disorders. Therefore, there is an increasing interest in developing KOR antagonists as novel potential treatments for such diseases.
Aiming to identify a new KOR-selective antagonist, we screened a library of novel cyclopeptides derived from LOR17, a KOR-selective, G protein-biased agonist we recently developed (Bedini et al., 2020).
Via competition binding assays in HEK-293 cells expressing human mu opioid receptor (hMOR), delta opioid receptor (hDOR) or hKOR, RDM1127 was identified as a highly KOR-selective ligand (K_i = 0.55±0.04 nM). Interestingly, within the same competition binding assays, RDM1127 failed to fully displace [^{3}H]-Diprenorphine from KOR; thus, suggesting a possible allosteric binding mode.
In HEK-293/hKOR cells, and in U87-MG human astrocytoma cells (endogenously expressing KOR), RDM1127 did not inhibit forskolin-induced cAMP accumulation when administered as a single agent. Conversely, in the same cell models, 1-10 μM RDM1127 counteracted the inhibition of forskolin-mediated cAMP production induced by the KOR-selective agonist U50,488, significantly decreasing both U50,488 IC_{50} and E_{max}. Similarly, in HEK-293/hKOR cells and in U87-MG human astrocytoma cells, 10 μM RDM1127 dampened KOR-dependent activation of ERK1/2 phosphorylation, significantly decreasing both U50,488 EC_{50} and E_{max}.
Thus, displaying a profile suggestive of negative allosteric modulation.
To confirm these observations, RDM1127-mediated effects on KOR-dependent astrocytoma cell migration were investigated via wound-healing assay: we found that U50,488 determined a significant, concentration-dependent increase in U87-MG astrocytoma cell migration (EC_{50} = 0.21±0.04 μM); interestingly, when 10 μM RDM1127 was co-administered, U50,488 potency and efficacy in promoting cell migration were significantly decreased by 4-fold and 25%, respectively.
To complete RDM1127 characterization, experiments are being carried out in HEK-293/hKOR and U87-MG cells to compare its pharmacological profile to that displayed by KOR antagonists (e.g.: norBNI, 5'GNTI); results will be presented at the conference.
RDM1127 emerges as a new, KOR-selective ligand displaying the profile of a negative allosteric modulator. Due to these features, RDM1127 may represent a useful research tool to further dissect the role of KOR modulation in the frame of depressive, anxiety and substance abuse disorders, and might provide an interesting starting point to develop potential drug candidates for the treatment of these diseases.

Support:
Work supported by QSPainRelief project funded from the European Union's Horizon 2020 research and innovation programme (grant agreement No 848068); RFO2020, RFO2021, RFO2022 and FARB (FFBO125290) from University of Bologna; Fondazione "Umberto Veronesi" Grant (Tryptoids Project).

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

Title: Cell-type-specific regulation of *Fosb* gene expression in nucleus accumbens moderates effects of chronic stress on sleep and diurnal rhythms

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Background: The transcription factor CREB (Cyclic AMP Response Element Binding Protein) regulates expression of numerous genes, including those encoding dynorphin (which acts at kappa-opioid receptors [KORs]) and deltaFosB. Interestingly, these CREB targets are thought to have opposing effects on susceptibility and resilience to stress, suggesting a potential mechanism for individual differences. There is considerable evidence that *Fosb* in nucleus accumbens (NAc) medium spiny neurons (MSNs) regulates resilience to chronic social defeat stress (CSDS). Promoting *Fosb* function in dopamine type 1 receptor-expressing MSNs (D1-MSNs) confers resilience to CSDS as measured with social interaction and sucrose preference tests, whereas repressing *Fosb* in D1-MSNs confers susceptibility. D1- and D2-MSNs also differentially regulate sleep architecture, such that DREADD-mediated inhibition of D1-MSNs increases duration of REM sleep. However, the ways in which changes in *Fosb* function in MSNs within the NAc affect CSDS-induced alterations in sleep architecture remains unknown. We hypothesized that enhancing *Fosb* function in D1-MSNs, through use of a construct that promotes histone acetylation at the *Fosb* promoter with a targeted zinc finger protein (ZFP) fused to p65, would prevent CSDS-induced alterations in sleep architecture, reflecting a pro-resilient phenotype.

Methods: Male Drd1-Cre mice were injected with a viral vector (AAV(DJ)-*Fosb*-ZFP-p65) that induces the endogenous *Fosb* gene in the NAc to physiologically relevant levels, or the corresponding control vector in which the ZFP lacks a functional domain (AAV(DJ)-*Fosb*-ZFP-ASSB). Two weeks later, mice were implanted with wireless transmitters to measure EEG, EMG, body temperature, and locomotor activity, followed by a week for recovery. Continuous telemetry was collected for 2 days of baseline, 10 days of CSDS, and 2 days of post-stress recovery. Active wake, REM sleep, and slow wave sleep (SWS) vigilance states, as well as activity and body temperature, were quantified as percent of baseline across all 14 days of the experiment. Data were analyzed using two-way repeated measures ANOVA and Sidak's post-hoc multiple comparisons tests.

Results: Elevated *Fosb* function (*Fosb*-ZFP-p65) in NAc D1-MSNs prevented stress-induced alterations in REM and SWS duration, and body temperature, observed in control mice. Repressed *Fosb* function (*Fosb*-ZFP-G9a) prevented stress-induced alterations in awake duration, and exacerbated SWS and REM duration changes observed in control mice.

Conclusions: Enhancement of *Fosb* function in D1-MSNs within the NAc mitigated the effects of CSDS on REM and SWS, reflecting a potential pro-resilient effect. We have previously observed that similarly, kappa opioid receptor (KOR) antagonism attenuates the effects of stress on REM bouts. Repression of *Fosb* function in D1-MSNs within the NAcc augmented the effects of CSDS on wake duration. Future studies will characterize the ways in which manipulation of *Fosb* function in D2-MSNs affect these same endpoints. Furthermore, we will also explore whether these ZFP-mediated *FosB* alterations are associated with subsequent changes in KOR and dynorphin expression.

Support: R01MH063266, R01MH051399
Conflict of Interest: The authors declare no conflict of interest.

Kappa Opioid Receptor and Endogenous Ligand Visualized Together in a New Mouse Line: (Pdyn-iCre x Ai6) x KOR-tdTomato

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The distributions of both the kappa opioid receptor (KOR) and its endogenous ligands dynorphin peptides (Dyn) have been well established but have yet to be visualized within a single animal. We generated a knock-in mouse line capable of displaying Dyn and the KOR simultaneously. Pdyn-iCre mice were crossed with the reporter Ai6 mice (both from Jackson Labs). Subsequently pDyn-iCre x Ai6 mice were cross-bred with KOR-tdT (KtdT) mice which have tdTomato fused to the C-terminus of the *Oprk1* gene [*eNeuro* **7** (2020) doi: 10.1523/ENEURO. 0028-20.2020]. CLARITY-cleared brains of KOR-tdT mice and pDyn-iCre x Ai6 mice reveal comparable distributions to that of KOR autoradiography and prodynorphin mRNA, respectively. Brain sections of pDyn-iCre x Ai6 x KtdT mice were examined. Areas that have high KtdT and Pdyn processes/terminals include retrorubral field (A8 dopamine cells), PVT and BST. Brain regions that have high KtdT and Pdyn cell bodies include Acb, LH, PVH, DR, LPB, BST, CPu, IPAC (interstitial nucleus of post limb of ac), dorsal tuberomammillary nucleus and superior vestibular nucleus. Areas with high KtdT only include claustrum, PAG, VTA, substantia innominata, SN, VP, deep layer cortex, linear nucleus of raphe, raphe cap, medial vestibular nucleus magnocell. Areas with high Pdyn only include middle layers of the cerebral cortex, CeA, subbrachial nucleus, retroethmoid nucleus, supraoptic nucleus and subfornical organ. This is the first time the KOR/DYNs system can be visualized within the same animal brain and also among the first in the GPCR field to visualize a receptor and its endogenous ligands in the same brain.

Supported by NIH grants P30DA013429 and R01DA041359 (to LYLC).
The authors report no conflicts of interest

Kappa opioid receptor modulation of nucleus accumbens microcircuits

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The dynorphin/kappa opioid receptor (KOR) system within the nucleus accumbens (NAc) contributes to negative affective states following withdrawal from illicit drugs, pain, and stress. In the NAc, parvalbumin fast-spiking interneurons (PV-FSIs) receive similar excitatory input as neighboring medium spiny neurons (MSNs) and inhibit MSN activity via feedforward inhibition, critically regulating NAc circuit dynamics. KORs presynaptically inhibit glutamate release onto MSNs, but dynorphin/KOR regulation of excitatory drive onto PV-FSIs remains unknown. Thus, we characterize KORs at glutamatergic synapses onto NAc PV-FSIs and feedforward action at MSNs. Using PV-cre/Ai9(tdTom), Drd1 tdTom BAC transgenic mice and whole cell electrophysiology, we show that activation of KORs induces long-term depression (LTD) of excitatory drive onto PV-FSIs that is mediated by PKA and calcium/calcineurin dependent endocytosis of AMPA receptors. We also report that KORs preferentially modulate midline nuclei of the thalamus afferents and not prefrontal cortex afferents onto NAcc PV-FSIs with concomitant depression of feedforward inhibition. To determine the potential involvement of this mechanism in the response to stress, mice were subjected to restraint stress for one hour, a manipulation shown to increase NAc dynorphin. In stressed animals the KOR agonist no longer elicited LTD. Interestingly, the LTD is rescued by administration of the KOR antagonist, nor-BNI, prior to the stress. This work provides a novel mechanism and synaptic locus by which KORs modulate NAc circuit activity and provides evidence for the recruitment and/or dysregulation of this mechanism following stress.

Support:
This study was supported by DA040630 (BAG).

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

Methamphetamine and fentanyl co- self-administration modifies fentanyl taking and exacerbates mesolimbic dopamine deficits

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The opioid epidemic currently affecting the United States has entered a new wave of mortality, with combined use of opioid and psychomotor stimulants as a major contributing factor to the number of overdose deaths. Combined use of fentanyl and methamphetamine may be due to a number of factors, including greater rewarding effects, decreased negative side effects, and/or feelings that combined use is somehow 'safer' than use of fentanyl alone. For those reasons, there is a clear need to examine the differences in the behavioral and neurobiological alterations that occur following chronic use of fentanyl, methamphetamine, and these two substances in combination. Male and female Long Evans rats were trained to self-administer 2.5 μg/kg/inf fentanyl. Following acquisition, rats were randomly assigned to either fentanyl alone or fentanyl + methamphetamine and were tested on a short access, fixed ratio 1 schedule of reinforcement (3 hr sessions, max. 20 infusions) for ascending doses of fentanyl or combined fentanyl + methamphetamine (1.25, 2.5, 5.0 μg/kg/inf fentanyl ± 0.1 mg/kg/inf methamphetamine, 5 days per dose). A separate group of male and female Long Evans rats were trained to self-administer 0.1 mg/kg/inf methamphetamine, and following acquisition were tested on a short access, fixed ratio 1 schedule of reinforcement (3 hr sessions, max. 20 infusions, 0.1 mg/kg/inf) for 15 days to match total methamphetamine exposure in the combined fentanyl and methamphetamine group.

Both male and female rats self-administering methamphetamine had greater rates of responding than those self-administering fentanyl or combined fentanyl and methamphetamine. At the highest doses tested, male rats showed greater rates of self-administration of combined fentanyl and methamphetamine than fentanyl alone, while in females, the rate of self-administration of combined fentanyl and methamphetamine was less than fentanyl alone. Additionally, in male rats, the latency to initiate responding was shorter in animals self-administering combined fentanyl and methamphetamine than those self-administering fentanyl alone, while the opposite was observed in female rats.

Following self-administration, coronal brain slices containing the nucleus accumbens were prepared for *ex vivo* fast scan cyclic voltammetry. Combined fentanyl and methamphetamine rats had decreased evoked dopamine release and uptake rate (Vmax) compared to saline and fentanyl alone animals. Further, evoked dopamine release was decreased across stimulation amplitudes and frequencies in combined fentanyl and methamphetamine animals, compared to fentanyl alone animals. Bath application of kappa opioid receptor agonist U50-488H revealed differences in kappa opioid receptor functionality between combination, fentanyl alone, and methamphetamine groups. Distinct sex differences were also observed, with females displaying enhanced kappa functionality compared to males.

Together, these results highlight the complexities of combined opioid and stimulant use, and suggest that there may be unique sex specific neuroadaptive processes specific to combined fentanyl and methamphetamine which are not sufficiently explained by the individual changes observed following use of fentanyl or methamphetamine alone.

Funding and Disclosure:
This research is financially supported by the National Institutes of Health grants F31 DA057815, T32 DA041349, U01 AA014091, and R01 DA048490. The authors declare no conflict of interest.

Extended κ-opioid receptor antagonism reduces opioid self-administration in dependent mice

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The dynorphin/κ-opioid receptor (κOR) system is a brain stress system that generally promotes dysphoria-, anxiety- and depression-like behavior. Dynorphin is upregulated in limbic brain regions during opioid dependence and is involved in opioid withdrawal-induced hyperalgesia, which is defined as an increase in pain sensitivity during opioid withdrawal. Hyperalgesia is hypothesized to contribute to drug taking and seeking through negative reinforcement. However, the direct role of the dynorphin-κOR system in opioid-related behaviors is not well understood. Here, we aimed to further our understanding of this system by evaluating the effect of two κOR antagonists on fentanyl vapor self-administration (FVSA) in mice. We hypothesized that κOR antagonism would decrease FVSA. To test this hypothesis, we trained male and female C57BL/6J mice to self-administer vaporized fentanyl and split them between short-access (ShA; 1 h sessions) and long-access (LgA; 6 h sessions) groups. Mice tested in LgA sessions escalated their fentanyl intake, whereas those tested in ShA sessions did not. We tested the short-acting κOR antagonist aticaprant (0, 0.3, 1, 3, 10, 30 mg/kg, oral), which failed to reduce fentanyl self-administration in both the LgA and ShA groups. Following three weeks of abstinence, a single treatment with the long-acting κOR antagonist norBNI (10 mg/kg, intraperitoneal) significantly reduced the re-escalation of fentanyl in mice in LgA but not in ShA conditions. These data suggest that extended blockade of κORs is necessary to decrease opioid self-administration in dependent mice or that κORs are involved in the transition to opioid dependence rather than on established escalated drug intake. Further research will determine the efficacy of chronic treatment with short acting κOR antagonists in reducing opioid self-administration and whether long acting κORs antagonists reduce previously escalated drug intake.

This work was supported by the NIDA IRP. The authors declare no conflicts of interest.

β-Arrestin 2 (arrb2) deletion has end-point dependent and sex-specific effects on the kappa opioid receptor (KOR)-mediated behaviors in mice

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We previously demonstrated by utilizing a mutant mouse line K4A that agonist-promoted KOR phosphorylation plays important roles in the selective KOR agonist U50,488H (U50)-induced anti-pruritic tolerance and conditioned place aversion (CPA) in a sex-dependent manner, without affecting acute U50-induced anti-pruritic and hypo-locomotor effects. K4A has all the four KOR phosphorylation sites mutated to alanine, thereby becoming KOR phosphorylation-deficient. Phosphorylation deficiency in K4A mice would lead to little recruitment of arrb2, resulting in little arrb2-mediated KOR regulation, downstream signaling and behaviors. To compare with K4A mice, here we examined effects of arrb2 deletion on KOR-mediated behaviors in arrb2 knockout [arrb2(-/-)] mice. We found that arrb2 deletion enhanced anti-pruritic effects produced by acute U50 in males, but not in females. In contrast, arrb2 deletion did not affect U50-induced CPA in either male or female mice and had no effects on anti-pruritic tolerance of U50 in male mice. Thus, these results collected in arrb2(-/-) mice to date showed some differences from our previous findings in K4A mice. The differences may be due to that arrb2(-/-) affects many GPCRs-mediated signaling, whereas K4A impacts only KOR-mediated signaling. We are continuing to examine the effects of arrb2 deletion on U50 tolerance in female mice and will also investigate the role of sex hormones in the sex differences observed. To our knowledge, this is the first to demonstrate sex differences in the role of arrb2 in KOR-mediated behaviors and among the first in the GPCR field.

Support: Work supported by P30DA013429 and R01DA041359 (to LYLC)

Conflict of interest: none

Trans-species Transcriptomics of Receptors in Dorsal Root Ganglia to Identify Potential Analgesic Targets.

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Two main sites for peripherally acting analgesics are nociceptive dorsal root ganglion neurons and their processes. Highly effective analgesia can be obtained by lesions or local anesthetic application delivered anywhere along the trajectory of these neurons, making them attractive targets for more precise pharmacological interventions. The *mu* opioid receptor exemplifies this analgesic locale: it is expressed by nociceptive DRG neurons and administration of mu agonists inhibits transmitter release from presynaptic terminals in dorsal spinal cord. We hypothesize that for receptor and ion channel genes, similar expression in DRG *across* species indicates a fundamental, preserved function related to neuronal processes. In contrast, variable expression across species may suggest a non-neuronal function with expression occurring in other DRG cells, likely satellite glial cells (SGCs). This type of differentiation may provide a preliminary evaluation for molecules that could be exploited for potential analgesic activity. In the present study, we examine the kappa opioid and adenosine A3 receptors, which are two potential candidates for peripherally acting analgesic agents. We evaluated several animal and human DRGs for (a) transcript expression and (b) cellular localization using multiplex fluorescence RNA Scope in situ hybridization. The Kappa receptor showed somewhat similar expression across human, mouse and rat with the exception being dog DRG, where it was not expressed. This prompted an in situ localization of human DRG to determine which cell types express the Kappa receptor. Using two different in situ probes we observed expression not in neurons but in satellite glial cells (SGCs). Most neurons were surrounded by Kappa fluorescent signal; both probes showed the same SGC pattern. The adenosine A3 receptor (ADORA3), another GPCR, also has been suggested as a peripheral analgesic drug target. The species expression pattern for *ADORA3* was also highly varied. The *ADORA3* expression was absent in mouse, expressed at low levels in rat and dog DRG, and was abundant in human DRG. Here again in situ hybridization disclosed expression restricted to SGCs. In contrast, ADORA1 was expressed in a subpopulation of DRG neurons. These data suggest peripherally targeted agonists for either receptor may not be an effective analgesic strategy and provide a first-line transcriptomic screen for potential peripheral molecular analgesic candidates.

Dorsal Hippocampus To Nucleus Accumbens Projections Drive Reinforcement Via Activation of Accumbal Dynorphin Neurons

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The hippocampus represents a key structure in the integration of emotional processing, learning and memory, and reward-related behaviors. While the ventral subdivision of the hippocampus (vHPC) is involved in processing emotional values of salient stimuli and goal-directed behaviors, the dorsal hippocampus (dHPC) plays a critical role in episodic, spatial, and associative memory. In addition, it has been shown that the dHPC is necessary for context- and cue-associated reward behaviors, including the expression of reward seeking. The nucleus accumbens (NAc), a central structure in the mesolimbic reward pathway, integrates the salience of aversive and rewarding stimuli and its activity is sufficient to drive aversive and appetitive behaviors. Recent evidence has demonstrated that dHPC→NAc pathway is necessary for expression of a conditioned place preference. However, despite years of groundbreaking research and identification of direct projections from the dHPC to the NAc, the sufficiency for dHPC→NAC inputs to drive reinforcement and reward associated behavior remains to be determined.

Here using a wide range of complementary and cutting-edge techniques including behavior, *in-vivo* manipulation using optogenetics, chemogenetics and fiber photometry recordings, we demonstrate that activation of excitatory projections from the CA1 subregion of the dHPC (dCA1) is sufficient to drive reinforcing behaviors. In addition, we provide strong evidence that this reinforcing behavior is driven by 1) a direct projection from the dCA1 to the NAc and 2) enhanced glutamatergic signaling within the NAc. Furthermore, we uncovered that while dCA1 stimulation increases the activity of both enkephalin- and dynorphin-containing medium spiny neurons in the NAc, the selective activity of dynorphin-containing neurons is necessary for the expression of this reinforcing behavior. Our findings shed light on a novel pathway governing reinforcement and further extend the role of the dHPC on reward seeking.

Funding:

NIH DA045463, DA042499 and DA041781 grants to JAM.

Nalfurafine is aversive in doses that produce equi-effective antinociception to U50,488

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Introduction
Nalfurafine is an unconventional kappa opioid receptor (KOPr) agonist that is thought to produce antinociception without inducing the aversion typically associated with classical KOPr agonists, such as U50,488 [1]. Here we assess this and provide evidence that nalfurafine does produce aversion in mice.

Methods
C57BL/6J mice (8-week-old; male/female) were used. KOPr-induced spinal antinociception was assessed by warm water tail-withdrawal. After obtaining baseline, animals received either U50,488 (1.25; 5; or 20mg/kg, i.p.), nalfurafine (0.015; 0.06; or 0.24mg/kg, i.p.), or saline (i.p.) and were retested 30 minutes later. Dose-responses were generated using least-squares linear regression analysis followed by calculation of 95% confidence interval (CI) [2]. The antinociceptive effect of U50,488 (5mg/kg) was compared to doses of nalfurafine with unpaired t-tests. Sex differences were investigated by extra sum-of-squares F test.
In a separate cohort of 8-week-old males, aversiveness of U50,488 and nalfurafine was established in a one-week conditioned place aversion (CPA) experiment where animals received either saline or KOPr agonist over four days before test day. CPA scores were calculated by: *(time spent in paired compartment—time spent in unpaired compartment)/total time* and scores at test were compared to habituation by paired t-test. CPA scores between KOPr agonists were determined with unpaired t-tests. Significance level: $p<0.05$ (two-tailed).

Results
A dose of 5mg/kg U50,488 decreased the latency to withdraw the tail by 26.5% [20.2-32.8%] (mean [95% CI]) which was statistically equi-effective to 0.06mg/kg nalfurafine, 36.3% [30.2-42.4%] ($p=0.64$). No sex-differences in latencies by nalfurafine ($p=0.56$) nor U50,488 ($p=0.75$) were found.
5mg/kg U50,488 induced significant CPA compared to baseline (mean difference±SEM, p-value) (0.11±0.05, $p<0.05$) ($t=2.21$, $df=11$), but surprisingly, so did 0.06mg/kg nalfurafine (0.14±0.02, $p<0.001$) ($t=5.80$, $df=11$). There was no significant difference between U50,488- and nalfurafine-induced CPA ($p=0.65$).

Conclusion
The results show that in mice there is no separation between the analgesic and aversive effects of nalfurafine.

Funding and disclosure of conflict of interest
EJK's research project is 50/50 funded by University of Bath and DevelRx Ltd.
No conflict of interest to declare.

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A single injection of kappa opioid receptor agonist inhibits contextual heroin cues by counter-conditioning, not by enhancing extinction

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Introduction

Relapse in addiction can be triggered by craving, drug re-exposure, conditioned cues, or stress. Dysphoria in stress is encoded by kappa opioid receptors (KOPrs). KOPr activation is suggested to potentiate drug-seeking throughout acquisition, maintenance of reinforcement, abstinence, and relapse [1]. However, KOPr signalling during different memory phases underlying reinstatement of drug-seeking remains understudied. A rat self-administration study demonstrated KOPr activation before extinction inhibited cocaine-seeking [2]; whether it was through enhanced extinction or counter-conditioning was not determined. We have investigated whether KOPr agonists decrease reinstatement in a contextual model of heroin-induced drug-seeking and the mechanisms involved.

Methods

C57BL/6J mice (8-week-old; male/female) were used. First, aversiveness of 5mg/kg KOPr agonist U50,488 was confirmed using conditioned place aversion (CPA). In separate animals, all mice acquired conditioned place preference to heroin (2 mg/kg) followed by extinction and heroin-primed (1 mg/kg) reinstatement. Animals received a single injection of U50,488 (5 mg/kg) or saline before the first extinction session and were either confined to the heroin-conditioned chamber (inducing counter-conditioning or extinction; 'confined') or could explore both conditioned and unconditioned chambers (selectively inducing extinction; 'unconfined'). Times in each chamber were recorded. U50,488-induced CPA (and preference scores after heroin-primed reinstatement) was analysed by unpaired t-test, drug effects over extinction/reinstatement by paired ANOVA with multiple comparisons (Sidak corrected). Significance level: $p<0.05$ (two-tailed).

Results

U50,488 (5mg/kg) induced CPA, confirming that this dose is aversive ($p<0.01$) (n=12/group). In the second experiment, animals that were injected with U50,488 during extinction and could freely move between rooms ('unconfined') did not show differences in preference during extinction and heroin-primed reinstatement compared to saline controls ($F(1,42)=1.3$, $p=.26$) (n=22/group). However, when U50,488 was given during extinction, contingent to the previously drug-paired chamber ('confined'), their preference during extinction/reinstatement was significantly lower compared to saline ($F(1,33)=4.7$, $p<0.05$). Post hoc analysis revealed this was largely driven by the inability of a drug-prime to induce reinstatement in the U50,488 group ($t(66)=2.2$, $p=0.056$) (n=17-18/group).

Conclusion

KOPr activation can selectively counter-condition learned heroin contexts and decrease drug-seeking without affecting extinction. Thus, pharmacologically induced aversion can decrease contextual heroin-seeking, but only when contingent to the drug-paired context.

Funding and disclosure of conflict of interest

EJK's research project is 50/50 funded by University of Bath and DevelRx Ltd.
No conflict of interest to declare.

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Title:
Dissecting selective responses of dynorphin-expressing neurons during voluntary alcohol consumption in the central amygdala

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Abstract:
Dynorphin signaling within the central amygdala (CeA) has been implicated in responses to both stress and alcohol. Disrupting CeA dynorphin-expressing neurons or kappa opioid receptor signaling attenuates aberrant drinking behavior that results from the interaction of stress and prolonged alcohol drinking. Yet, how cellular activity of dynorphinergic neurons in the CeA relates to active alcohol drinking is not well-understood. Our preliminary evidence using fiber photometry showed that CeA dynorphin-expressing neurons (CeA^{Dyn}) are strongly engaged during voluntary alcohol consumption. The goals of the current studies was two-fold: 1) Replicate these initial results and increase the number of subjects to investigate potential sex differences and 2) to test hypotheses explaining the nature of this alcohol-specific signal. To accomplish these goals, activity of the Cre-dependent calcium sensor, GCaMP7f, in the CeA of prodynorphin-Cre (Pdyn-Cre) mice was recorded and time-locked to bouts of licking during 2-hr, 20% alcohol, water, or 0.5% sucrose drinking. We replicated initial findings that CeA dynorphin neurons show a greater increase in calcium transients during bouts of licking for alcohol than for water or sucrose, indicating these neurons are uniquely engaged during alcohol consumption. Furthermore, this increase was larger in females than in males. To accomplish the second goal, a separate cohort of Pdyn-Cre mice received 2-hr access to multiple substances in series while recording CeA^{Dyn} GCaMP7f activity time-locked to bouts of licking. First, since ethanol has a bitter taste component, while sucrose and water do not, we examined responses of CeA^{Dyn} neurons to two concentrations of quinine. Next, we tested whether the signal was related to the value of ethanol by investigating activity during water drinking after water value was increased by deprivation. Then we tested whether the response was due to the combination of both sweet and bitter tastes unique to ethanol by recording responses to sucrose adulterated with quinine. We next tested the response to saccharin since it has been shown to exhibit taste characteristics similar to ethanol and consumption has been shown to involve CeA subpopulations even where sucrose consumption does not. Finally, we also tested responses to citric acid, since these mice are on a genetic background of alcohol-preferring B6 mice, which consume more of this sour substance than other strains. Collectively, these studies aimed to further characterize and explain the unique response of CeA^{Dyn} neurons to ethanol consumption. These findings begin to explore fundamental CeA cell-population mechanisms that may underlie the complex interactions between stress and alcohol in AUD.

Funding: Supported by NIAAA grants (P50 AA010761, R01 AA02653, U01 AA014095, U01 AA020929, U01 AA020930, F31 AA027420, F32 AA029026, K01 AA025110) and VA Medical Research (BX000813).

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

Dynorphin signaling in the ventromedial prefrontal cortex regulates state transitions during acute threat exposure.

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Dynorphin (Dyn) is expressed in the ventromedial prefrontal cortex (vmPFC), a region critical for suppressing threat reactivity. However, little is known about how Dyn coordinates vmPFC activity during fear. Our lab has previously shown that vmPFC Dyn cells respond to threats and release Dyn within the vmPFC. Knockdown of vmPFC Dyn promotes passive defensive behaviors that persist beyond the threat, suggesting that Dyn may prevent vmPFC circuits from constraining fear-associated states. We expanded on this to test whether downregulation of vmPFC Dyn affects vmPFC processing of threats. To assess this, we used single-cell calcium imaging to compare pan-neuronal vmPFC activity in freely-moving mice with or without intact vmPFC Dyn signaling during fear conditioning. Dyn knockdown reduced the degree of both excitation and inhibition of vmPFC neurons throughout fear conditioning, but not at baseline, suggesting that a lack of Dyn constrains vmPFC activity during fear. Next, we determined how Dyn knockdown affected vmPFC state transitions during fear by applying principal components analysis to visualize the state space throughout fear conditioning. In control mice, vmPFC activity at baseline separated from activity following the first shock and the rest of the session, indicating that vmPFC networks transition to fear-related states. In vmPFC Dyn knockdown mice, threat-induced vmPFC state transitions were blunted. Further, a classifier predicted these states from neuronal activity in control mice but not mice with vmPFC Dyn knockdown, suggesting that Dyn may help the vmPFC compartmentalize fear states. Finally, we found that vmPFC network synchrony in fear is diminished in mice lacking vmPFC Dyn, indicating that Dyn promotes vmPFC synchrony in response to threats. This suggests that impaired vmPFC synchrony and state transitions may be a substrate through which Dyn signaling constrains passive defensive behaviors. Together, this experiment reveals how the Dyn system regulates cortical networks during fear states and may offer insight into potential treatments for stress- and anxiety-related disorders.

This work was supported by the National Institute of Mental Health Intramural Research Program. Dr. Flores-Garcia is supported by an NIH Center for Compulsive Behavior Fellowship and an NIH Post-Doctoral Research Associate Training Fellowship. Dr. Tejeda is supported by a Brain and Behavior Research Foundation NARSAD Young Investigator Award. The authors declare no conflict of interest.

Difelikefalin, a peripherally restricted KOR (kappa opioid receptor) agonist, produces diuresis through a central KOR pathway

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Difelikefalin (KORSUVA^{TM}) is the first and only kappa opioid receptor (KOR) agonist approved by the FDA and is indicated for the treatment of pruritis in adult patients with chronic kidney disease undergoing hemodialysis. Due to its hydrophilic D-amino acid peptidic structure, difelikefalin is thought to activate KORs and produce antipruritic and analgesic effects by an action restricted to the periphery. These studies investigated the cardiovascular and renal responses to difelikefalin, and using the KOR antagonist norbinaltorphimine (norBNI), examined whether any difelikefalin-induced changes in the renal excretion of water and/or electrolytes were mediated through a central or peripheral KOR pathway. The effects of norBNI pretreatment on nalfurafine, a KOR agonist that crosses the blood-brain barrier, were also examined. We hypothesized that difelikefalin would alter urine output differently than nalfurafine, given that KOR agonists produce diuresis via activating central KORs to inhibit vasopressin release. Following catheterization, conscious Sprague-Dawley rats were infused i.v. with isotonic saline and pretreated with norBNI centrally via an intracerebroventricular (ICV) cannula or peripherally via an intravenous catheter. After stabilization, difelikefalin or nalfurafine was administered as an i.v. bolus and urine output, heart rate, and mean arterial pressure (MAP) were recorded for 90 minutes. As compared to control levels, i.v. difelikefalin produced a significant increase in urine output, and significant decrease in urinary sodium and potassium excretion, urine osmolality, and MAP. ICV norBNI pretreatment markedly attenuated the increase in urine output caused by difelikefalin and nalfurafine but did not inhibit the electrolyte effects. However, i.v. norBNI pretreatment prevented all responses to difelikefalin and nalfurafine. Together, these findings demonstrate that difelikefalin and nalfurafine utilize central KOR pathways to elicit diuresis and a decrease in MAP but enhance renal tubular electrolyte reabsorption through a peripheral KOR pathway. These data provide important insight into two clinically useful KOR agonists.

Support: Work supported by LSUHSC SOM Research Enhancement Program grant to DRK.

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

CVL-354, a novel, brain penetrant and selective kappa opioid receptor antagonist

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Kappa opioid receptors (KOR) have strong activity throughout many key regions of the brain where their activity is believed to underlie psychological phenomena, including motivation incentivized by reward or reinforcement mediated via addictive substances, in addition to anxiety. As a regulator of both neuronal excitability and synaptic transmission of key circuits involved in reward and mood, KORs present a unique therapeutic target for clinical investigation into the treatment of major depressive disorder and substance abuse disorders. In vitro pharmacology studies were conducted to evaluate binding affinity and functional antagonism ([cAMP]) of CVL-354 at human KORs and human mu opioid receptors (MORs). Pharmacokinetic studies were run in mouse, rat and nonhuman primate to determine brain:plasma ratios. In mouse, target engagement studies were conducted to investigate the relationship between drug exposure and occupancy of CVL-354 at both the mKOR and mMOR to determine in vivo selectivity. Opioid-induced thermal insensitivity measured in the tail flick assay was utilized to determine in vivo pharmacodynamic selectivity for mKOR over mMOR. Additionally, CVL-354 reversal of KOR agonist-induced deficits in motivation were measured in the progressive ratio responding task. In vitro binding data demonstrated that CVL-354 has 31-fold binding affinity for hKOR over hMOR indicating selectivity for KORs. Furthermore, CVL-354 was determined to be an antagonist at both KOR (IC_{50} = 0.04 nM) and MOR (IC_{50} = 9.10 nM) and, importantly, did not demonstrate agonist activity at either receptor up to 1uM (0.01 nM - 1 μM tested). Pharmacokinetic studies in mouse, rat and nonhuman primate demonstrated that CVL-354 is brain penetrant and suggested greater brain penetration in higher species. In vivo target engagement studies in mouse revealed 27-fold selectivity for KOR (IC_{50} = 2.2 nM) over MOR (IC_{50} = 59.7 nM), similar to human in vitro binding studies. The thermal sensitivity assay demonstrated functional antagonism at MOR via producing analgesia ($p<0.05$) and qualitatively suggested ~10- fold pharmacodynamic selectivity. The effects of CVL-354 on progressive ratio responding were evaluated. Administration of the KOR agonist, spiradoline, in mice induces an anhedonic-like phenotype in which the animals are less motivated to seek rewards in the progressive ratio paradigm ($p<0.05$). One-way ANOVA (tail flick and progressive ratio responding assays) were used in addition to Dunnett's post hoc tests comparing treatment to control groups as appropriate, to demonstrate dose-dependent reversal of spiradoline induced anhedonia behaviors (ED50=0.09mg/kg, ~2nM). In summary, we have characterized CVL-354 as a novel, potent receptor-selective and brain-penetrant kappa opioid receptor (KOR) antagonist that demonstrates the ability to reverse KOR agonist induced deficits in motivation in vivo.

Support:
Work was supported by Cerevel Therapeutics.

Conflict of interest:
All authors are employees of Cerevel Therapeutics and may hold stock and/or stock options in the company.

Development of novel diketopiperazine and dipeptide analogs as selective KOR ligands as potential pain therapy

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Abstract:

In our laboratory, we have identified two chemotypes with selective affinity to the kappa opioid receptor (KOR). The next generation of molecules with favorable Multiparameter Optimization (MPO) and Ligand Lipophilicity (LLE) profiles were prepared using medicinal chemistry approaches. Using the Thermal Pace Preference Test (TPPT), we have shown that the tested KOR ligand blocks the antinociceptive effect of U50488, a known KOR agonist. Multiple reports suggest that modulation of KOR signaling is a promising therapeutic strategy in treating neuropathic pain (NP). As a proof-of-concept study, we tested the same compound in a rat model of NP and recorded its ability to modulate sensory and emotional pain-related behaviors. Observed *in vitro* and *in vivo* results suggest that these ligands can be used to develop compounds with potential applications as pain therapeutics.

Kappa Opioid Receptor Activation Increases Energy Expenditure, Body Temperature, and Feeding Through Central Regulation of Brown Adipose Tissue

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Energy balance and thermal regulation are closely linked, and maintenance of body temperature represents a major energy expenditure for endotherms. Signaling through opioid systems can modulate both body temperature and feeding behavior, positioning these neuromodulators as key regulators of energy balance. Here, we focus on the kappa opioid receptor (KOR), as its role in regulating metabolism is poorly understood. Activation of the KOR promotes food intake, leading to postulation that KOR signaling plays a central role in managing energy intake. The kappa opioid system has also been proposed as a pharmacologic target for treating obesity. To test this, we examined how the diverse effects of the kappa opioid system on feeding, metabolism, and thermoregulation are interconnected. Combing pharmacology, genetic tools, quantitative thermal imaging, and metabolic phenotyping, we show that activation of kappa receptors in the central nervous system led to activation of brown adipose tissue, hyperthermia, and increased energy expenditure. Surprisingly, preventing brown fat activation blocked the increase in food intake. This result indicates that restoration of energy balance is likely the driver for kappa receptor-induced feeding rather than direct modification of function of neural circuits regulating feeding by the kappa receptor. We also find KOR activation-mediated elevation of brown fat thermogenesis is disrupted by a chronic high fat diet, suggesting a potential contributor to high-fat diet induced weight gain. Thermogenesis in mice with diet induced obesity improved after a brief change to regular chow diet and modest weight loss. Taken together our findings support the conclusion that central nervous system KOR signaling is a key regulator or BAT and promotes feeding secondary to effects on BAT activation.

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

Funding: K08MH119538 and R21EY031269 (Norris)

Effects of the kappa-opioid receptor in the nucleus accumbens shell on ethanol drinking: Influence of sex, subregion targeted, and prior ethanol intake

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Although activation of the kappa-opioid receptor (KOR) is canonically believed to promote drug use, evidence on the direction of its effects on ethanol intake has been mixed. Remarkably, although the nucleus accumbens (NAc) shell is a region especially implicated in motivated behavior, studies have thus far not found KOR activation in the NAc shell to influence ethanol drinking. Heterogeneity may have influenced these results, as there is not only heterogeneity in the behavioral effects of the KOR along the rostro-caudal axis of the NAc shell, but there is also heterogeneity in ethanol drinking phenotypes between subjects. We have previously found that KOR activity in the rostral NAc shell increases approach behavior while KOR activity in the caudal shell instead promotes avoidance behavior. Moreover, we have found that while most male rats drinking under the intermittent access paradigm can be classified as lower ethanol drinkers, females can instead be lower- or higher-level drinkers. Thus, to determine if KOR activity in the NAc shell could in fact influence ethanol drinking, we used microinjections in male and female Long-Evans rats and examined how the effects of these injections might depend on subject sex, level of ethanol intake, and NAc subregion. Using the selective KOR agonist, U-50,488 (8.0 nmol or 0.8 nmol) compared to vehicle (0.3 uL), we found that although KOR stimulation in the middle NAc shell had no effect on ethanol drinking, KOR activity in the caudal NAc shell promoted ethanol drinking in males and higher-drinking females. Interestingly, in the rostral NAc shell, KOR activation enhanced ethanol drinking in higher-drinking females but decreased intake in males and lower-drinking females. Conversely, using the selective, long-acting KOR antagonist, nor-binaltorphimine (18.1 mmol), KOR blockade in the rostral NAc shell stimulated ethanol drinking in lower-drinking rats, with no effect on higher-level drinkers. To determine if these effects of KOR manipulation were substance-specific, we injected male and female sucrose-drinking rats with U-50,488 and found no effect on sucrose intake from injection into either the rostral or caudal NAc shell. To determine if the differential effects of KOR manipulations in the rostral NAc shell on ethanol drinking could be due to changes in receptor expression, we performed quantitative real-time PCR on NAc shell tissue from female rats with a history of ethanol or water drinking. While there was no effect of ethanol on gene expression of the endogenous KOR ligand, dynorphin, there was an upregulation of levels of KOR mRNA in the rostral NAc shell following a history of ethanol drinking, and this expression was positively and significantly correlated with level of ethanol intake. These findings demonstrate that the effects of KOR stimulation depend on subject sex, NAc subregion, and substance consumed, and they suggest that, possibly due to an upregulation in KOR expression, the level of prior ethanol intake may even reverse the effect of KOR stimulation on subsequent ethanol drinking. Thus, the KOR may represent a promising pharmacotherapeutic target to treat alcohol use disorder in specific subtypes of alcohol drinkers.

Support: This research was supported by NIH Grant R01 AA028228 (ANK and JRB).

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

Live-cell time-resolved fluorescence microscopy/spectroscopy assess ethanol and kappa-opioid receptor (KOP) antagonists effect on KOP and lipid dynamics

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The kappa-opioid receptor (KOP) specific antagonist LY-2456302 (Aticaprant) has been reported to reduce alcohol intake in mice when administered alone or in combination with the general opioid receptor antagonist naltrexone (NTX) [Zhou Y et. al. J Pharm Pharmacol 2022; 9(1): 1]. Understanding KOP antagonists' action on ethanol (EtOH)-induced effects is of importance for developing effective KOP-mediated treatments for alcohol use disorder (AUD). In this work, we applied advanced time-resolved fluorescence microscopy/spectroscopy techniques, such as Fluorescence Lifetime Imaging Microscopy (FLIM) and Fluorescence Correlation Spectroscopy (FCS), to characterize in live cells ethanol effects on lipid and KOP dynamics in the plasma membrane and examine whether these effects can be suppressed by the selected KOP antagonists. In our study, FLIM is used to characterize in live cells ethanol/drug-induced changes in the intracellular environment by measuring the fluorescence lifetime of the enhanced green fluorescence protein (eGFP) fused with KOP at the C-terminal end (KOP-eGFP), while FCS is used to read-out the lateral dynamics of plasma membrane lipids and KOP-eGFP, and characterize KOP-eGFP homo-oligomeric states. Our data show that ethanol increases plasma membrane fluidity and reduces (i) KOP oligomeric forms and (ii) KOP confinement in the plasma membrane. Pretreatment with NTX suppressed ethanol effects on plasma membrane fluidity and KOP oligomeric states, which was not observed for LY-2444296. Ca^{2+} imaging also showed that NTX suppresses EtOH-induced KOP-mediated Ca^{2+} influx under K^{+} depolarization.

Support: National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health (R01AA028549), the Swedish Research Council (2016-01922, 2018-05337 and 2022-03402), Karolinska Institutet Research Funds (2020-01591 and 2020-02325) and Strategic Research Program in Neuroscience (StratNeuro).

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

A novel, short-acting kappa opioid receptor antagonist blocks the analgesic effects of U50,488 and attenuates symptoms of spontaneous oxycodone withdrawal in rats.

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A large body of preclinical evidence has demonstrated that the neuropeptide dynorphin (DYN), which acts at kappa opioid receptors (KOR), is a key player in opioid withdrawal (Koob, 2009; Bruchas et al., 2010). Chronic opioid exposure increases DYN and KOR activation and is thought to produce negative affective states including anhedonia, anxiety-like, and aversive behaviors. Importantly, KOR antagonism has been shown to reduce opioid withdrawal signs and escalation of opioid self-administration in rodent models. However, KORA compounds used in these preclinical models (e.g., norBNI, JDTic) have extremely long KOR antagonist actions—on the order of weeks. As such, the development of a selective and short-acting KOR antagonist has the therapeutic potential to facilitate discontinuation of opioid use with a pharmacological profile better suited for clinical development. CVL-354 is a potent, selective and relatively short-acting KOR antagonist that was tested in a rat model of spontaneous oxycodone withdrawal.

Adult male Sprague Dawley rats were used. To determine dose and time course effects of CVL-354 KOR antagonism, rats were treated with CVL-354 (0.0 – 1.0 mg/kg, SC) followed by the KOR agonist, U50,488 (30 mg/kg, SC) and nociceptive responses were measured in the Tail Flick (TF) and Hot Plate (HP) thermal pain assays. To determine the effects of CVL-354 on oxycodone somatic withdrawal signs, rats were subcutaneously implanted with iPRECIO minipumps (Alzet, model SMP 200) programmed to deliver an escalating dose regimen of oxycodone or saline 2x/day for 14 days. For oxycodone, the escalating dose regimen was 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg/infusions (2 infusions/day; 7:00-9:00AM/PM), with the 0.5 mg/kg dose administered for 2 days and the remaining doses administered for 3 days each. After the 14-day escalating dose oxycodone (or saline) regimen, spontaneous opioid withdrawal signs emerged, including diarrhea, ptosis, wet dog shakes, teeth chattering, and body flattening. To determine the effects of CVL-354 on somatic withdrawal, rats were administered CVL-354 (0.0, 0.1, 0.3, 1.0 mg/kg, SC) at 6-h (Wdrl d0) and 24-h (Wdrl d1) after cessation of drug delivery. Opioid withdrawal can also alter locomotor activity, with results dependent on factors such as novelty and area of the test arena. As such, we tested the effects of CVL-354 on oxycodone withdrawal-induced alterations in locomotor activity in Open Field chambers (Med Associates) on Wdrl d1, immediately after somatic withdrawal measurements. Activity was digitally recorded and later scored using DeepLabCut (Mathis et al., 2018). As a control for attenuation of spontaneous withdrawal signs, we administered the a2 noradrenergic agonist lofexidine (0.64mg/kg) to separate rats. At the end of behavioral testing on Wdrl d1, rats were euthanized via rapid decapitation to collect plasma for ELISA-based corticosterone analysis. Statistical analyses used were: One-way ANOVA (somatic withdrawal, corticosterone) and two-way ANOVA with repeated measures on time (open field test, tail flick and hot plate tests). Dunnett's posthoc tests comparing treatment to control groups were done when appropriate.

A dose range of 0.1-1.0 mg/kg CVL-354 blocked U50,488-induced analgesia in the TF and HP tests at 1- and 4-h, but not at 24-h. Within this same dose range, CVL-354 reduced spontaneous oxycodone somatic withdrawal signs ($p<0.01$). In neither the pain nor the somatic withdrawal assays did CVL-354 have an effect on its own. Lofexidine, the current standard of care for mitigation of acute opioid withdrawal symptoms, also significantly reduced somatic withdrawal signs suggesting predictive validity of this model ($p<0.01$). Locomotor activity was significantly decreased during spontaneous oxycodone withdrawal compared to activity in control rats ($p<0.05$). Lofexidine exacerbated withdrawal-induced decreases in locomotor activity ($p<0.01$), whereas CVL-354 had no effect. Finally, spontaneous oxycodone withdrawal resulted in significantly elevated levels of unbound plasma corticosterone compared to control rats ($p<0.01$). Interestingly, lofexidine pre-treatment significantly potentiated plasma corticosterone levels compared to oxycodone withdrawn rats pre-treated with vehicle ($p<0.01$), whereas CVL-354 (0.3mg/kg) pre-treatment showed a trend to decrease oxycodone withdrawal-induced plasma corticosterone levels.

These data demonstrate that CVL-354 has KOR antagonist actions in thermal pain assays for at least 4, but less than 24, hours, and that it is effective at reducing somatic withdrawal signs in a model of spontaneous oxycodone withdrawal model. Intriguingly, lofexidine treatment suppressed locomotor activity and exacerbated levels of the stress biomarker, corticosterone. CVL-354 did not produce these undesirable effects, suggesting that KOR antagonism may provide better overall efficacy for mitigation of opioid withdrawal symptoms.

Keywords: Kappa opioid receptor, kappa opioid receptor antagonist, opioid dependence, pharmacotherapy, animal model, withdrawal

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Support: No funding source to disclose

Disclosures: GLS, PI, SD, SC, GG, SC are employees of Cerevel Therapeutics and may hold stock and/or stock options in the company. **MN, GC and EC** have nothing to disclose.

Kappa opioid receptor control over monoaminergic transmission is differentially modulated by ethanol consumption along the NAc shell rostral-caudal axis

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Kappa-opioid receptor (KOR) activation in the nucleus accumbens (NAc) shell produces both hedonic and anhedonic behaviors. Specifically, KOR activation in the rostral NAc shell promotes positive affect behaviors but leads to aversive behaviors in the caudal NAc shell. Similarly, preliminary data suggest that KOR activation in the caudal NAc shell augments ethanol intake, while activation in the rostral subregion attenuates consumption. These opposing effects of KOR activation may be driven by mechanisms such as differences in monoamine projection densities, or differences in total number of KORs along the rostral-caudal axis. Furthermore, KOR modulation of monoamine transmission may also vary by region, an effect that may be further modulated by ethanol intake. Given that KORs modulate both serotonin and dopamine transmission in the NAc shell, the current study aimed to investigate how chronic long-term volitional ethanol consumption alters this interaction. Rats were given access to two-bottle choice intermittent access paradigm; one ethanol and one water bottle or two water bottles on alternating days, three days per week for 24 hours, over 8 weeks. Approximately 17 hours following the final drinking session, *ex-vivo* fast scan cyclic voltammetry was used to measure dopamine and serotonin kinetics in both the rostral and the caudal subregion of the NAc shell. Furthermore, KOR agonist U50,488 (U50) was used to measure KOR modulation of monoamine release and uptake kinetics. While control rats did not show regional differences in dopamine release, dopamine release was greater in the rostral subregion of ethanol drinking rats compared to the caudal subregion, with significantly greater release in ethanol drinking male rats. Dopamine uptake was greater in the rostral subregion compared to the caudal subregion in both control and ethanol drinking rats. No differences were observed across the rostral-caudal axis in serotonin release or uptake and ethanol drinking rats showed no differences in serotonin release and uptake compared to control rats. These results indicate that ethanol exposure augments dopamine release and uptake in a sex and region-specific manner with no effect of ethanol consumption on serotonin kinetics. Male ethanol drinking rats showed greater KOR inhibition of dopamine release compared to male control rats, although this effect was not region specific. KOR activation significantly decreased dopamine release across the rostral-caudal axis of both ethanol drinking and control female rats without regional or treatment differences. KOR activation reduced dopamine uptake in a region independent manner in all control rats and female ethanol drinking rats, but in male ethanol drinking rats only the rostral subregion showed a reduction in dopamine uptake, with no change in caudal dopamine uptake. KOR-mediated inhibition of serotonin release and uptake did not vary by region in ethanol drinking or control rats. However, we did observe a trend whereby KOR inhibition of both serotonin release and uptake was greater in ethanol drinking rats compared to control rats. Overall, these results suggest that ethanol exposure augments the inhibitory effects of KORs in both monoamine systems; however, KOR effects on dopamine transmission following ethanol consumption are greater in magnitude and are regionally specific. Overall, the potential shift in synergistic interactions between the three systems (KOR, dopamine, and serotonin) may ultimately influence behavioral outcomes. These findings elucidate potential neural mechanisms that underlie alcohol use disorder, a crucial step in the development of potential treatments.

Support: This research was supported by NIH grant R01 AA028228 (ANK and JRB)

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

Kappa opioid receptors reduce serotonin uptake and escitalopram efficiency in the substantia nigra pars reticulata

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The kappa opioid receptor (KOR) and serotonin systems are strongly implicated in disorders of negative affect, such as anxiety, addiction, and depression. KORs expressed on axon terminals have been shown to inhibit the release of neurotransmitters, including serotonin. The substantia nigra pars reticulata (SNr) receives the densest serotonergic innervation in the brain and has high KOR expression; however, the influence of KORs on serotonin transmission in this region is yet to be explored. The recent implication of the SNr in the regulation of affective behaviors, such as avoidance and drug reinstatement, highlights the importance of understanding how the serotonin and KOR systems interact within this brain region. Therefore, we used *ex vivo* fast-scan cyclic voltammetry (FSCV) to investigate the effects of a KOR agonist, U50, 488 (U50), and a selective serotonin reuptake inhibitor, escitalopram, on serotonin release and reuptake in the SNr. We observed for the first time ex vivo that escitalopram dynamically alters release and uptake of serotonin over time. In addition, U50 caused a reduction in serotonin release and reuptake and when applied prior to escitalopram blunted both the release and uptake effects of escitalopram. Here, we show that the KOR influences serotonin signaling in the SNr via augmentation of release and reuptake, and short-term activation of the KOR alters serotonin responses to escitalopram. These interactions between KORs and serotonin may contribute to the complexity in the responses to selective serotonin reuptake inhibitors (SSRIs) and other treatments for disorders of negative affect. In conclusion, the KOR system may be a promising pharmacological target, alongside traditional antidepressant treatments.

Funding and Disclosures: This research was funded by the National Institute on Alcohol Abuse and Alcoholism, under the grant numbers U01AA014091, P50AA026117 and T32AA007565, and the National Institute on Drug Abuse, under the grant numbers R01DA048490 and R01DA054694. The authors declare no conflict of interest.

Mapping kappa and mu opioid receptor expression in the amygdala

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Throughout the brain, neurons expressing the kappa opioid receptor (KOR) functionally oppose neurons expressing the mu opioid receptor (MOR). While KOR and MOR are both found in subregions of the amygdala, the structural and functional consequences of having these two types of opioid receptors in this brain region are not fully understood. Using in situ hybridization and viral tracing, we have begun evaluating the circuitry of opioid receptor-expressing neurons in the amygdala. With this work we hope to gain a greater understanding of the neural circuits by which KOR and MOR modulate amygdalar activity.

Support
Work supported by NINDS R01NS096705.

Conflict of Interest
The authors declare no conflict of interest.

Unique Morphological and Electrophysiological Characteristics Define Dynorphin Neurons in the Medial Prefrontal Cortex.

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

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