KAPPA THERAPEUTICS

CONFERENCE

April 3-7, 2017 Philadelphia, PA

April 2017

PROGRAM BOOK

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General Information

The 4th Conference on the Therapeutic Potential of Kappa Opioids in Treating Pain and Addiction.

Conference Venue

The Hilton Philadelphia at Penn's Landing
201 S. Christopher Columbus Blvd, Philadelphia, PA

Internet Access in Meeting Rooms

"Hilton-PSAV" password "kappa2017"

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 located in the Hall (outside of General Sessions in Ballroom) and will be open

Monday, April 3	5 pm - 7 pm
Tuesday, April 4	7 am - 5 pm
Wednesday, April 5	7 am - 5 pm
Thursday, April 6	7 am - 5 pm

Meals

Continental Breakfast and Coffee Breaks will be provided in Columbus Ballroom Foyer. Buffet lunch will be provided in the Grand Ballroom C/D for all registrants.

Hilton Philadelphia at Penn's Landing Meeting Space

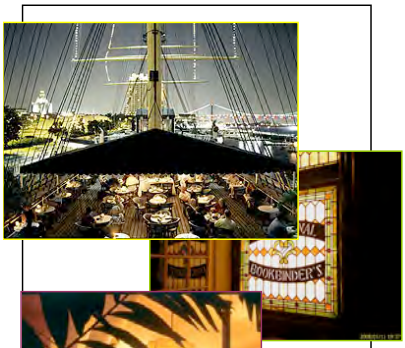
Instructions for Presenters Posters

Poster boards are 4 feet x 6 feet. Pushpins will be provided. Posters must be hung before lunch on Wednesday, April 5.

Your poster number is listed in the Program

Oral presentations

We will have a Macintosh computer with the latest Operating System and 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) for uploading at the registration desk.



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Tastes of the culinary traditions of this storied island paradise
(Moderate Price; Group Capacity 250)
http://www.cubalibrerestaurant.com ♦ 10 S. 2nd Street

Farmicia (215) 627-6274
Delicious, organic cuisine in Old City
(Moderate Price; Group Capacity 30 people)
http://www.farmiciarestaurant.com ♦ 15 S. 3rd Street

Hilton Philadelphia's Restaurant Recommendations

Old City/Penn's Landing – BY BLOCK DISTANCE

ONE BLOCK

Keating's River Grill @ *the Hilton Philadelphia,* 201 S. Columbus Blvd. 215-521-6509 casual dining, casual dress, moderate prices, breakfast, lunch and dinner. The restaurant serves regional cuisine with a view and an outdoor deck overlooking the Delaware River.

Moshulu 401 S. Columbus Blvd at Lombard Circle 215-923-2500 Casual dress, moderate/expensive. The restaurant serves creative American cuisine with international influences, the restaurant is the ship Moshulu a 1904 four-masted sailing vessel on the Delaware River. Serves Lunch, Sunday Brunch and Dinner daily. Located ½ block from the Hilton

The Chart House 555 S. Columbus Blvd at Lombard Circle, 215-625-8383 casual dress, moderate prices. The restaurant serves a variety of seafood with a view of the Delaware River or Center City. Serves Sunday Brunch and Dinner daily. Located 1½ block from Hilton

TWO BLOCKS

Positano Coast 214 Walnut St. at 2nd St. 215-238-0499 casual dress, moderate prices. The restaurant serves Northern Italian, in the fashion of Positano Italy, which is a family style. Serves Lunch, Brunch on Sundays and Dinner. Located 2 blocks from the Hilton

Zahav 237 St. Place near Ritz 5 movie 215-625-8800 Casual dress, moderate prices. The restaurant serves creative modern Israeli cuisine. Dinner Daily Located 2 blocks from the Hilton

Buffalo Billiards and Metropolitan Lounge 118 Chestnut St. between Front and 2nd Sts. 215-574-7665. 14 billiard tables to shoot pool and lots of comfy chairs to sit back and relax for Happy Hour $3 ALL Drafts, Half off Apps. Located 2 blocks from the Hilton

Spasso's 34 S. Front St. between Chestnut and Market Sts. 215-592-7661 casual dress, price inexpensive to moderate prices. The restaurant serves northern and southern Italian cuisine and is great for families. Serves lunch and dinner. Located 2½ blocks from Hilton

Brazil's 112 Chestnut St. between Front and 2nd St. 215-413-1700, 9:30PM to 2:00AM Wednesday thru Saturday Salsa dancing. Located 2½ blocks from the Hilton

Prime Stache 110 Chestnut Streets 267-886-8354 American bistro style menu and carefully selected craft beer and cocktails. Dinner and Cocktails daily. Lunch on weekends. Sunday Brunch. Located 2 blocks from Hilton

THREE BLOCKS

City Tavern 138 S. 2nd St. @ Walnut St. 215-413-1443 casual dress, moderate prices, children's menu, lunch, Sunday brunch and dinner The restaurant serves colonial American cuisine with modern flair and the staff dresses in 18th Century attire. Located 3 blocks from the Hilton

Double Shot 211 Chestnut St. between 2nd and 3rd Sts. 215-351-5171. Espresso Bar serves coffee, tea pastries, sandwiches and has Internet access. Hours: Monday-Thursday 7:00 AM to 8PM, Saturday 9:00AM to 11:00PM, Sunday Closed. Located 3 block from the Hilton

Cuba Libre 10 S. 2nd St. between Chestnut and Market Sts. 215-627-0666 casual dress, moderate prices, Saturday and Sunday brunch, lunch and dinner. Serving Cuban cuisine known for its' Mojitos. On Friday and Saturday night the restaurant pushes some of the tables aside and has salsa dancing after 9:00pm. Located 3 blocks from the Hilton

Karma 114 Chestnut St. between Front St. and 2nd St. 215-924-1444 casual dress, moderate/inexpensive. The restaurant serves traditional Indian cuisine. Located 3 blocks from the Hilton

Panorama 14 N. Front St. at Market St. 215-922-7800 Business causal, moderate prices. The restaurant serves creative northern Italian cuisine and is the best wine bar in the city. Serves lunch Monday thru Friday, Dinner daily. Located 3 blocks from Hilton

4th Conference on the "Therapeutic Potential of Kappa Opioids"

April 3-6th, 2017
Hilton Penn's Landing, Philadelphia, PA

Monday, April 3rd

5 - 7 PM Registration (Columbus Ballroom Foyer)

7 - 8 PM Opening Reception (Columbus Ballroom Foyer)

Tuesday, April 4th

7 - 8 AM Continental Breakfast & Registration (Columbus Ballroom Foyer)

8:00 AM Welcome: Charles Chavkin / Lee-Yuan Liu Chen (Columbus Ballroom)

Oral Session 1: Receptor Structure (Charles Chavkin, Chair)

8:15 AM Ray Stevens (University of Southern California) **Structural studies of peptide receptor-ligand interactions**

8:35 AM Seva Katritch (University of Southern California) **Structure based discovery of new ligand chemotypes for opioid receptors**

8:55 AM Bryan Roth (University of North Carolina) **New insights into KOR structure and function**

9:15 AM Selena S. Schattauer (University of Washington) **norBNI activation of c-Jun Kinase alters the structural composition of the kappa opioid receptor-signaling complex**

9:35 AM Discussion

9:45 AM Coffee Break (Columbus Ballroom Foyer)

Oral Session 2: Dynorphin Circuits (Tom Kash, Chair) (Columbus Ballroom)

10:15 AM Ream Al-Hasani (Washington University) **In vivo detection of optically released opioid peptides**

10:35 AM Taylor A. Gentile (Temple University) **The role of hypocretin (orexin) and dynorphin in reward and anxiety following chronic cocaine administration and withdrawal**

10:55 AM Michael Bruchas (Washington University) **Stress-induced reinstatement of nicotine preference requires dynorphin/kappa opioid activity in the basolateral amygdala**

11:15 AM Hugo A. Tejeda (NIDA-IRP) **Pathway and cell-specific kappa-opioid receptor modulation of excitatory-inhibitory balance differentially gates D1 and D2 accumbens neuron activity**

11:35 AM Discussion

12:00 PM Buffet Lunch (Grand Ballroom)

Oral Session 3: Drug and Alcohol Abuse (Michael Bruchas, Chair) (Columbus Ballroom)

2:00 PM Yan Zhou (Rockefeller University) **Effects of mesyl salvinorin B, naltrexone, nalmefene or nor-BNI on alcohol escalation, "relapse" and "binge" drinking in male and female mice**

2:20 PM Brendan Walker (Washington State University) **The role of ventral striatal kappa-opioid receptors in the motivational / emotional phenotypes of alcohol dependence**

2:40 PM Anushree N. Karkhanis (Wake Forest School of Medicine) **Adolescent social isolation-potentiated kappa opioid receptor function augments cocaine seeking in adulthood**

3:00 PM Katherine Holleran (Wake Forest School of Medicine) **Negative affect-like behavior and kappa opioid receptor function in the NAc during protracted abstinence from ethanol in mice**

3:20 PM Coffee Break

3:50 PM Jay McLaughlin (University of Florida) **Variable opioid activity arising from ring substitution in CJ-15,208 and characterization of an analog that blocks stress-induced reinstatement of extinguished cocaine-conditioned place preference**

4:10 PM Yu-Jun Wang (Shanghai Institute of Materia Medica) **Role of dynorphin/kappa opioid system in the formation of aversive memory associated with morphine withdrawal**

4:30 PM Kevin Freeman (University of Mississippi) **Comparison of the punishing effects of nalfurafine and salvinorin A on cocaine and oxycodone self-administration in rhesus monkeys**

4:50 PM Discussion

Student / Postdoc Mixer (6 – 7 PM) ***(meet in the hotel lobby at 5:45 pm or at the pub)***
*(*Cavanagh's Headhouse, *421 S 2nd St, Philadelphia, 10 min walk)*

Dinner (no host, maps to local restaurants provided)

Wednesday, April 5th

7 - 8 AM Continental Breakfast & Registration (Columbus Ballroom Foyer)

Oral Session 4: Human Studies (Bill Carlezon, Chair) (Columbus Ballroom)

8:00 AM Jeffrey M. Miller (Columbia University) ***In Vivo* Kappa Opioid Receptor Binding Assessed by PET Imaging in Major Depressive Disorder**

8:30 AM Stuart Collinson (Tioga Pharmaceuticals) **Asimadoline, a Selective Kappa-Opioid Receptor Agonist for the Treatment of Atopic Dermatitis: Preliminary Safety Results of a Proof-of-Concept Trial**

9:00 AM Ronald Marcus (Cerecor) **A Randomized, Double-Blind, Placebo-Controlled Study Examining the Selective Kappa Antagonist, CERC-501, in a Human Laboratory Model of Smoking Behavior**

9:30 AM Elliot Ehrich (Alkermes) **Treatment of Resistant Depression with a Samidorphan/Buprenorphine (ALKS 5461), a Balanced Agonist-Antagonist Opioid Modulator**

10:00 AM Coffee Break

10:30 AM Mary Jeanne Kreek (Rockefeller University) **Repeated administration of a novel selective KOP-r antagonist, LY2456302, in normal volunteers and persons with cocaine dependence diagnosis: Neuroendocrine and behavioral profile**

11:00 AM Ivy Carroll (Research Triangle Institute) **Design, synthesis, and in vitro pharmacological evaluation of novel kappa opioid receptor antagonists**

11:30 AM Discussion / Data Blitz / Short talks

[Participants wanting to comment are welcome to show a data blitz slide]

12:00 PM Buffet Lunch (Grand Ballroom)

Oral Session 5: KOR Agonists and Antagonists (Ivy Carroll, Chair)

2:00 PM Bill Clarke (University of Texas) **Functional selectivity of U50,488 analogues at Kappa Opioid Receptors (KOR) expressed in peripheral pain-sensing neurons**

2:20 PM E. Andrew Townsend (University of Mississippi) **Nalfurafine decreases the reinforcing effects of oxycodone while producing additive thermal antinociception in male rats**

2:40 PM Bernhard Wünsch (University of Münster) **Conformationally restricted κ-opioid receptor agonists**

3:00 PM Philip Mosier (Virginia Commonwealth University) **Structure–activity relationships of cyclized ML140 analogues at the kappa opioid receptor, and why butyl is not necessarily futile**

3:20 PM Coffee Break

Oral Session 6: Dynorphin / KOR in the Stress Response (Sara Jones, Chair)

3:50 PM Bill Carlezon (McLean Hospital & Harvard Medical School) **Kappa opioid receptor antagonism mitigates stress effects on sleep and circadian rhythms in male mice**

4:10 PM Elyssa Margolis (UCSF) **Corticotrophin releasing factor alters kappa opioid receptor function in the ventral tegmental area**.

5 – 7 PM **Poster Session** (Grand Ballroom D)

Dinner (no-host, maps to local restaurants provided)

Thursday, April 6th

7 - 8 AM Continental Breakfast & Registration (Columbus Ballroom Foyer)

Oral Session 7: KOR and Pain Behaviors (Lee-Yuan Liu-Chen, Chair)

8:00 AM Nicolas Massaly (Washington University) **Pain-induced alterations in motivational states are mediated via upregulation of the accumbal kappa opioid receptor system**

8:20 AM Andrea Bedini (University of Bologna) **LOR17 is a functionally selective KOR agonist eliciting potent analgesic effects in animal models of nociceptive and neuropathic pain**

8:40 AM Salina D. Johnson (University of Washington) **Sex differences in kappa opioid receptor-mediated C57BL/6 mouse pain behaviors**

9:00 AM M. Imad Damaj (Virginia Commonwealth University) **Role of kappa receptors in chemotherapy-induced neuropathy and emotional-like deficit behaviors in mice.**

9:20 AM Discussion

9:30 AM Coffee Break

Oral Session 8: Kappa Opioid Receptor Interactions and Trafficking (Elena Chartoff, Chair)

10:00 AM Vladana Vukojević (Karolinska Institute) **Spatial organization and dynamics of opioid receptor variants (kappa, mu_{wt} and mu_{N40D}) in the plasma membrane at the nanoscale level**

10:20 AM Abigail M. Polter (George Washington University) **Acute stress induces constitutive activation of kappa opioid receptors.**

10:40 AM Giovanni Tangherlini (University of Munster) **Development of new imaging probes to investigate the role of κ-opioid receptors in multiple sclerosis**

11:00 AM Admire Munanairi (Washington University) **Spinal KOR Activation Attenuates Itch by Inhibiting GRPR Function**

11:20 AM Alex Willhouse (Temple University) **Characterization of a knockin mouse line expressing KOR-tdTomato fusion protein**

11:40 AM Discussion

Buffet Lunch 12:00 – 2 PM (Grand Ballroom)

Oral Session 9 (continued): Effects of KOR Activation and Antagonism (Irwin Lucki, Chair)

2:00 PM Christoph Schwarzer (Medical University of Innsbruck) **Preclinical evidence for a rAAV based gene-therapy of temporal lobe epilepsy targeting Kappa opioid receptors**

2:20 PM Antony D. Abraham (University of Washington) **Kappa opioid receptor activation on dopamine neurons disrupts behavioral inhibition**

2:40 PM Benjamin B. Land (University of Washington) **Kappa opioid receptors modulate mammalian target of rapamycin (mTOR) through p38 MAP kinase**

3:00 PM Jordan G. McCall (Washington University) **Intrinsic properties of central amygdala dynorphin neurons**

3:20 PM Coffee Break

3:50 PM Caroline A. Browne (Uniformed Services University of the Health Sciences) **Antidepressant activity of the buprenorphine analogue BU10119**

4:10 PM Lee-Yuan Liu-Chen (Temple University) **Receptor phosphorylation and mTOR pathway are involved in KOR agonist-induced aversion**

4:30 PM Discussion / Data Blitz / Late Breaking Abstracts

5:00 PM Presentation of the ***2017 Toni Shippenberg Young Investigator Awards*** (C Chavkin)

[mentors may nominate trainees by e-mail to CC; awards will be selected by vote of the Program Committee]

Closing at 5:30 PM

Dinner (no-host)

Friday, April 7th

Checkout & Departure

Posters (Wednesday, 5-7 PM) (Grand Ballroom D)

Double-sided 4' x 6' corkboards and push-pins will be provided. Please put up your poster during a break in the program after lunch on Wednesday before 5PM and take it down at the end of the session.

Refreshments will be provided.

POSTER

1. Christoph Abels, Dirk Schepmann, Dieter Metze, Tobias Lotts, Ulrich Knie, Sonja Ständer, Bernhard Wünsch, Michael Soeberdt **Anti-inflammatory activity of a topically applied, potent and selective, peripherally restricted κ-opioid receptor agonist in mouse models of skin inflammation**

2. Michael Soeberdt, Peter Molenveld, Roy P. M. Storcken, Renaud Bouzanne des Mazery, Geert Jan Sterk, Reshma Autar, Marjon G. Bolster, Clemens Wagner, Sebastianus N. H. Aerts, Frank R. van Holst, Anita Wegert, Giovanni Tangherlini, Bastian Frehland, Dirk Schepmann, Ulrich Knie, Bernhard Wünsch, Christoph Abels **Design and synthesis of enantiomerically pure decahydroquinoxalines as potent and selective κ-opioid receptor agonist**s

3. Anderson RI, Kash TL, Becker HC **Pharmacological and chemogenetic evidence for a role of the dynorphin/kappa opioid receptor system in binge-like ethanol consumption**

4. Kelly A Berg, Miryam Pando, Teresa Chavera, and William P Clarke **DOR-KOR heteromers, expressed in peripheral nociceptors, maintain functional competency under prolonged inflammatory conditions**

5. Daniel W. Bloodgood & Thomas L. Kash **Alcohol drinking induced alterations in dynorphin signaling in the extended amygdala**

6. Yi-Ting Chiu, Chongguang Chen and Lee-Yuan Liu-Chen **Kappa opioid receptor is phosphorylated by G protein-coupled receptor kinases and protein kinase C**

7. Dunn AD, Dunn AM, Reed BR, Butelman ER, Kreek MJ **Novel library of N-phenylethyl-N-3-hydroxyphenylethyl-amines with differing tertiary N substitutions: characterization of kappa opioid receptor effects**

8. Fontaine HM, Abraham AD, Song AJ, Land BB, Chavkin C **Prior stress and ventral tegmental area dopamine neuron inhibition potentiates reward**

9. Antony D. Abraham, Selena S. Schattauer, Benjamin B. Land, Charles Chavkin **The peroxiredoxin-6 (PRDX6) inhibitor MJ33 blocks the long-lasting antagonism of kappa opioid receptors by nor-BNI and blocks analgesic tolerance to morphine**

10. Selena S. Schattauer, Benjamin B. Land, Kathryn L. Reichard, Shao En Ong, Charles Chavkin **Kappa and Mu Opioid receptor activation stimulates the production of reactive oxygen (ROS) via PRDX6 and JNK.**

11. Reichard, KL, Schauttauer, S, Burgeno, L, Steger, J; Abraham, A; Land, BB; Chavkin, C **NorBNI inactivates Dopamine D2 receptors on VTA nerve terminals by stimulating ROS production through a JNK/PRDX6 mechanism.**

12. Allisa J. Song, Antony D. Abraham, Selena S. Schattauer, Sanne M. Casello, Benjamin B. Land, Charles Chavkin. **Characterization of kappa opioid receptor (KOR)-expressing and dynorphin-containing neurons in the mouse brain**

13. Jennifer S. Steger, Salina D. Johnson, Charles Chavkin. **Chemogenetic inhibition of the lateral septum in male C57BL/6 mice induces social aggression and consequent social defeat behaviors in both male and female intruders**

14. Aubrie A. Harland, Tarsis Brust, Huiyong Ma, Kimberly M. Lovell, Kevin J. Frankowski, Laura M. Bohn, and Jeffrey Aubé **Structure-Activity Relationship Exploration of a Bisamide Series of Kappa Opioid Receptor Agonists**

15. Lansu, K., Karpiak, J., Liu, J., Huang, X-P., Kroeze, W.K., Jin, J., Shoichet, B.K., and Roth, B.L. **In silico design of novel probes for the atypical opioid receptor MRGPRX2**

16. Margolis EB, Van Orden LJ, Martin WJ **Electrophysiological characterization of BTRX-335140, a novel selective kappa opioid receptor antagonist, in ventral tegmental area dopamine neurons in rat**

17. Zan GY, Wang YJ, Wang Q, Long JD, Chai JR, Lu YC, Hang A, Deng YZ, Liu JG **Kappa opioid receptor activation in the amygdala mediates depressive-like behaviors following morphine abstinence through p38 MAPK.**

18. Zaidi SA, Katritch V. **Exploring the role of Sodium binding pocket in kappa opioid receptor activation**

19. Tao Che, Ivy F. Carroll, Els Pardon, Jan Steyaert, Bryan L. Roth **Rational identification of functionally selective kappa-opioid receptor ligands**

20. Patel N, Zheng Z, Huang X, Mangano T, Zou R, Chen X, Zaidi S A, Roth B, Stevens R, Katritch V. **Structure based discovery of new antagonist and biased agonist chemotypes for Kappa Opioid Receptor**

ABSTRACTS for ORAL PRESENTATIONS

Vsevolod Katritch[1], Zhong Zheng[1], Saheem Zaidi[1], Nilkanth Patel[1], Raymond C. Stevens[1], Xi-Ping Huang[2,4], Thomas J. Mangano[2,4], Rodger Zuo[2,4], Xin Chen[2], Bryan L. Roth[2,3,4]

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[2]Department of Pharmacology and [3]Division of Chemical Biology and Medicinal Chemistry and [4]National Institute of Mental Health Psychoactive Drug Screening Program, University of North Carolina Chapel Hill Medical School, 4072 Genetic Medicine Building, Chapel Hill, North Carolina 27514, USA

Structure based discovery of new ligand chemotypes for opioid receptors

An expanding repertoire of high resolution structures of opioid receptors presents a unique opportunity for in silico discovery of new chemotypes as tool and lead compounds with desired selectivity and functional profiles. We employed a multi-template virtual screening strategy, using the crystal structure of KOR and corresponding ligand-optimized atomistic models, to discover several new KOR chemotypes with sub-micromolar activities and distinct functional features. The initial prospective screening achieved a 32% hit rate and identified six new promising fragment- and lead-like scaffolds, while the follow up round of structure-activity relationship (SAR) yielded eleven additional hits in sub-micromolar affinity range (best K_i = 90 nM and the best ligand efficiency *LE*=0.53 kcal/mol per heavy atom). Functional characterization shows KOR antagonist or agonist activity for a number of new ligands, while compound #**81** was identified as an agonist with a G-protein biased profile, relevant for therapeutic applications. A similar approach is now being applied to screening for ligands with bifunctional activity as DOR antagonists and MOR agonists, as well as allosteric modulators. In general, although opioid and other peptide- and protein-binding GPCRs present special challenges for virtual ligand screening (VLS), these examples show utility of VLS in discovery of new opioid leads with potential for reduced tolerance and side effects.

Supported by P01 DA035764 and R21/R33 DA038858 from NIDA.

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

Selena S. Schattauer, Shao En Ong, Charles Chavkin

Department of Pharmacology, University of Washington School of Medicine, Seattle, WA

Long-lasting kappa receptor antagonism by norBNI is mediated by a JNK/PRDX6 mechanism that blocks guanyl nucleotide exchange from the receptor associated Gαi protein.

Inactivation of opioid receptors limits therapeutic efficacy, and mechanisms of arrestin-independent desensitization of GPCRs remain poorly understood. Prior studies demonstrated that the long-acting antagonism of KOR by norBNI and acute analgesic tolerance to morphine was mediated by cJun-N-terminal Kinase (JNK) activation. However the phosphorylation substrates and mechanisms were not defined and the molecular mechanisms responsible for kappa opioid receptor inactivation by norBNI treatment have been somewhat controversial. We used a Silac-based proteomic approach to isolate and identify proteins whose association with myc-tagged KOR expressed in HEK293 cells were affected by norBNI activation of JNK. NorBNI pretreatment (6 hr) significantly enhanced the association of KOR with $G\alpha_i\beta\gamma$. These results were confirmed by western analysis following co-immunoprecipitation and found to be dependent on c-Jun N-terminal kinase activation (JNK). Western analysis identified a similar increase in MOR- $G\alpha_i\beta\gamma$ association following morphine treatment (3 hr), which was also JNK-dependent. A subsequent proteomic study identifying proteins whose association with FLAG-$G\alpha_{i3}$ identified an enzyme peroxiredoxin 6 (PRDX6). To test the effect of norBNI-stimulated JNK on PRDX6 activity, we performed phospholipase enzyme activity assays with different cellular fractions. PRDX6 locally increased calcium-independent phospholipase A2 activity at the plasma-membrane in a JNK-dependent manner, and MJ33 (an inhibitor of PRDX6 phospholipase A2 activity) blocked the norBNI-enhanced association KOR and $G\alpha_i\beta\gamma$. Furthermore, norBNI-stimulated PRDX6 activity resulted in generation of reactive oxygen species. To determine if the palmitoylated cysteine on Gαi was a target of PRDX6, we used co-immunoprecipitation combined with the acyl-biotin exchange assay. NorBNI administration reduced the palmitoylation of receptor-associated Gαi, and the loss of palmitoylation was blocked by MJ33. In contrast, palmitoylation of KOR or Gαi not associated with KOR was unchanged. These data demonstrate an unexpected role for PRDX6 recruitment in receptor signaling and implicate G protein depalmitoylation in receptor inactivation. We propose that the loss of this lipid modification distorts the receptor-G protein association, thereby preventing agonist-induced nucleotide exchange and that this form of GPCR inactivation may be a general mechanism of GPCR regulation.

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

Ream Al-Hasani[1], Jenny M. Wong[2], Jordan G. McCall[1], Omar S. Mabrouk[2], Gavin Schmitz[1], Kirsten Porter-Stransky[3], Brandon Aragona[3], Robert T. Kennedy[2], Michael R. Bruchas[1].

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In vivo detection of optically released opioid peptides

We recently used an optogenetic approach to demonstrate that stimulation of dynorphinergic cells in the ventral nucleus accumbens shell (vNAcSh) elicits robust aversive behavior and photostimulation of dorsal NAcSh dynorphin (dNAcSh) cells induces a place preference and is positively reinforcing. Both of which appear to be dependent on kappa opioid receptor (KOR) activation. To follow these recently published findings, we are investigating how KOR is able to mediate these opposing behaviors in two distinct regions of the NAcSh. We are using an opto-microdialysis approach which combines optogenetics with microdialysis for use in awake, freely moving mice. This system allows quantification of neuropeptide release while directly modulating cell-type specific neuronal firing in the NAcSh. We have identified that the amount of dynorphin and met-enkephalin released during optogenetic stimulation is equal in the dNAc and vNAc. Interestingly, release of leu-enkephalin and dopamine is only detectable following photostimulation in the dNAc release. To further understand the circuitry driving the opposing unique behaviors and distinct neuropeptide release profiles, we are mapping the projections to and from discrete regions with the dyn-reporter mouse (dyn-Cre^{tdTomato}) and using tracing approaches (Rabies, canine adenovirus and cholera-toxin B). Thus far we have identified projections from the lateral septum, dorsal and ventral tegmental area (VTA). Together these experiments will help us understand how these distinct populations of dynorphin neurons in the NAcSh are engaged, altered, and recruited in stress and reward-related behaviors.

Supported by NIDA K99/R00 Pathway to Independence Award DA038725 (RA), R01-DA037152 EUREKA NIDA (MRB), NIDA R01DA037152 (MRB), NIMH F31MH101956 (JGM)

Disclosure: The authors have no conflicts of interest to disclose

The role of hypocretin (orexin) and dynorphin in reward and anxiety following chronic cocaine administration and withdrawal

Taylor A. Gentile[1], Steven J. Simmons[1], Mia Watson[1], Jessica Shaw[2], Rodrigo España[2], John W. Muschamp[1]

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Lateral hypothalamic orexins (hypocretins) have a role in arousal, reward processing, attention, and impulsivity. Dynorphin, the endogenous ligand of the kappa opioid receptor (KOR), co-localizes with orexin, and has critical roles in producing negative affective states through interactions with brain stress circuits. Orexin-dynorphin neurons project to structures that govern motivated behavior, including the bed nucleus of the stria termanalis (BNST), amygdala, locus coeruleus and ventral tegmental area (VTA). Orexin and dynorphin transmission modulates cell excitability through opposing signaling mechanisms; while orexins bind predominantly excitatory orexin-1 and -2 G_s- coupled receptors, dynorphins bind inhibitory G_i-protein coupled kappa opioid receptors (KORs). Several mental illnesses, including anxiety and substance abuse disorders, may be due in part to alterations in orexin-dynorphin signaling. The present experiments were conducted to explore the role of orexin and dynorphin activity in models of cocaine reward as well as the negative effects associated with cocaine withdrawal. To accomplish this we measured alterations in lateral hypothalamic orexin and dynorphin content in response to chronic cocaine administration and withdrawal using enzyme-linked immune-sorbent assays and cfos immunohistochemistry. Further, effects of chronic cocaine administration on reward and anxiety were assessed in using intracranial self-stimulation, conditioned place preference, and the elevated plus maze. Collectively, these studies will provide a comprehensive evaluation of how endogenous orexin and dynorphin peptide content becomes altered within substrates known to regulate positive and negative mood states at various phases of cocaine addiction.

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Stress-Induced Reinstatement of Nicotine Preference Requires Dynorphin/Kappa Opioid Activity in the Basolateral Amygdala

The dynorphin (DYN)/kappa-opioid receptor (KOR) system plays a conserved role in stress-induced reinstatement of drug seeking for prototypical substances of abuse. Due to nicotine's high propensity for stress-induced relapse, we hypothesized that stress would induce reinstatement of nicotine seeking-like behavior in a KOR-dependent manner. Using a conditioned place preference (CPP) reinstatement procedure in mice, we show that both foot-shock stress and the pharmacological stressor yohimbine (2 mg/kg, i.p.) induce reinstatement of nicotine CPP in a norbinaltorphimine (norBNI, a KOR antagonist)-sensitive manner, indicating that KOR activity is necessary for stress-induced nicotine CPP reinstatement. After reinstatement testing, we visualized robust c-fos expression in the basolateral amygdala (BLA), which was reduced in mice pretreated with norBNI. We then used several distinct but complementary approaches of locally disrupting BLA KOR activity to assess the role of KORs and KOR-coupled intracellular signaling cascades on reinstatement of nicotine CPP. norBNI injected locally into the BLA prevented yohimbine-induced nicotine CPP reinstatement without affecting CPP acquisition. Similarly, selective deletion of BLA KORs in KOR conditional knock-out mice prevented foot-shock-induced CPP reinstatement. Together, these findings strongly implicate BLA KORs in stress-induced nicotine seeking-like behavior. In addition, we found that chemogenetic activation of Gαi signaling within CaMKIIα BLA neurons was sufficient to induce nicotine CPP reinstatement, identifying an anatomically specific intracellular mechanism by which stress leads to reinstatement. Considered together, our findings suggest that activation of the DYN/KOR system and Gαi signaling within the BLA is both necessary and sufficient to produce reinstatement of nicotine preference.

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Pathway and Cell-Specific Kappa-Opioid Receptor Modulation of Excitatory-Inhibitory Balance Differentially Gates D1 and D2 Accumbens Neuron Activity

Abstract
Endogenous dynorphin signaling via the kappa-opioid receptor (KOR) in the nucleus accumbens (NAcc) powerfully mediates negative affective states and stress reactivity. Excitatory inputs from the hippocampus and amygdala play a fundamental role in shaping the activity of both NAcc D1 and D2 MSNs, which encode positive and negative motivational valences, respectively. However, a circuit-based mechanism by which KOR modulation of excitation-inhibition balance modifies D1 and D2 MSN activity is lacking. Here, we provide a comprehensive synaptic framework wherein presynaptic KOR inhibition decreases excitatory drive of D1 MSN activity by the amygdala, but not hippocampus. Conversely, presynaptic inhibition by KORs of inhibitory synapses on D2 MSNs enhances integration of excitatory drive by the amygdala and hippocampus. In conclusion, we describe a circuit-based mechanism showing differential gating of afferent control of D1 and D2 MSN activity by KORs in a pathway specific manner.

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Effects of mesyl salvinorin B, naltrexone, nalmefene or nor-BNI on alcohol escalation, "relapse" and "binge" drinking in male and female mice

Mesyl Salvinorin B (MSB) is a potent selective kappa opioid receptor (KOP-r) agonist that has potential for development as an anti-psychostimulant agent with fewer side-effects (e.g., sedation, depression and dysphoria) than classic KOP-r agonists. First, we investigated whether MSB alone or in combination with naltrexone (mu-opioid receptor [MOP-r] antagonist), or nor-BNI (KOP-r antagonist) alone altered voluntary alcohol drinking in both male and female C57BL/6J mice subjected to 3 weeks of intermittent access chronic escalation drinking (IACED, 15% alcohol vs water in a two-bottle choice with 24-h access every other day) – a model of alcohol "dependency" with high intake (20-30 g/kg/day). We found that: [1] single, acute administration of MSB (0.3-3 mg/kg) or naltrexone (1-2 mg/kg) alone dose-dependently reduced alcohol intake and preference in both male and female mice. The effect was specific to alcohol and without anhedonic effect, suggested by the lack of any effect of MSB or naltrexone on sucrose or saccharin intake; [2] MSB reduced alcohol drinking in a KOP-r dependent manner, as the selective KOP-r antagonist nor-BNI (5 mg/kg) blocked the effects of MSB (3 mg/kg) on alcohol drinking in female mice (the dose of nor-BNI alone did not alter alcohol intake per se); [3] when alcohol was presented again after a 1-week abstinence from IACED, female mice displayed significant increases in alcohol intake (alcohol deprivation effect [ADE] or relapse-like drinking), and pretreatment with MSB (3 mg/kg) prevented the ADE; [4] MSB (a longer-lasting relative to its parent compound Sal A) did not alter spontaneous locomotor activity in either male or female mice, different from many traditional KOP-r agonists with sedative effects; [5] upon investigation of potential synergistic effects between MSB and naltrexone, we found that acute administration of a combination of naltrexone (1 mg/kg) + MSB (0.3 mg/kg, the dose 10 times lower than the effective dose) reduced alcohol intake profoundly after the 3-week IACED, and repeated 5 administrations of this combination led to less tolerance development than repeated MSB (3 mg/kg) alone in both males and females; and [6] single, acute nor-BNI (1-10 mg/kg) reduced alcohol intake in males but not females after the 3-week IACED. Finally, using the drinking-in-the-dark (DID) model with limited access (4 h/day, 15% alcohol in one bottle) to mimic "binge" drinking, we also evaluated the pharmacological effect of MSB, naltrexone and nalmefene (selective MOP-r antagonist with partial KOP-r agonism), and found that naltrexone (0.5-5 mg/kg) and nalmefene (0.5-2 mg/kg) decreased DID, while MSB (1-3 mg/kg) had no effect in either sex. Our study suggests that the novel KOP-r agonist MSB, both alone and in combination with naltrexone, shows potential in alcoholism treatment models.

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The role of ventral striatal kappa-opioid receptors in the motivational / emotional symptoms of alcohol dependence.

Alcohol dependence is accompanied by physiological withdrawal symptoms and negative affect during withdrawal, which have been hypothesized to be the basis for an alcohol self-medication hypothesis where alcoholics escalate their alcohol use to alleviate withdrawal. Therefore, attenuation of withdrawal-induced negative affect could reduce escalated alcohol consumption. The kappa-opioid receptor/dynorphin (KOR/DYN) system has been implicated in alcohol withdrawal-induced escalated drinking and negative affect. Specifically, KOR-antagonists attenuate both escalated alcohol drinking and negative affect in dependent rodents. Previous data from this lab has demonstrated that the KOR antagonist nor-binaltorphimine (nor-BNI) reverses alcohol withdrawal-induced escalation of rat 22-kHz ultrasonic vocalizations (USVs) when administered into the lateral ventricles. Measurement of 22-kHz USVs is an ethologically valid strategy used to assess negative affective states in rats. Previous evidence has implicated KORs in the nucleus accumbens (Acb) shell in escalated alcohol consumption observed in alcohol dependent rodents during acute withdrawal. Therefore, this study aimed to assess the effects of intra-Acb nor-BNI on escalated alcohol self-administration, USVs, immobility in the forced swim test (FST), open-arm time in the elevated plus maze (EPM) and physiological withdrawal signs during acute withdrawal. Male Wistar rats were trained to self-administer alcohol, after which Acb shell guide cannula were surgically implanted. After recovery, animals underwent dependence induction via inhalation of intermittent alcohol vapor for 4 weeks. Following dependence induction, rats were again allowed to self-administer alcohol during acute withdrawal until stability was achieved. Nor-BNI (0, 2, or 6 ug) was infused into the Acb shell and dose-dependently decreased escalated alcohol consumption and 22-kHz USVs (in the absence of changes in FST and EPM performance), but had no effect on physiological withdrawal scores. The results demonstrate that KOR/DYN activation in the Acb in alcohol-dependent rats is necessary for ethologically-valid withdrawal-induced negative affect-like states and escalated alcohol self-administration, but not physiological withdrawal signs. These data further demonstrate that the neurobiological substrates of motivation/negative affect that drive maladaptive behavioral phenotypes in alcohol dependence can be dissociated from those related to physiological withdrawal and provide possible strategies for developing novel pharmacotherapies to treat alcohol use disorders.

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Adolescent social isolation-potentiated kappa opioid receptor function augments cocaine seeking in adulthood

Exposure to chronic stress during early-life augments drug- and cue-induced cocaine craving as well as severity of withdrawal symptoms, potentially contributing to cocaine addiction in humans. Similarly, in rodents, adolescent social isolation (aSI) results in elevated cocaine seeking compared to rats that are group housed during adolescence (aGH). aSI results in a chronic upregulation of KOR function in adulthood. Additionally, acute KOR activation time-dependently modulates cocaine reward processing. Thus, the goal of this study was to compare the reinforcing efficacy of cocaine in aSI and aGH animals and to investigate the underlying mechanisms involved in driving the augmented cocaine seeking in aSI rats. All experiments were conducted following a 6-week housing manipulation, during which rats were either housed in groups (aGH; 4 rats/cage) or individually (aSI; 1 rat/cage). Systemic administration of cocaine (15 mg/kg; i.p.) resulted in an augmented locomotor response to cocaine in aSI compared to aGH rats. Jugular catheters were implanted and after a recovery period of 2 days, rats were given access to levers to self-administer cocaine using various schedules of reinforcement. Self-administration using a progressive ratio schedule showed an enhanced motivation to self-administer cocaine in aSI compared to aGH rats (mean final ratio: aGH, 77; aSI, 268; 2.25 mg/kg/infusion). Threshold analysis, during which the cocaine dose administered for each lever press is systematically reduced in ten minute bins, showed that rats exposed to aSI were willing to “pay a greater price” to maintain their level of cocaine intake compared to aGH (lever presses/mg of cocaine: aGH, 200; aSI, 500). Administration of nor-binaltorphimine (nBNI; 10 mg/kg; i.p.), a KOR antagonist, reduced this enhanced motivation to self-administer cocaine selectively in aSI rats (lever presses/mg of cocaine post nBNI: aGH, 198; aSI, 210). Interestingly, cocaine consumption in the two groups was not different. This suggests that the reinforcing value of cocaine is greater for aSI compared to aGH rats, thereby increasing the risk of developing cocaine use disorder. The KOR inhibition-induced attenuation of cocaine seeking suggests that KORs may contribute to the potentiated motivation to self-administer cocaine. Thus, KORs are promising therapeutic targets to treat cocaine addiction especially in individuals exposed to childhood adversities and are therefore predisposed to cocaine addiction.

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Negative affect-like behavior and kappa opioid receptor function in the NAc during protracted abstinence from ethanol in mice

Alcohol abuse and alcoholism are often accompanied by disorders of negative affect – such as depression and anxiety, particularly during periods of withdrawal or abstinence. It has long been hypothesized that increased activity of the kappa opioid receptor (KOR) system instigated by extensive alcohol (ethanol, EtOH) exposure contribute to these affective states, and may confer vulnerability to relapse. KOR activation inhibits dopamine (DA) release, and in general hypodopaminergia is thought to be characteristic of withdrawal from EtOH. Indeed, our lab and others have shown that KORs appear to show supersensitivity after four weeks of chronic intermittent ethanol (CIE) vapor exposure. Immediately and 72 hours after final EtOH exposure, the DA-decreasing effect of KOR activation by the agonist U50,488 was augmented compared to control animals as measured by fast scan cyclic voltammetry (FSCV) in mouse brain slices containing the nucleus accumbens (NAc). Additionally, basal stimulated DA release is reduced and DA reuptake is faster in the NAc, contributing to putative hypodopaminergia. At the 72h time point, negative affect-like behavior in the marble burying task is observed, and this behavior is rescued to baseline levels with the administration of the KOR antagonist, norbinaltorphimine (norBNI). In this study, we observed negative affect-like behavior in the novelty-suppressed feeding test (NSFT) after 10 days of abstinence from 4 weeks of CIE. Further, decreased stimulated DA release in the NAc after CIE is maintained as long as 3 weeks after EtOH exposure. However, the sensitivity of KORs to agonist stimulation are returned to baseline levels after 3 weeks of abstinence from CIE. We then wondered if previous exposure to the 4 week CIE paradigm would alter the KOR response to agonist stimulation after a single acute 16h EtOH vapor exposure after protracted withdrawal. We observed that animals with a history of CIE exposure are resistant to augmented KOR function after a single EtOH exposure following 2 weeks of abstinence, but control animals show a marked increase in KOR responsiveness after only a single 16h EtOH exposure. Together these data suggest that animals exposed to CIE have altered DA dynamics and abnormal, though varying, function of KORs during both early and late abstinence periods.

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Variable opioid activity arising from ring substitution in CJ-15,208 and characterization of an analog that blocks stress-induced reinstatement of extinguished cocaine-conditioned place preference.

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Unlike linear peptides, the macrocyclic tetrapeptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) is stable to proteases and exhibits mixed opioid receptor agonism and kappa opioid receptor (KOR) antagonism, making it a promising lead compound for potential development of treatments for pain and drug abuse. As part of our exploration of the structure-activity relationships of this peptide, we incorporated different substitutions on the aromatic residues and assessed the opioid activity profiles of the resulting analogs. The substitutions only modestly altered binding affinity for the KOR, as determined with radioligand binding assays *in vitro*. In contrast, when examined *in vivo* after intracerebroventricular (i.c.v.) administration in the mouse 55 ^{o}C warm water tail withdrawal assay, the magnitude of antinociception and opioid antagonist activity varied widely depending on the substitution. Most notably, incorporation of a fluorine on either phenylalanine residue abolished KOR antagonism. An analog with a different substitution exhibited potent antinociception, with an ED_{50} (and 95% C.I.) value of 2.25 (1.52-3.26) nmol, i.c.v. and subsequent antagonism of all three opioid receptors lasting less than 6 hours. Pretreatment with this analog dose-dependently prevented stress-induced reinstatement of extinguished cocaine-conditioned place preference and a 30 nmol i.c.v. dose did not significantly impair respiration in the Comprehensive Lab Animal Monitoring System (CLAMS). However, this dose of the compound did exhibit modest sedation in the rotorod assay and conditioned place aversion after acute administration. Together, these results advance our understanding of the structure-activity relationship of these macrocyclic tetrapeptides and our search for potential treatments with improved liability profiles that can be used to prevent relapse to drug-seeking behavior.

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Role of dynorphin/kappa opioid system in the formation of aversive memory associated with morphine withdrawal

Aversive memories of drug withdrawal can generate a motivational state leading to compulsive drug taking. Dynorphin/Kappa opioid sytem is widely implicated in the regulation of emotion and motivation. Both human and animal studies demonstrated that kappa receptor activation produced dysphoria effect and anxiety- or prodepressive-like effects. However, the role of dynorphin/kappa opioid receptor system in the formation of aversive memories of drug withdrawal was unclear. In the present work, we found that two pairing with naloxone after morphine exposure produced significant aversion for the withdrawal-associated environment in morphine treated mice. Pretreatment with kappa receptor antagonist norBNI before pairing with morphine withdrawal markedly attenuated conditioned place aversion. The underlying mechanism was being elucidated. Thus, our preliminary data demonstrated that dynorphin/kappa opioid receptor system contributed to aversive memory formation of drug withdrawal.

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Comparison of the punishing effects of nalfurafine and salvinorin A on cocaine and oxycodone self-administration in rhesus monkeys

We previously reported that salvinorin A can punish cocaine self-administration in rhesus monkeys in a dose-dependent manner when it is administered contingently with cocaine injections in a drug vs. drug choice procedure. We have extended this work by testing the ability of nalfurafine, an atypical kappa agonist that does not produce dysphoric or psychotomimetic effects, to punish cocaine self-administration in drug vs. drug choice and progressive-ratio designs. Salvinorin A, when combined with cocaine, punished cocaine choice in a dose-dependent manner. However, nalfurafine was not effective as a punisher of cocaine choice, even at doses that produced overt sedation. When tested under a progressive-ratio schedule of reinforcement, both salvinorin A and nalfurafine, when administered contingently with cocaine and oxycodone, respectively, reduced drug self-administration in a dose-dependent manner. These results indicate that salvinorin A can reduce drug self-administration in a manner that is consistent with a punishment mechanism, but that nalfurafine may reduce drug-taking via another behavioral mechanism (e.g., direct effects). Potential roles for varying pharmacodynamic and kinetic profiles in the differences observed for the kappa agonists will be discussed.

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In Vivo Kappa Opioid Receptor Binding Assessed by PET Imaging in Major Depressive Disorder

Abstract: Background: The pathophysiology of major depressive disorder (MDD) remains inadequately understood, and existing treatments often fail. In animal models, endogenous kappa opioids are stimulated by corticotropin releasing factor and mediate pathological responses to stress. The relationship of the kappa opioid receptor (KOR) to life stress and psychopathology is understudied in humans.

Methods: KOR binding *in vivo* was quantified by PET imaging with [^{11}C]GR103545 in 13 healthy volunteers and 10 unmedicated patients with current MDD. We examined the relationship between KOR binding and diagnosis, depression severity, life stress measures, and salivary cortisol levels during a modified Trier Social Stress Test (mTSST), in four *a priori* regions of interest (ROIs: amygdala, hippocampus, ventral striatum, raphe nuclei) using linear mixed-effects models. Regional [^{11}C]GR103545 volumes of distribution (V_T) were estimated using Likelihood Estimation in Graphical Analysis with a metabolite-corrected arterial input function. Whole-brain voxelwise analyses were also performed.

Results: [^{11}C]GR103545 V_T did not differ between MDD and healthy volunteers in the 4 *a priori* ROIs ($p=0.48$). A trend-level inverse relationship was observed between V_T and cortisol areaunder-the curve during the mTSST ($p=0.085$). Within the MDD group, [^{11}C]GR103545 V_T was unrelated to childhood trauma ($p=0.72$), number of recent negative life events ($p=0.65$), or depression severity assessed with the Beck Depression Inventory (BDI) in these ROIs ($p=0.57$). Whole-brain voxelwise analyses will be presented as well.

Conclusions: KOR binding does not differentiate MDD subjects from healthy volunteers when considered categorically in unmedicated MDD in *a priori* regions of interest. The kappa opioid system appears to be coupled with hypothalamic-pituitary-adrenal (HPA) axis tone in humans. Further study on the role of KOR in the pathophysiology of depression is warranted.

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Asimadoline, a Selective Kappa-Opioid Receptor Agonist for the Treatment of Atopic Dermatitis: Preliminary Safety Results of a Proof-of-Concept Trial

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Kappa-opioid receptor agonists may be broadly effective for the treatment of chronic itch. Suppression of scratching behavior has been demonstrated in murine models after intradermal injection of histamine, substance P, and intracisternal injection of morphine, among other pruritogens. The kappa-agonist nalfurafine (Remitch®), is approved in Japan for uremic and cholestatic pruritus. Asimadoline is a novel, orally active, kappa-opioid receptor agonist with approximately 500-fold greater affinity for the human kappa-receptor as compared to delta- or mu-opioid receptors. Asimadoline is being investigated in a double-blind, placebo-controlled study in subjects with atopic dermatitis and itch severity of ≥40 on a 100mm Visual Analog Scale (VAS) of Itch Intensity. A seven-day blinded (to subject) placebo run-in period is followed by a four-week randomized, controlled trial (RCT) of asimadoline 2.5mg orally twice daily (BID) compared with identical placebo. The RCT is followed by a four-week open-label extension (OLE). Up-titration to 5mg BID in the RCT and OLE is permitted, based on standardized criteria. The primary efficacy outcome measure is change in average worst VAS from baseline, taken at the end of the placebo run-in period, versus the end of the RCT. Additional outcome measures include the EASI, SKINDEX-10, TIS (three-item severity, with photo-documentation), Patient-Oriented Eczema Measure, 5-D pruritus scale, safety laboratory results, and adverse events. Blinded safety results are available for 208 subjects. There have been no drug-related serious adverse events, and no drug-related early terminations. Incidence of adverse drug reports (ADRs) is 8.2% (23 events in 17 subjects; none severe), which compares favorably to the ADR incidence reported with nalfurafine (25% for the commercialized dose). Detailed safety results will be presented. Efficacy results will be available in Q3 2017.

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Title: A Randomized, Double-Blind, Placebo-Controlled Study Examining the Selective Kappa Antagonist, CERC-501, in a Human Laboratory Model of Smoking Behavior

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Background: Animal data indicate that selective kappa opioid receptor antagonists produce antidepressant and anxiolytic effects, decrease drug self-administration and escalation, and reduce behaviors and signs of withdrawal from nicotine and other drugs. CERC-501 is an orally active, highly selective kappa antagonist. The aim of the current study was to determine if treatment with CERC-501 could alleviate nicotine withdrawal, craving and the urge to smoke following the McKee Smoking Lapse Model.
Methods: Otherwise healthy, non-treatment seeking adult cigarette smokers were enrolled into this multi-site, within-subject, randomized, double-blind, placebo-controlled crossover study. Participants completed two randomized treatment blocks, CERC-501 (15 mg, p.o, once daily) and matched placebo, administered on an outpatient basis for 7 days. On the 7th day of dosing in each block, participants were admitted as inpatients for an 18-hr cigarette abstinence period followed by a smoking session in the laboratory. The primary outcome measures were 1) time to first cigarette, and 2) the number of cigarettes self-administered during a 60-min *ad lib* period.
Results: A total of 71 participants were enrolled (38 F, 33 M) of which 56 completed both treatment blocks. CERC-501 was well tolerated. CERC-501 did not significantly alter the latency to start smoking after deprivation (CERC-501: 15.5 mins; placebo: 18.8 mins) or the number of cigarettes self-administered during the 60-min ad lib period (CERC-501: 3.3 cigarettes; placebo: 3.1 cigarettes). There were no significant effects of CERC-501 compared to placebo on measures of cigarette craving, mood, anxiety, nicotine withdrawal or subjective effects of cigarette smoking relative to placebo.
Conclusions: CERC-501 did not affect smoking behaviors or measures of nicotine withdrawal and craving or mood during a period of smoking abstinence in smokers not seeking treatment. These data are not consistent with preclinical studies and do not support a role for CERC-501 in the treatment of acute nicotine withdrawal.

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Treatment of Resistant Depression with a Samidorphan/Buprenorphine (ALKS 5461), a Balanced Agonist-Antagonist Opioid Modulator

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Currently approved anti-depressive agents function primarily as serotonin or serotonin/norepinephrine re-uptake inhibitors (SSRIs and SNRIs). Despite the widespread availability of SSRIs and SNRIs, a significant proportion of patients with major depressive disorder (MDD) fail to respond or experience only a partial clinical response with these agents. Anti-depressive agents with alternate or complementary mechanisms of action are therefore urgently needed. Both pre-clinical and human research has revealed evidence of underlying endogenous opioid dysregulation in the context of MDD. Recent clinical findings in the treatment of endogenous opioid dysregulation in MDD will be reviewed including an update on the development of ALKS 5461 for the adjunctive treatment of patients with MDD and an inadequate response to SSRIs or SNRIs.

Disclosure: These studies were funded by Alkermes.

Repeated administration of a novel selective KOP-r antagonist, LY2456302, in normal volunteers and persons with cocaine dependence diagnosis: Neuroendocrine and behavioral profile

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There is only limited data available on the impact of selective KOP-r antagonism in humans. This study examined neuroendocrine and behavioral effects of a novel short-acting selective KOP-r antagonist, LY2456302 (now CERC-501), in normal volunteers and in volunteers diagnosed with cocaine dependence (DSM IV).

Procedures: Recruiting, informed consents and procedures were approved by the Rockefeller University IRB, and by an Investigator-initiated FDA IND (MJK). LY2456302 (5 mg capsules, produced under cGMP) were kindly provided by Eli Lilly Co..

Participants: Adult males and females, normal volunteers (NV; n=40), or with a cocaine dependence diagnosis (CD; n=30). After admission and overnight stress minimization, participants underwent the following protocol: Day 1: baseline, Days 2-5: LY2456302, 10 mg daily, orally. This dose of LY2456302 is sufficient to occupy KOP-r in humans, from target engagement studies. Neuroendocrine measures were obtained on Day 1 (baseline), and Days 2 and 5 (e.g., blood prolactin and cortisol levels; -30 to +480 min from LY2456302 administration). Behavioral measures, including scales for mood and craving, were also obtained.

Results: There were no substantial changes in vital signs. Only mild to moderate adverse events were observed (e.g., nausea or pruritus). LY2456302 did not cause changes to prolactin serum levels. LY2456302 caused a transient increase in cortisol levels in NV and CD, from 120 min after administration. LY2456302 did not produce changes in mood or “drug effects” VAS scales, or in the cocaine craving questionnaire-brief (CCQ-brief).

Conclusions: Repeated daily administration (4 days) of an active dose of the novel KOP-r antagonist LY2456302 was achieved in male and female NV and volunteers with cocaine dependence diagnosis, with only mild to moderate adverse events. Under stress-minimized inpatient conditions, LY2456302 did not produce robust subjective effects. The lack of prolactin-releasing effects of LY2456302 suggests that it does not have KOP-r partial agonist effects in humans.

Support:

Adelson Medical Research Foundation, and NIH-CCTS grants to the Rockefeller University Hospital.

Conflict of Interest:

The authors declare no conflict of interest

Carroll FI, Kormos CM, Ondachi PW, Runyon SP, Navarro HA, Thomas JB, Mascarella SW

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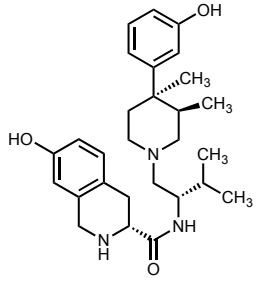
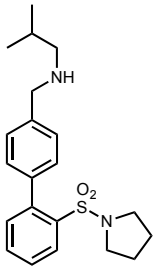
Design, synthesis, and in vitro pharmacological evaluation of novel kappa opioid receptor antagonists

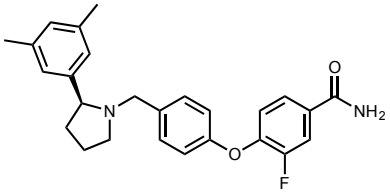
The opioid receptors belong to the super family of G-protein coupled receptors (GPCRs). The majority of research efforts have been directed towards the μ opioid receptor (MOR). However, over the last several years it has become increasingly clear that the entire family of opioid receptors (μ, δ, and κ) are actively involved in a host of biological processes. For example studies with selective κ opioid receptor (KOR) antagonists have shown that this system is involved in brain processes that relate to stress, fear, and anxiety as well as reward seeking behavior. For example in animal model paradigms KOR antagonists reduce immobility in the forced-swim assay, reduce exploratory behavior in the elevated plus maze and fear-potentiated startle, reduce stress-induced reinstatement of cocaine self-administration, block stress-induced potentiation of cocaine place preference conditioning, decrease dependence-induced ethanol self-administration, attenuate the expression of both the physical and effective signs of nicotine-induced withdrawal, and prevent prepulse inhibition mediated by a selective KOR agonist. At present three KOR antagonists (JDTic, PF4455242 and LY2456302) have reached clinical studies and LY2456302 is the only KOR antagonist still in clinical development. Here we present our research directed toward the design, synthesis, and in vitro evaluation of the novel KOR antagonist having the general structure **1**. RTI-5989-473, which has a K_e value of 0.058 nM at the KOR receptor in a [^{35}S]GTPγS binding assay with 5900- and 27,000-fold selectivity relative to the MOR and DOR, respectively, is the most potent and KOR selective antagonist developed thus far. Four other compounds have K_e values of 0.14-0.37 nM at the KOR and are more than 292-fold selective for the KOR relative to both the MOR and DOR. The SAR of these compounds plus several more will be presented.



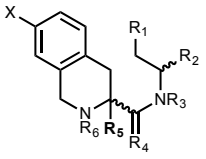
JDTic



PF-4455242



LY2456302



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

Zamora, J. C[1], Chavera, T.[1], Jennings, E. M[1], Johnson, S.N[2], Prisinzano, T. E[2], Berg, K.A[1], Clarke, W. P[1]

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Functional selectivity of U50,488 analogues at Kappa Opioid Receptors (KOR) expressed in peripheral pain-sensing neurons.

Functional selectivity describes the ability of agonists to differentially regulate individual signaling pathways coupled to a given receptor. By fine-tuning the structure of a ligand, the activity of therapeutically efficacious signaling can be enhanced whereas signaling that leads to adverse effects can be minimized. Here, we evaluated the ability of three structural analogues (SJ-1-147, SJ-1-163, and SJ-1-171) of the selective KOR-agonist U50,488, to differentially activate pathways known to regulate peripheral KOR-mediated antinociception. In both primary cultures of adult rat peripheral sensory neurons and in CHO cells transiently expressing rat KOR, all three analogues inhibited PGE2-mediated cAMP accumulation with similar potencies and efficacies to U50,488. However, unlike U50,488, all three analogues did not increase the activity of extracellular signal regulated kinase (ERK). In primary cultures of rat peripheral sensory neurons, U50,488 inhibited PGE2/Capsaicin-mediated CGRP release. However, the concentration response curve (CRC) was U-shaped, but rendered monotonic in the presence of the ERK inhibitor, U0126. By contrast, the CRCs for inhibition of CGRP release were monotonic (not U-shaped) for the test compounds, which is consistent with the loss of ERK signaling by the structural modifications. In a rodent model of thermal nociception, intraplantar (i.pl.) injection of peripherally-restricted doses of U50,488 produced antinociception with an inverted U-shaped dose response curve (DRC). By comparison, injection (i.pl.) of SJ-1-147 produced antinociception with a peak magnitude similar to U50,488, however, the DRC for 1-147 was monotonic. Overall, our data suggest that structural modifications of a KOR ligand can selectively alter its efficacy for individual signaling cascades, which may lead to improved therapeutic outcomes for peripherally-mediated analgesia.

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Disclosure: The authors have no conflicts of interest to disclose

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Nalfurafine decreases the reinforcing effects of oxycodone while producing additive thermal antinociception in male rats.

AIMS: Strategies to reduce the misuse of mu opioid agonists are critically needed. One approach for developing safer mu opioid analgesics involves combining the medication with a deterring agent that produces effects counter to reinforcement if overconsumption occurs. Kappa opioid receptor agonists may be useful in this application, as they have been shown to produce both reductions in drug reinforcement and antinociceptive effects. However, use of traditional kappa agonists is limited by their dysphoric and psychotomimetic side effects. It is currently unclear whether nalfurafine, an atypical kappa agonist available clinically, can reduce the abuse-related effects of a mu opioid agonist (i.e., oxycodone) while augmenting its antinociceptive effects.
METHODS: In Experiment 1, a progressive-ratio (PR) self-administration procedure was used to compare the reinforcing effects of intravenous oxycodone (0.056 mg/kg/inj) available alone or as a mixture with increasing doses of co-administered nalfurafine (0.32-3.2 µg/kg/inj). The proportions of oxycodone to nalfurafine for the three nalfurafine doses tested in Experiment 1 were 175:1, 56:1 and 18:1. In Experiment 2, full PR dose-effect functions were determined for oxycodone alone, nalfurafine alone, and for oxycodone/nalfurafine mixtures with these same fixed proportions of oxycodone to nalfurafine. Experiment 3 compared thermal antinociception dose-effect curves produced in a hot-plate test by oxycodone, nalfurafine, and the three mixtures using the same route of administration as Experiments 1 and 2 (intravenous).
RESULTS: Nalfurafine dose-dependently decreased the reinforcing effects of oxycodone in Experiment 1. In Experiment 2, rats earned significantly fewer injections of the 18:1 mixture relative to oxycodone alone. Furthermore, nalfurafine alone and the 18:1 mixture did not function as reinforcers at any dose tested. In Experiment 3, oxycodone and nalfurafine produced dose-dependent antinociception when administered alone, and the mixtures produced additive antinociceptive effects.
CONCLUSIONS: These results suggest that the addition of nalfurafine could decrease the abuse liability while augmenting the analgesic effect of oxycodone.

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

Conformationally restricted κ-opioid receptor agonists

Bernhard Wünsch

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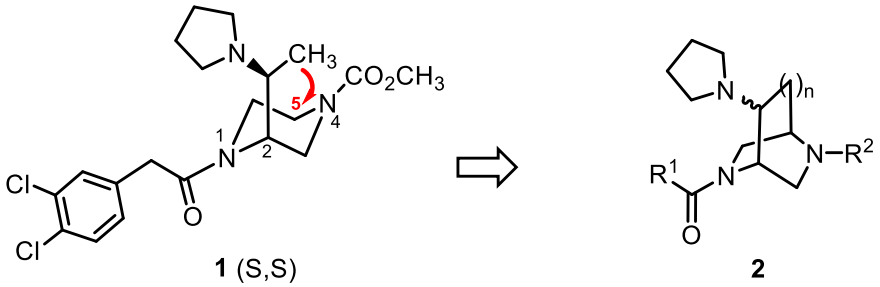
Activation of the three classical opioid receptors, μ-, δ-, and κ-opioid receptors, leads to strong analgesia. The clinically used strong analgesics are more of less selective μ receptor agonists and therefore associated with severe side effects such as respiratory depression, euphoria, constipation and development of tolerance and dependency. Activation of κ receptors is not associated with the dangerous μ agonistic side effects including respiratory depression and addiction, but centrally mediated dysphoria, sedation and strong diuresis have been reported as κ agonistic side effects. In addition to analgesic effects, it has been shown that the κ receptor is involved in the development of depression, autoimmune disorders and neurological diseases. In particular, it seems to play a crucial role in multiple sclerosis.

In this project, we aim at conformationally restricted κ agonists with an additional N-atom outside the κ pharmacophore allowing the fine-tuning of the pharmacokinetic properties of the ligands. Moreover, the conformational restriction should allow the analysis of the bioactive conformation of the ethylenediamine pharmacophore of κ agonists.

The piperazine derivative **1** belonging to the ethylenediamine class of κ agonists (K_i = 0.31 nM) represents the starting point of this project. The conformational flexibility of the side chain will be restricted by connecting the methyl moiety of the pyrrolidinylethyl side chain with the 5-position of the piperazine scaffold. In the lecture, the synthesis and pharmacological evaluation of bicyclic κ agonists of type **2** will be discussed. The substituent R^2 at the second piperazine N-atom outside the κ pharmacophore will allow diverse modification at this position.



1 (S,S) **2**

The project was supported by the DFG (Deutsche Forschungsgemeinschaft)

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

Mosier PD[1], Ma H[2], Brust T[3], Frankowski KJ[2], Bohn LM[3], Aubé J[2].

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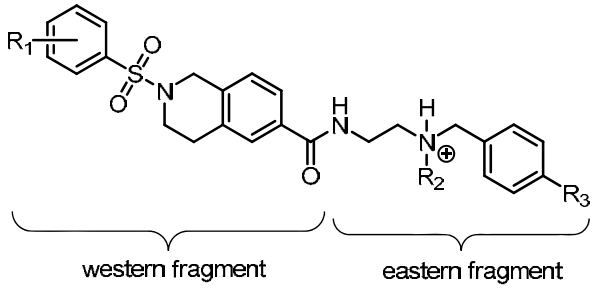
Structure–activity relationships of cyclized ML140 analogues at the kappa opioid receptor, and why butyl is not necessarily futile.

To provide further insight into the molecular recognition and signaling properties of the kappa opioid receptor (KOR), molecular modeling techniques combined with competitive binding and functional assays were employed to understand how a series of synthetically accessible antagonist probes, cyclized tetrahydroisoquinoline analogues of ML140, are able to both bind to the KOR with high affinity and inhibit KOR signaling.



Compounds were assessed for their affinity (K_i) and potency (IC_{50}) in [^{35}S]GTPγS-, ERK- or β-arrestin2-based assays and were shown to have a range of intercorrelated potencies. Interestingly, replacement of *i*-propyl with *t*-butyl at the R_2 position generally enhanced the compounds' potencies in all functional assays. Computationally, the compounds were docked to three populations of KOR models that were based on either 1) the crystal structure of the KOR in complex with antagonist JDTic (PDB: 4DJH), 2) homology models based on the crystal structure of the MOR in complex with antagonist β-FNA (PDB: 4DKL), or 3) homology models based on the activated structure of MOR in complex with BU72 (PDB: 5C1M). Interestingly, the models best able to explain the SAR for this set of compounds were KOR homology models built from the MOR–β-FNA structure, indicating that a) the conformation of the receptor in the JDTic–KOR co-crystal structure does not readily recognize this class of compounds, and that b) the functional state of the receptor influences its ability to recognize functionally similar ligands, corroborating previous studies of GPCR–ligand interaction. Analyses of the docking solutions revealed that the 'western fragment' interacts primarily with the extracellular half of TM2, while the 'eastern fragment' binds to a centrally-located region of TM3 and TM6, whose movement is thought to be essential in the activation process of class A GPCRs. This binding mode also places the R_2 substituent within a hydrophobic pocket circumscribed by Y3.33, M3.36 and I6.55, and the additional methyl group of *t*-butyl compared to *i*-propyl enhances the hydrophobic interaction with the receptor, presumably increasing its affinity and potency. Taken together, these results represent potentially significant insights into the activation processes of the KOR.

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

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Kappa opioid receptor antagonism mitigates stress effects on sleep and circadian rhythms in male mice

Stress plays a critical role in the neurobiology of mood and anxiety disorders. Sleep and circadian rhythms are affected in many of these conditions. Here, we examined the effects of chronic social defeat stress (CSDS), an ethological form of stress, on sleep and circadian rhythms. We exposed male mice implanted with wireless telemetry transmitters to a 10-day CSDS regimen previously shown to produce anhedonia (a depressive-like effect) and social avoidance (an anxiety-like effect). Electroencephalography (EEG), electromyography (EMG), body temperature, and motor activity data were collected continuously during the CSDS regimen and a 5-day recovery period. CSDS affected numerous metrics, including Paradoxical (Rapid-Eye Movement [REM]) and Slow Wave (non-REM [NREM]) sleep, as well as the circadian rhythmicity of core body temperature and motor activity. The magnitude of the effects tended to increase with repeated stress, and some effects (REM bouts, NREM time) persisted after the CSDS regimen had ended. CSDS was also associated with altered mRNA levels of circadian rhythm-related and stress-associated genes within brain areas that regulate motivation and emotion. Pretreatment with the long-lasting kappa-opioid receptor (KOR) antagonist JDTic (30 mg/kg, IP) before the CSDS regimen attenuated effects on sleep and circadian rhythms, or hastened their recovery. Our findings show that CSDS produces persistent disruptions in sleep, thereby mimicking key attributes of stress-related conditions as they appear in humans. The ability of KOR antagonists to mitigate these disruptions is consistent with previously reported anti-stress effects. Studying homologous endpoints across species may facilitate the development of improved treatments for psychiatric illness.

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Disclosure: Dr. Carlezon has a US patent on the use of kappa-opioid antagonists to treat depression. Within the last 2 years he has served as a consultant for Cerecor. Dr. Wells has nothing to disclose.

Margolis, EB[1,2], Driscoll JD[3,1], Fields HL[1,2,3].

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Corticotrophin Releasing Factor Alters Kappa Opioid Receptor Function in the Ventral Tegmental Area.

Stress can activate neural circuits associated with fear, anxiety, attention, motivation, and memory. Depending on the degree or type, stress can be associated with either positive (rewarding) or negative (aversive) behavioral outcomes. During stress, corticotrophin releasing factor (CRF) is released into the ventral tegmental area (VTA), which increases dopamine signaling in VTA terminal regions including the nucleus accumbens (NAc). CRF also leads to kappa opioid receptor (KOR) activation in brain areas that are involved in stress, resulting in conditioned place aversion (Land et al. 2008, Bruchas et al. 2009). Understanding the interaction of CRF and the kappa opioid system may help to dissociate the negative deleterious aspects of stress from those that allow for positive physiological adaptation. We previously showed that the KOR agonist U69593 selectively hyperpolarizes VTA dopamine neurons that project to the amygdala or prefrontal cortex but not those projecting to the NAc (Margolis et al., 2006; 2008). Here we used whole cell *ex vivo* electrophysiology to examine how CRF modulates responses to U69593 in VTA neurons. In current clamp (I=0 pA), baseline KOR responses were evaluated with U69593 (1 uM), then CRF was bath applied for 5-7 min and washed out for at least 5 min before a second U69593 application. Surprisingly, 13/22 neurons that showed initial U69593 hyperpolarizations later showed U69593 induced depolarizations (9 neurons) or no longer responded (4 neurons) following CRF application; the remaining 9/22 maintained their U69593 induced hyperpolarizations. Excitations were independent of the magnitude of the initial U69593 response and the effect of CRF itself on the cell. In a second set of recordings, where neurons were not selected based on their initial sensitivity to U69593, 8/27 neurons were excited by U69593 after CRF exposure and 12/27 were inhibited. In summary, CRF exposure robustly altered KOR induced signaling in a subset of VTA neurons, causing a switch from inhibition to excitation. These results provide insight into how stress may profoundly alter neuronal responses to KOR activation in mesolimbic neurons.

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

Pain-Induced Alterations In Motivational States Are Mediated Via Upregulation Of The Accumbal Kappa Opioid Receptor System.

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The mesolimbic pathway, involved in reinforcing and motivational properties of rewards, undergoes long term changes in the presence of pain. The kappa opioid receptor (KOR), controlling dopamine release in the nucleus accumbens (NAc), can be altered by pain and involved in motivational alterations for reinforcers. Indeed, KOR stimulation in the NAc shell cold spot (NacShCS) decreases the liking of sucrose. Furthermore, accumbal release of dynorphin, the KOR endogenous agonist, mediates aversive behaviors. Thus, changes in the KOR system could lead to the pain-induced negative effects. In this work, we uncover these pain-induced neurobiological alterations in the NAcShCS and their consequences on motivational behaviors. We first showed that both expression and function of KORs are enhanced in the NAc shell 48 hours after the induction of inflammatory pain using western blot and autoradiographic GTPgammaS assay. Patch clamp recordings confirmed that pain potentiates the excitability of dynorphin neurons in the NacShCS. Furthermore, we demonstrated that KOR activation is both necessary and sufficient to induce the pain-induced decrease in motivation using long term antagonist, NorBNI, or short-acting agonist, U50488, microinjections. In addition, silencing dynorphin neurons in the NacShCS, using a HSV-dynorphin-Gi-DREADD, reverses the pain-induced alterations in motivation. Lastly, we showed that light-induced release of dynorphin, using ChannelRhodopsin, induces a real time place aversion that can be reversed by NorBNI microinjection in the NacShCS. Two days after pain induction this dynorphin-induced aversion can no longer be blocked by NorBNI pretreatment, revealing pain-induced adaptations in the KOR system. To summarize we show in the present work that the dynorphin-KOR system in the NacShCS is enhanced in the presence of pain. The uncovering of the kappa opioid system adaptations induced by pain provides insight in another lead on the understanding of how pain affects the mesolimbic pathway and triggers changes in motivational properties of stimuli.

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

All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University and Washington University in St. Louis.

LOR17 is a functionally selective KOR agonist eliciting potent analgesic effects in animal models of nociceptive and neuropathic pain

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Kappa opioid receptor (KOR) agonists are studied as alternatives to mu opioid receptor (MOR) analgesics; however, relevant side effects, including dysphoria and sedation, limit their clinical use. An accumulating body of evidence suggests that KOR agonists determine antinociception and anti-itch effect via G-protein signaling whereas their arrestin-3-dependent induction of p38MAPK leads to different adverse effects.
Thus, G-protein biased KOR agonists displaying limited p38MAPK activation are considered promising therapeutics possibly devoid of the above described adverse effects.
Therefore, we aimed at detailing functional selectivity and analgesic effects of LOR17, a novel KOR selective agonist that we recently identified.
Similarly to U50,488, LOR17 inhibited adenylyl cyclase in HEK-293 cells expressing recombinant hKOR, in U87-MG human astrocytoma cells and in human astrocytes endogenously expressing this receptor. Conversely to U50,488, LOR17 activated early (5-15min), G protein-dependent ERK1/2 phosphorylation in HEK-hKOR, U87-MG cells and human astrocytes, without triggering, in the same cells, late (60min), arrestin-dependent ERK1/2 or p38MAPK phosphorylation. Moreover, U50,488 induced p38MAPK-dependent increase in cell proliferation and IL-1β mRNA levels in human astrocyte cell models whereas LOR17 was not effective in a wide concentration range (10nM-100μM).
LOR17 and U50,488 caused KOR-mediated antinociception in mice in the warm-water tail-withdrawal test (LOR17-ED_{50}=10.07±0.36mg/kg; U50,488-ED_{50}=9.93±0.37mg/kg) and in acetic acid-induced visceral pain (LOR17-ED_{50}=5.74±0.46mg/kg; U50,488-ED_{50}=8.24±0.59mg/kg); however, only LOR17 fully, and dose-dependently, reverted thermal hypersensitivity in a mouse model of oxaliplatin-induced neuropathy (ED_{50}=6.63±0.23mg/kg). LOR17-mediated pain relief was maintained, in neuropathic mice, following prolonged administration, whilst it did not alter motor coordination, locomotor and exploratory activities and did not induce anhedonia-related behaviors.
LOR17 emerges as a novel, KOR-selective agonist not activating p38MAPK and the subsequent cellular responses *in vitro*, displaying potent analgesic effects in different pain models and lacking of relevant behavioral side effects. LOR17 may represent an interesting starting point to develop innovative analgesics to treat both nociceptive and neuropathic pain.

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

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Sex Differences in Kappa Opioid Receptor-Mediated C57BL/6 Mouse Behaviors

Historically, most research investigating the causes and circuitry of anxiety and depression-like behaviors has been conducted in male mice. As women in the human population are disproportionately affected by depression and anxiety disorders, it is important to look at these behaviors in female animal models as well. The kappa opioid receptor (KOR) system is activated by social and physical stressors, and in males has both analgesic and dysphoric effects that are mediated by distinct intracellular pathways. The analgesic effects are mediated by the G beta-gamma subunit of the inhibitory G protein associated with KOR, whereas aversion and potentiation of drug reward are mediated through the GRK3/beta-arrestin/p38 MAPK pathway. To examine possible sex differences in these KOR-mediated signaling pathways, we looked at several different behavioral measures including locomotion, analgesia, conditioned place aversion, and stress-induced potentiation of cocaine conditioned place preference. Robust conditioned place aversion by the KOR agonist U50,488 (2.5 mg/kg) and stress-potentiated cocaine conditioned place preference were observed in females. Suppression of locomotor activity by U50,488 (5 mg/kg) did not differ between males and females. However, analgesic responses significantly differed between sexes, as only males consistently increased their latency to tail-flick after either U50,488 administration (10 mg/kg), or forced swim stress. Females showed a variable analgesic response that was cycle-dependent. During estrus, a period of low circulating estradiol, females showed similar withdrawal latencies to males, whereas in non-estrus phases there was no significant U50,488-induced analgesia. Ovariectomized females showed an analgesic response similar in magnitude to males, confirming a critical role for estradiol in KOR-mediated analgesia. In parallel studies, we examined intracellular kinase signaling after KOR agonism in males and females. 30 min after U50,488 (10 mg/kg), there were no sex differences in p38 signaling, consistent with the lack of differences observed in p38-mediated behavior. However, early-phase ERK 1/2 phosphorylation (15 min post-U50,488), which is G-beta-gamma mediated, increased only in males, consistent with the observed differences in analgesia between males and females. Bruchas (2011) and Schindler (2012) previously reported that stress-induced activation of KOR caused a p38-dependent translocation of SERT from an endosomal compartment to the cell surface of nerve terminals projecting from the dorsal raphe nucleus to the ventral striatum. In the present study, we used rotating disk electrode voltammetry (RDEV) and biotinylation of synaptosomal surface proteins. We find that repeated forced swim stress increased serotonin transporter (SERT) surface expression and 5HT uptake kinetics in synaptosomes prepared from the ventral striata of both male and female C57BL/6 mice. Overall, our results demonstrate that while the p38 MAPK-mediated effects of KOR activation are the same in both male and female mice, the beta-gamma-mediated effects, including analgesia, are sex-hormone dependent. These studies provide additional context for the therapeutic utility of KOR agonists in males and females, and may have important implications in the etiology of sex-dependent effects in stress disorders.

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Role of Kappa Receptors in Chemotherapy-Induced Neuropathy and Emotional-like Deficit Behaviors in Mice.

While chemotherapy has played a significant role in the survival of cancer patients, exposure to drugs such as taxanes often results in chemotherapy-induced peripheral neuropathy (CIPN) and mood-related changes. Indeed, in addition to CIPN, paclitaxel treatment in patients precipitates and maintains long-term changes in mood such as anxiety and depression. The mechanism of this long-term change in mood is unknown.

Male C57BL/6J treated with i.p. paclitaxel (8 mg/kg every other day for a total of 32 mg/kg) resulted in the development and maintenance of CIPN. Paclitaxel also induced anxiety-like behavior, as assessed in the novelty suppressed feeding and light/dark box tests. In addition, paclitaxel-treated mice displayed depression-like behavior during the forced swim test and an anhedonia-like state in the sucrose preference test. Paclitaxel-induced anhedonia-related effect in this test was fully reversed by nor-BNI, a selective kappa long-term antagonist. However, nor-BNI failed to reverse paclitaxel-induced allodynia. Moreover, in mice-paclitaxel treated mice, the kappa agonist U-50,488-induced conditioned place aversion (CPA) was enhanced compared to the vehicle-treated animals.

Our findings suggest that paclitaxel produces over-activation of dynorphin/KOR signaling in the brain ultimately producing changes in affective-like behaviors in mice. The results from this study suggest that blockade of the KOR may be useful in the treatment of taxanes mood-related changes.

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Spatial organization and dynamics of opioid receptor variants (kappa, mu_{wt} and mu_{N40D}) in the plasma membrane at the nanoscale level

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Dynamic regulation of opioid receptor localization in the plasma membrane is a fundamental control mechanism through which opioid receptor activity is finely tuned. This mechanism, which may have important pharmacological implications, is difficult to analyze nondestructively and our progress towards its clarification is tightly related to the concomitant development of advanced analytical techniques with high spatial and temporal resolution. We use here two methods with single-molecule sensitivity, Fluorescence Correlation Spectroscopy (FCS) and Photo-Activated Localization Microscopy (PALM), to quantitatively characterize at the nanoscale level the spatial organization and dynamics of kappa (KOP) and mu opioid receptors (MOP_{wt} and its naturally occurring variant MOP_{N40D}). Our study shows that the investigated opioid receptors are partially associated with cholesterol- and GPI-enriched domains and largely excluded from ganglioside GM1-enriched nano-domains. We also observed a dynamic equilibrium between opioid receptors associated in larger molecular assemblies (domains) and freely imbedded in the lipid bilayer. Cholesterol dynamically regulates the fraction of KOP and MOP_{wt} receptors associated with domains; this effect was not observed for MOP_{N40D}, which lacks an important glycosylation site. Importantly, these receptors seems to influence the domain size/occupancy and the fraction of associated molecules: the size and the population density of the domain is receptor specific – with the largest and most populated nano-domains being observed for KOP (~105 nm in radius; 9-10 detected molecules/domain), whereas smallest and least populated domains were observed for MOP_{N40D} (~82 nm in radius; 7-8 detected molecules/domain). Moreover, KOP showed the smallest and MOP_{N40D} the largest fraction of receptors that reside outside of these domains. Our experimental study, supported using ensemble-averaged Monte Carlo simulations, shows that the complex, dynamically regulated lateral organization of opioid receptors, and G protein-coupled receptors in general, can be quantitatively characterized in detail using the advanced approaches presented.

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Acute stress induces constitutive activation of kappa opioid receptors.

Dopaminergic neurons in the ventral tegmental area (VTA) are an important locus for the convergent effects of stress and drugs of abuse. We previously identified a long-term potentiation of GABAergic synapses on these neurons (LTP_{GABA}) that is blocked by stress through activation of kappa opioid receptors (KORs, Graziane et al, Neuron, 2013). Our recent work shows that a brief swim stress blocks LTP_{GABA} for five days. Blocking KORs with norBNI even after stress restores LTP_{GABA} and prevents reinstatement of cocaine self-administration (Polter et al, Biological Psychiatry, 2014). In this study, we examine the mechanism by which KORs are persistently activated by acute stress and the role of this activation in stress-induced reinstatement of drug seeking.

Here we show that the block of LTP_{GABA} by stress is due to persistent changes in the KOR. While bath application of an inverse agonist (norBNI, 100 nM) rescues LTP_{GABA} in slices from stressed animals, a neutral antagonist (6-β-naltrexol, 10 μM) does not. These results suggest that LTP_{GABA} is blocked by constitutive activity of KORs rather than by persistently elevated dynorphin. In support of this, the ability of norBNI to rescue LTP_{GABA} in the slice was blocked by the JNK inhibitor SP600125, indicating that the effect of norBNI is non-competitive.

In contrast to norBNI, which rescues LTP_{GABA} after stress, we find that *in vivo* 6-β-naltrexol only rescues LTP_{GABA} when administered prior to stress. These data suggest that activation of the KOR by dynorphin during stress leads to persistent, dynorphin-independent activation of the receptor. We also find that treatment of rats with norBNI, but not 6-β-naltrexol, 24 hours after stress prevents reinstatement of cocaine self-administration. Our results show that a single exposure to acute stress causes long-lasting changes in inhibitory plasticity through constitutive activity of KORs. These studies demonstrate a novel mechanism of KOR regulation and highlight a potential target for treatment of stress-induced drug seeking behavior.

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Development of new imaging probes to investigate the role of κ-opioid receptors in multiple sclerosis

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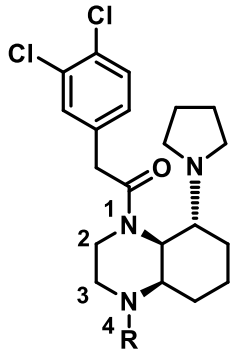
Numerous studies demonstrated that κ-opioid receptors, G-protein coupled receptors belonging to the opioid receptor family, are involved in several autoimmune disorders and neurological diseases. In particular, in multiple sclerosis they seem to play a key role, even though their exact function in this pathological process is not well understood. Therefore, the development of novel κ-selective imaging probes is essential to better interpret the biological pathways related to this pathology.
The aim of this project is the synthesis and the characterization of new imaging probes based on quinoxaline-derived κ agonists, as shown in Figure 1.
Therefore, a novel synthetic route was developed to achieve stereoisomerically defined perhydro-quinoxalines **1**. Moreover several substituents in 4-position have been introduced to modulate both the κ-opioid receptor affinity and the pharmacokinetic properties. The most promising ligands have been used as starting point for the development of PET tracers, by introducing a radioactive ^{18}F-atom in different positions of the side chain at 4-position. In this respect, the radiosynthetic routes were established and *in vivo* small animal-PET studies were performed with *wt*-mice.
Since our SAR studies confirmed a high tolerance of the receptor towards a large variety of moieties in 4-position, we plan to attach a fluorescent dye at that position, which would expand the imaging possibilities.
Additionally, new κ agonists of type **1** were tested *in vivo* in experimental autoimmune encephalomyelitis (EAE), a known mouse model for multiple sclerosis, to evaluate these κ agonists with respect to their potential to inhibit disease perpetuation.

Figure 1:

Basic scaffold of novel κ agonists bearing a F-containing substituent or a fluorescent dye at 4-position



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Spinal KOR Activation Attenuates Itch by Inhibiting GRPR Function

Chronic itch is a major unmet problem which cannot be treated with anti-histamines. Nalfurafine, a κ-opioid receptor (KOR) agonist, has been approved for treating uremic pruritus in Japan. However, its broad application has been hindered by side effects associated with KOR agonists. The mechanisms underlying KOR-mediated itch inhibition are unknown. Gastrin-releasing peptide (GRP) in sensory neurons and its receptor, gastrin-releasing peptide receptor (GRPR), an itch-specific receptor expressed in the dorsal horn of the spinal cord, are primarily required for mediating nonhistaminergic itch. GRPR is crucial for the development of chronic itch in mouse models. In this study, we found that KOR is co-expressed with GRPR in the spinal cord, and that KOR inhibits GRPR function via a $G_{\alpha i}$-protein independent process. Furthermore, spinal KOR activation suppressed GRP-induced scratching behavior and nonhistaminergic acute/chronic itch. Interestingly, inhibition of protein kinase C (PKC) blocked KOR-mediated itch inhibition and GRPR function, suggesting that KOR negatively regulates GRPR via PKC-mediated phosphorylation events. Our data also revealed that KOR activation induces the translocation of a Ca^{2+}-independent PKCδ from the cytosol to the plasma membrane, mediating GRPR desensitization via phosphorylation. This study shows a unique mechanism by which spinal KOR activation suppresses itch, providing other targets for anti-itch therapy.

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Characterization of a knockin mouse line expressing KOR-tdTomato fusion protein

The κ-opioid receptor (KOR), one of the three opioid receptors, is a rhodopsin-like G-protein-coupled receptor. KOR has a host of functions, involving analgesia, antipruritic effect, diuresis, and dysphoria. Due to lack of specific antibodies for immunohistochemistry (IHC), it has been difficult to characterize the distribution of KOR protein at a resolution higher than that of autoradiography of radioligand binding to the KOR. To circumvent this problem, we generated and characterized a line of knock-in mice expressing KOR fused at the C-terminus with the red fluorescent protein tandem dimer Tomato (tdT). Treatment of homozygous (KtdT/KtdT) mice with the selective KOR agonist U50,488H inhibited scratching behaviors elicited by compound 48/80 and reduced novelty-induced locomotor activity, suggesting intact KOR neuronal circuitry. Binding of [3H]U69,593 to brain membranes showed higher KOR levels in KtdT/KtdT mice than in wildtype mice. By qRT-PCR, we found that KOR mRNA levels in the brain were higher in KtdT/KtdT mice than in wildtype mice. IHC with antibodies against tdTomato performed on coronal brain sections of KtdT/KtdT mice revealed that distribution of KOR-tdT immunoreactivity corresponded well with that of autoradiography of [3H]U69,593 binding to the KOR. The highest levels of KOR-tdT were found in the claustrum and endopiriform nucleus. KOR-tdT was also observed throughout all layers of the neocortex except layer IV, dorsal and ventral striatum, nucleus accumbens, interstitial nucleus of the posterior limb of the anterior commissure, substantia nigra, ventral tegmental area, and periaqueductal gray. These mice thus represent a powerful, and heretofore unparalleled, tool for accurate, high-resolution mapping of KOR throughout the nervous system and its relationship to neurotransmitters and receptors of interest.

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Preclinical evidence for a rAAV based gene-therapy of temporal lobe epilepsy targeting Kappa opioid receptors

The high incidence of drug-resistant focal epilepsies poses a persistent challenge in medicine. Certain patients benefit from surgical removal of the epileptogenic focus. However, a large cohort of patients cannot be treated sufficiently at present. We and others have demonstrated the importance of endogenous peptides like dynorphins in seizure control. Since long-term treatment is needed in epilepsy, viral vector derived, locally restricted expression of dynorphins may be suitable to fill the treatment gap. We now aimed to evaluate the potential of prolonged dynorphin overexpression as treatment of focal epilepsy in a pharmaco-resistant model of temporal lobe epilepsy (TLE).
rAAV expressing either human preprodynorphin (pDyn-AAV) or a truncated form of GFP (ΔGFP-AAV) were tested in a pharmaco-resistant model of TLE induced by injection of kainic acid into the dorsal hippocampus of mice. Dynorphins expressed in the epileptogenic focus suppressed generalized seizures and hippocampal paroxysmal discharges (hpds) up to 6 months after injection (longest time interval investigated). By contrast ΔGFP-AAV displayed 1-3 generalized seizures per day and frequent hpds. Moreover, treatment of mice 1 or 2 weeks after kainic acid injection conserved spatial learning ability (as tested by Barnes maze) up to 6 months, while ΔGFP treated animals lost this ability already after 1 or 2 months.
Dynorphin expression through a rAAV serotype 1 vector was observed in neurons, but not glial cells in epilepsy. Moreover, microdialysis experiments showed stimulation dependent release of mature dynorphins. No effects of overexpression of dynoprhins in one dorsal hippocampus was observed in naive animals.

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The authors have a patent application pending.

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Kappa opioid receptor activation on dopamine neurons disrupts behavioral inhibition

The dynorphin/kappa opioid receptor (KOR) system has been previously implicated in the regulation of cognition, but the neural circuitry and molecular mechanisms underlying KOR-mediated cognitive disruption are unknown. Here, we used an operational test of cognition involving timing and behavioral inhibition and found that systemic KOR activation impairs performance in the differential reinforcement of low response rates (DRL) task of both male and female C57BL/6 mice. Systemic KOR antagonism also blocked repeated forced swim stress-induced disruptions of DRL performance. In contrast to the KOR-mediated disruptions of DRL performance, KOR activation did not alter total responding in a fixed ratio task and promoted extinction of a fixed ratio response. Together, these experiments demonstrated that when reinforcement probability was ambiguous, KOR activation promoted compulsive responding. Local inactivation of KOR by injection of the long-acting antagonist norBNI in the ventral tegmental area (VTA), but not the prefrontal cortex (PFC) or dorsal raphe nucleus (DRN), prevented disruption of DRL performance caused by systemic KOR activation. Cre-dependent genetic excision of KOR from dopaminergic, but not serotonergic neurons, also blocked KOR-mediated disruption of DRL performance. At the molecular level, we found that these disruptive effects did not require arrestin-dependent signaling, because neither global deletion of G-Protein Receptor Kinase 3 (GRK3) nor cell-specific deletion of GRK3/arrestin-dependent p38α MAPK from dopamine neurons blocked KOR-mediated DRL disruptions. We then found that the G-biased KOR agonist nalfurafine produced disruptions in DRL performance. Together, these studies demonstrate that KOR activation in VTA dopamine neurons disrupts behavioral inhibition in a GRK3/arrestin-independent manner and suggest that selective G-biased KOR agonists may retain cognitive disrupting properties.

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Kappa opioid receptors modulate mammalian target of rapamycin (mTOR) through p38 MAP kinase

Exposure to stress can exacerbate depressive symptoms and trigger depressive episodes, and the molecular mechanisms by which stress can induce depression are not completely defined. Both the mammalian target of rapamycin (mTOR) and Kappa-opioid receptor (KOR) systems have been independently associated with stress-induced depression, but no study has attempted to link these two systems. One potential kinase that could provide this bridge is p38 MAPK, which has been shown to underlie the pro-depressant effects of stress and KOR stimulation, and p38 MAPK has been associated with mTOR in limited studies. In these experiments, we tested the relationship between KOR activation and phospho-mTOR (p-mTOR) expression *in vitro* and in the spinal cord, hippocampus, and prefrontal cortex. Surprisingly, in HEK 293 cells, incubation of U50,488 significantly increased p-mTOR expression that peaked at 15 min post-administration and lasted up to 60 min. In cells pretreated with the p38 MAPK inhibitor SB203580, the increase in p-mTOR was completely blocked, suggesting the acute response was dependent on p38 MAPK. In spinal cord extracts following in vivo treatment, we also observed an increase in p-mTOR 30 min after systemic U50,488 injection. This effect was not evident in GRK3$^{-/-}$ animals, again suggesting that p38 MAPK was required for acute mTOR signaling because KOR-induction of phosho-p38 MAPK is GRK3/arrestin dependent. In brain, U50,488 (10 mg/kg) produced a bi-phasic response, where p-mTOR is significantly increased at 30 min in both hippocampus and prefrontal cortex but significantly decreased at 28 hr in hippocampus only. This decrease in p-mTOR signaling was norBNI and GRK3 dependent, although norBNI itself produced a small decrease in p-mTOR expression. Two-day, repeated forced-swim stress also reduced p-mTOR in the hippocampus, recapitulating the pharmacological treatment. After 9-10 injections of U50,488 (10 mg/kg) over 5 days, p-mTOR expression in the PFC was significantly reduced, and measurements of dendritic spine morphology were also decreased, consistent with other morphological changes evident during depression-like states. These KOR/p38 MAPK-dependent decreases seen at 28 hr and chronically over 5 days are consistent with synaptic pruning classically associated with depression, and point to a critical role for the KOR system in promoting depression-like states through mTOR.

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Intrinsic properties of central amygdala dynorphin neurons

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The central amygdala (CeA) is a critical anatomical substrate for emotional regulation in response to stress, pain, and alcohol-related behaviors. While many cell-types have been identified in the CeA, much less is understood about the unique properties of these molecularly defined neurons. We focus on a subset of neurons in the CeA expressing the neuropeptide dynorphin, the endogenous ligand of the kappa opioid receptor. To genetically identify dynorphinergic (Dyn+) neurons, we crossed a Cre-dependent tdTomato reporter mouse to a mouse expressing Cre recombinase under the same promoter as preprodynorphin. In this model, only dynorphinergic cells express tdTomato, allowing complete visualization of dynorphinergic circuitry throughout the brain and enabling visually-guided, targeted whole-cell recordings in amygdala slices. Here, we report distinct patterns of c-fos expression in Dyn+ neurons following stress, pain, and alcohol exposure. We characterize the intrinsic properties of these neurons including the input resistance, resting membrane potential, and firing profiles of these neurons compared to neighboring Dyn- CeA neurons. Furthermore, the morphology of CeA Dyn+ neurons is defined by filling the cells with Neurobiotin. We also identify incoming spontaneous and optically-evoked synaptic transmission to these neurons. In particular, we examine the strength of genetically-defined excitatory inputs from the parabrachial nucleus and subsequent modulation by metabotropic glutamate receptors. To determine the long-range connectivity of Dyn+ CeA neurons, we utilize cell-type selective expression of reporter viruses, in tandem with traditional retrograde tracers, to identify these molecularly-defined projections throughout the brain. Together these data provide a base knowledge for further cell-type selective manipulation and observation *in vivo*. Understanding the mechanisms by which the dynorphin/kappa opioid system regulates emotional processing in the context of stress, chronic pain, and alcohol abuse will provide valuable insight into potential therapeutic targets for these neurological and neuropsychiatric disorders.

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The authors declare no conflict of interest.

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Antidepressant activity of the buprenorphine analogue BU10119

Buprenorphine (BPN), a potent kappa opioid receptor (KOR) antagonist and mu opioid receptor (MOR) partial agonist, has been shown to rapidly and effectively alleviate symptoms in treatment-resistant major depressed patients. Previously, using two rodent models of stress-induced depression, chronic mild stress and chronic social defeat, we demonstrated that BPN rapidly produces effects that are comparable to established antidepressants. Using the forced swimming test (FST) as a screen for antidepressant effects, KORs were shown to mediate the antidepressant effects of BPN, whereas in the novelty-induced hypophagia test, it was established that blockade of MORs was necessary for BPN's anxiolytic action. Although BPN's therapeutic benefits in opioid addiction and pain are mediated via MOR partial agonism, this characteristic may complicate the development of BPN as a treatment modality for depression. The goal of these studies was to evaluate the behavioral effects of a novel BPN analogue, BU10119, which was modified to reduce signaling efficacy at MORs without altering antagonism of KORs. As BPN produces protracted effects lasting for days following a single administration, BU10119 was screened in male C57BL/6J mice using the FST, 24 h post administration. BU10119's effects on immobility in the FST were compared with those of the selective KOR antagonist CERC-501 and the selective MOR antagonist cyprodime. BU10119 produced an inverted U-shape curve in the FST. Bonferroni multiple comparisons tests revealed that immobility scores were reduced at 1 and 3 mg/kg ($p<0.05$), but not at 10 mg/kg. Similarly, CERC-501 reduced immobility scores in the FST at 1 ($p<0.05$) and 3 mg/kg ($p<0.01$). Conversely cyprodime did alter immobility scores 24 h post administration. Although more research is required, taken together these studies suggest that BU10119 has comparable effects to BPN on tests for antidepressant effects, producing similar temporal and behavioral profiles in the FST that are mediated via KOR antagonism.

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Receptor phosphorylation and mTOR pathway are involved in KOR agonist-induced aversion

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KOR agonists are potentially useful as analgesics, antipruritic agents and water diuretics; however, their development for clinical use has been limited by dysphoria. Nalfurafine, U50,488H and 2-*O*-methoxymethyl salvinorin B (MOM-SalB) are structurally distinct selective KOR agonists. Nalfurafine is used in Japan for treatment of uremic pruritus in hemodialysis patients; significantly, at the therapeutic doses dysphoria was not observed as an adverse reaction. Here, we took a "bedside to bench" approach to investigate possible mechanisms underlying the aversion caused by KOR activation. We found that nalfurafine produced analgesia and anti-scratch at doses lower than that causing conditioned place aversion (CPA), whereas MOM-SalB and U50,488H produced CPA at doses lower than the A_{50} values for anti-scratch and analgesia. Lack of CPA by nalfurafine was not due to its action on the MOR. At a dose ~A_{50} in the anti-scratch test, U50,488H, but not nalfurafine, significantly increased the baseline threshold in the intracranial self-stimulation. Thus, our findings on nalfurafine mimicked clinical observations. At the KOR level, in mouse brains, at the lowest doses that produce maximal anti-scratching effects, U50,488H and MOM-SalB promoted robust KOR phosphorylation at T363 and S369, but nalfurafine did not. Shotgun phosphoproteomic experiments provide an unbiased view of changes in downstream phosphorylation events upon KOPR activation by monitoring tens of thousands phosphosites simultaneously. We found that in the mouse striatum, over 393 phosphosites were found to be different between U50,488H and nalfurafine treatment (ANOVA with *post hoc* Dunnett's test $p<0.05$). Among these, U50,488H activated the mTOR pathway, whereas nalfurafine did not. Inhibition of the mTOR pathway by rapamycin abolished U50,488H-induced CPA, without affecting antinociceptive and anti-pruritic effects. These results indicate that the mTOR pathway is involved in U50,488H-induced CPA and lack of CPA by nalfurafine may be related to its inability to activate the mTOR pathway. In addition, our mouse models may be useful in screening for KOR agonists with lower propensity to cause dysphoria.

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ABSTRACTS for POSTER PRESENTATIONS

Anti-inflammatory activity of a topically applied, potent and selective, peripherally restricted κ-opioid receptor agonist in mouse models of skin inflammation

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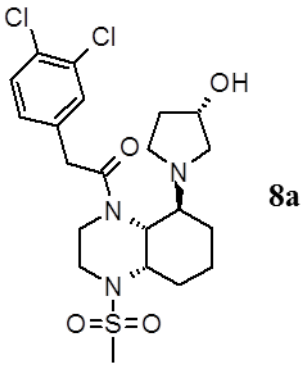
(4a*R*,5*S*,8a*S*)-Configured decahydroquinoxaline **8a** was discovered as potent (K_i = 0.63 nM) and highly selective (>500fold selectivity *vs.* μ and δ receptors) κ-opioid receptor (KOR) agonist (EC_{50} = 1.8 nM in the [^{35}S]GTPγS assay) in a hit-to-lead program aiming for peripherally restricted κ agonists. Application of **8a** (5 mg/kg, i.v.) in a screening PK study combined with monitoring of CNS effects revealed that it was mostly restricted to the periphery (brain/plasma ratio of 0.02, 1 h after administration) without showing any clinical signs. Subsequently we investigated anti-inflammatory effects of **8a** following topical administration in two dermatitis models *in vivo*.

In the topical arachidonic acid (AA)-induced ear swelling model in mice **8a** at 0.1 to 3 mg/ear and vehicle (acetone/ethanol (1:1)) were each administered 30 min before and 15 min after AA (5 mg/ear). **8a** at 1 and 3 mg/ear caused significant ($p<0.05$) reduction of ear swelling relative to vehicle.

In addition, **8a** was tested in a mouse model of oxazolone-induced dermatitis. Mice were sensitized on day 0 and challenged on days 5 and 7-12. On days 7-18, vehicle and **8a** (0.1 to 2.5 mg in 20 μl acetone/ethanol (1:1)) was daily applied on right ear 3 h prior oxazolone challenge on days 7-12. Dosing continued without oxazolone through day 18. At the dose of 2.5 mg **8a** caused significant ($p<0.05$) reduction of ear swelling on days 8-19. Lower doses of **8a** showed little or no effect. Histological analysis showed dose dependent effects of **8a** on ear thickness, epidermal thickness and dermal infiltrate ($CD4^+$ and $CD8^+$ T-lymphocytes). Effects of the highest dose were almost comparable with the effect of 0.6 mg hydrocortisone. Topical application of a potent and selective, peripherally restricted KOR agonist showed significant anti-inflammatory effects in two murine dermatitis models. Based on these findings KOR agonists should be further investigated as treatment for inflammatory skin diseases.



8a

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Design and synthesis of enantiomerically pure decahydroquinoxalines as potent and selective κ-opioid receptor agonists

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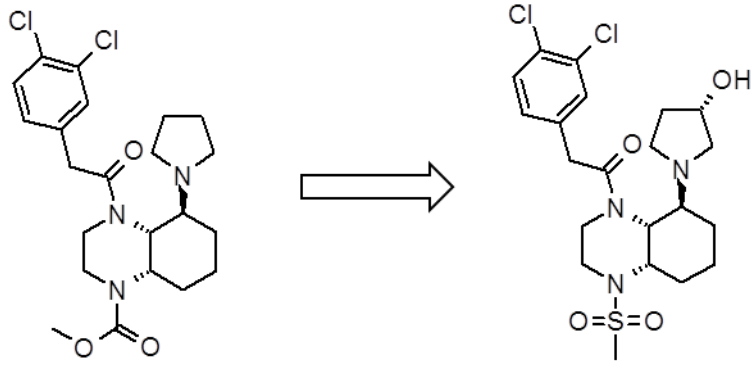
Racemic *cis-trans* decahydroquinoxaline **5** was identified as highest affinity κ-opioid receptor ligand of all four possible diastereoisomers. Its κ affinity resides almost exclusively in the (4a*R*,5*S*,8a*S*)-configured enantiomer **5a**. Initially **5a** was obtained via a synthetic route that was neither diastereoselective nor enantioselective. In order to develop novel κ agonists restricted to the periphery, a diastereo- and enantioselective synthesis of (4a*R*,5*S*,8a*S*)-configured decahydroquinoxalines **5**-**8** was developed. Physicochemical and pharmacological properties were fine-tuned by structural modifications in the arylacetamide and amine part of the pharmacophore as well as in the amine part outside the pharmacophore. A total number of 23 analogs was synthesized and evaluated for their binding affinity towards κ, μ and δ receptors. Functional activity was determined applying a [^{35}S]GTPγS assay. Decahydroquinoxalines **5**-**8** show single-digit nanomolar to subnanomolar κ-opioid receptor affinity, full κ agonistic activity in the [^{35}S]GTPγS assay, and high selectivity over μ and δ receptors. Methanesulfonamide **8a** containing the (*S*)-configured hydroxypyrrolidine ring was identified as potent (K_i = 0.63 nM) κ agonist (EC_{50} = 1.8 nM) with >500fold selectivity *vs.* μ and δ receptors.



5a

κ affinity: K_i = 0.25 nM
GTPγS EC_{50} = 2.0 nM
107% κ activation at 1 μM

8a

κ affinity: K_i = 0.63 nM
GTPγS EC_{50} = 1.8 nM
106% κ activation at 1 μM

Supported by Deutsche Forschungsgemeinschaft (DFG).

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Pharmacological and chemogenetic evidence for a role of the dynorphin/kappa opioid receptor system in binge-like ethanol consumption

Kappa opioid receptors (KORs) have received recent attention as putative therapeutic targets for treatment of alcohol use disorders. Binge drinking has been associated with numerous long-term health consequences and may facilitate the transition to alcohol (ethanol) dependence. Although previous studies have reported that blockade of KORs in the central nucleus of the amygdala (CeA) reduces elevated ethanol consumption associated with dependence, the role of KORs in binge-like drinking has not been examined. Accordingly, the present study employed both pharmacological and chemogenetic approaches to determine whether the dynorphin/KOR system also contributes to ethanol intake in non-dependent subjects using the drinking-in-the-dark (DID) model of binge-like consumption. Experiment 1 assessed the effects of systemic KOR activation and blockade in adult male C57BL/6J mice. For all drinking sessions, water bottles were replaced with 20% ethanol starting 3 hours into the dark cycle. On Days 1-3, mice received vehicle injections prior to a 2-hr drinking session, and on Day 4, mice received systemic injections of the KOR agonist U50,488 (0 or 5mg/kg) or the short-acting KOR antagonist LY2444296 (0 or 10 mg/kg) 30 min prior to a 4-hr ethanol consumption test. Both drugs were also tested in a separate cohort of mice with a control solution (0.5% sucrose instead of ethanol) using the same DID procedure. Experiment 2 examined effects of site-specific KOR blockade in C57BL/6J mice implanted with guides aimed at the CeA. Drinking was measured for 3 days (2-hr access to 20% ethanol) before and after a bilateral infusion of the long-acting KOR antagonist nor-binaltorphimine (vehicle or 2.5 µg/side). For Experiment 3, Pdyn-ires-Cre mice received microinfusions of virus containing an inhibitory DREADD (hM4Di) into the CeA. A separate cohort of Pdyn-ires-Cre mice received infusions of blank control virus. Mice received vehicle injections on Days 1-3. To assess effects of inactivating prodynorphin-containing neurons in the CeA, on Day 4, mice received either vehicle or clozapine-N-oxide (CNO; 3 mg/kg) 30 min prior to a 4-hr ethanol drinking test. In Experiment 1, KOR activation (U50,488) resulted in elevated ethanol consumption whereas KOR blockade (LY2444296) resulted in decreased consumption relative to vehicle. These effects were selective for ethanol; neither drug significantly altered sucrose intake. In Experiment 2, nor-binaltorphimine administered in the CeA resulted in reduced ethanol consumption relative to vehicle. In Experiment 3, administration of CNO reduced binge-like ethanol consumption selectively in mice infused with the DREADD virus, but not the control virus. Thus, converging data from pharmacological and chemogenetic approaches indicate that inhibition of the dynorphin/kappa opioid receptor system reduces ethanol intake in a rodent model of binge-like drinking, further supporting this system as a target for treatment of excessive alcohol use.

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DOR-KOR heteromers, expressed in peripheral nociceptors, maintain functional competency under prolonged inflammatory conditions

Opioid receptor systems expressed by peripheral pain-sensing neurons (nociceptors) are under dual regulatory control by cyclooxygenase (COX) and lipoxygenase (LOX) dependent arachidonic acid (AA) metabolites. Neither DOR, KOR nor DOR-KOR heteromers are functionally active for antinociception under basal conditions, but become responsive following exposure to inflammatory mediators (e.g., carrageenan, bradykinin (BK) or AA) that produce COX-dependent AA metabolites. In addition, we have found that both DOR and KOR become non-responsive for antinociception due to production of the LOX-dependent AA metabolites, 12- and 15-HETE. Here we sought to determine if 12- and 15-HETE altered DOR-KOR heteromer signaling and antinociception in primary cultures of adult rat peripheral sensory neurons (ex vivo model) and in the carrageenan model of inflammation (in vivo). Ex vivo, we compared the effects of 12- and 15-HETE on inhibition of PGE_2-stimulated cAMP accumulation by the DOR agonist, DPDPE, the KOR agonist, U50488 and the DOR-KOR heteromer agonist, 6'-GNTI. Addition of 12- and 15-HETE blocked DPDPE- and U50488-mediated inhibition of PGE_2-stimulated cAMP accumulation. By contrast, 12- and 15-HETE had no effect on 6'-GNTI-mediated signaling. We next compared the abilities of DPDPE, U50488 and 6'-GTNI to inhibit carrageenan induced thermal allodynia in the rat hind paw. When administered 15 min after intraplantar (i.pl) injection of carrageenan (500 ug), all agonists completely reduced carrageenan-induced thermal allodynia. When injected (i.pl.) 3h or 24h after carrageenan administration, neither DPDPE nor U50488 produced antinociceptive responses. However, responsiveness was restored following i.pl. injection of the 12/15-LOX inhibitors, baicalein and luteolin. Interestingly, the heteromer agonist, 6'-GNTI, completely inhibited the nociceptive response when administered (i.pl.) 3h or 24h (longest period tested) after carrageenan. Taken together, in striking contrast to DOR and KOR, DOR-KOR heteromers appear to remain functionally competent for a prolonged period of time under inflammatory conditions, suggesting that they may be suitable targets for peripherally restricted analgesics.

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Disclosure: The authors have no conflicts of interest to disclose

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Alcohol drinking induced alterations in dynorphin signaling in the extended amygdala

The extended amygdala, principally comprised of the Central Amygdala (CeA) and the Bed Nucleus of the Stria Terminalis (BNST), has been shown to be important for regulating the negative reinforcing aspects of drugs of abuse. Behavioral pharmacology studies have shown that systemic administration of KOR antagonists (Walker & Koob 2008) and site-specific infusion into the CeA (Kissler et al. 2014) decrease alcohol self-administration in dependent animals. KORs are present on presynaptic CeA terminals in the BNST and inhibit GABA release (Li et al. 2012). Unpublished data from the lab shows that chronic intermittent exposure to ethanol vapor enhances KOR inhibition of GABA release in the BNST. One potential result of this upregulation would be increasing alcohol drinking through disinhibition of outputs from the BNST to the VTA which have previously been shown to regulate binge-like drinking behavior (Rinker et al. 2016). Here we mechanistically test this hypothesis by genetically manipulating expression of KOR, dynorphin, and the vesicular GABA transporter (VGAT) in dynorphin cells in CeA neurons. Knockout of CeA KORs resulted in decreased ethanol consumption in the Drinking in the Dark (DID) and Intermittent Access (IA) paradigms without affecting total fluid intake. Additionally, this manipulation decreased ethanol preference without affecting general consumatory or anxiety-like behavior. However, the dynorphin knockdown and knockdown of VGAT in CeA dynorphin neurons was without effect. Ongoing experiments will examine the result of CeA ➔ BNST pathway-specific KOR knockout on excessive drinking behavior.

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

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Kappa opioid receptor is phosphorylated by G protein-coupled receptor kinases and protein kinase C

We reported previously that the selective agonist U50,488H promoted phosphorylation of the mouse kappa opioid receptor (KOPR) at S356, T357, T363 and S369 in cells and generated and characterized phospho-KOPR specific antibodies recognizing pS356/pT357, pT363 and pS369. In this study, we characterized the protein kinases involved in KOPR phosphorylation with immunoblotting using the phospho-KOR specific antibodies and their regulation of KOPR-mediated cellular functions. We found that multiple protein kinases (GRKs2, 3, 5 and 6 and PKC) and Gi/o_{α} proteins were involved in U50,488H-caused KOPR phosphorylation. PKC activation induced agonist-independent KOPR phosphorylation yielding much higher phosphorylation at S356/T357, yet much lower and slower phosphorylation at T363 than U50,488H. GRKs, but not PKC, were involved in U50,488H-induced KOPR internalization and desensitization of G protein-dependent extracellular signal regulated kinase 1/2 (ERK1/2) activation. PKC activation promoted agonist-independent KOPR internalization. Thus, GRKs and PKC produce different phosphorylation patterns, which result in different functional outcomes. To the best of our knowledge, this is the first demonstration that PKC and GRK6 are involved in KOPR phosphorylation and that GRKs- and PKC-induced KOPR phosphorylation has different functional consequences.

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Novel library of *N*-phenylethyl-*N*-3-hydroxyphenylethyl-amines with differing tertiary *N* substitutions: characterization of kappa opioid receptor effects

A recent report of different N-substituted derivatives of *N*-phenylethyl-*N*-3-hydroxyphenylethyl-amine indicated that select compounds of this class demonstrate preferential binding and GTP-gamma-S stimulation at the kappa opioid receptor, compared to other opioid receptors or dopamine receptors (Spetea…Schmidhammer, 2012. J Med Chem 55(22):10302). In our search for novel compounds with specific kappa opioid receptor activation profiles, we utilized this compound class as a scaffold to prepare derivatives, chiefly incorporating novel substituents (including cyclic, linear, and branched chain moieties) at the central amine. We investigated these compounds first in *in vitro* assays, including inhibition of [^{3}H]U69,593 binding to the kappa opioid receptor in membranes from heterologously expressing cell lines (including HEK 293 and U2OS cells). Further studies on select compounds investigated interaction with the mu and delta opioid receptors and the nociceptin receptors. For compounds which bound to the kappa opioid receptor (IC_{50} < 5,000 nM), we further investigated functional activation at the kappa opioid receptor. We tested both [^{35}S]GTP-gamma-S stimulated binding and beta-2-arrestin coupling (using an enzyme complementation assay). Across this set of novel derivatives, we observed a variety of efficacy and potency profiles for both signaling pathways, resulting in varying degrees of “bias”. A subset of the 56 total compounds have been tested *in vivo* using rotarod and prolactin assays. Results are consistent with arrestin-mediated incoordination and G protein-mediated prolactin signaling through the kappa opioid receptor. The variety of kappa opioid receptor signaling profiles for this library of compounds will be useful for probing the roles of biased signaling in the effects of kappa opioid receptor agonists.

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Prior stress and ventral tegmental area dopamine neuron inhibition potentiates reward

Repeated stress causes the release of endogenous dynorphins, which activate kappa opioid receptors (KORs) and have been shown to encode the dysphoric component of stress. Due to these dysphoric properties, KOR activation was initially proposed as a physiological counter to the rewarding effects of drugs of abuse; surprisingly however, prior pharmacological or behavioral activation of KOR *enhanced* drug reward, increased rates of drug escalation, and induced relapse after periods of abstinence. While subsequent research has suggested potentiation of reward is dependent on a downstream effector, p38 MAPK activation in dopaminergic and serotonergic neurons, the precise circuit-level mechanisms underlying this pro-addictive effect of stress-induced KOR activation remain unclear. To test the hypothesis that a transient reduction of glutamatergic or dopaminergic tone is sufficient to potentiate drug-induced reward, we utilized a virally-delivered, Cre-dependent inhibitory channelrhodopsin (Step Waveform inhibitory Channelrhodopsin; $SwiChR_{CA}$) to optically inhibit dopaminergic ventral tegmental area neurons (DAT^{VTA}) or glutamatergic dorsal raphe nucleus neurons ($VGluT3^{DRN}$). Following an initial preference test, mice were subjected to two days of conditioning consisting of a morning session, in which $SwiChR_{CA}$-mediated inhibition of $VGluT3^{DRN}$ or DAT^{VTA} neurons preceded exposure to the cocaine-paired (15 mg/kg) context, and an afternoon session, consisting of exposure to the saline-paired context. The following day, mice were allowed to freely explore the conditioned place preference CPP apparatus to assess preference for the drug-paired context. We found that transient inhibition of dopaminergic VTA neurons prior to cocaine conditioning significantly potentiated cocaine place preference, and that inhibition of glutamatergic DRN neurons also reduced cocaine place preference. A complementary set of studies evaluated the ability of stress to potentiate optically-evoked reward produced by these two populations. In these studies, we phasically stimulated (20Hz stim for 1s/10s) DAT^{VTA} or $VGluT3^{DRN}$ neurons during confinement to one context of a two-chamber apparatus, tethered the animals with no light stimulation in the other context, and conducted a preference test each day. Mice exhibiting a stable preference for the optically-paired chamber after two days of training were subjected to a modified Porsolt swim stress procedure (rFSS), terminating 10 min prior to a final preference test. rFSS enhanced preference generated by stimulation of DAT^{VTA} or $VGluT3^{DRN}$ neuron populations. Current studies are employing pharmacological and genetic tools to evaluate the necessity of KOR for this effect. In summary, these data suggest that stress-induced transient reductions in dopaminergic tone mediated by KOR are sufficient to potentiate reward. Together, these data identify circuit-level requirements for the potentiation of reward.

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Conflict of Interest: None to disclose.

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The peroxiredoxin-6 (PRDX6) inhibitor MJ33 blocks the long-lasting antagonism of kappa opioid receptors by nor-BNI and blocks analgesic tolerance to morphine

Kappa opioid receptor (KOR) antagonists are in development for the treatment of mood and substance use disorders, but their therapeutic utility in humans has been challenged by the unusually long-acting pharmacological properties of the highly selective, prototypical KOR antagonists. Reversal of norbinaltorphimine (nor-BNI) antagonism of the analgesic effects of KOR agonists requires 3-4 weeks in rodents. Prior studies demonstrated that the long-duration was not correlated with antagonist affinity or tissue clearance rates (Bruchas, 2008; Melief 2010; Melief 2011); rather we previously demonstrated that the duration of KOR antagonism *in vivo* was positively correlated with c-Jun N-terminal kinase (JNK) activation in spinal cord. A similar, JNK-mediated mechanism of mu receptor (MOR) inactivation was found for morphine, but not acute analgesic tolerance to highly efficacious opioid agonists including fentanyl. Together, these studies showed that KORs and MORs can be inactivated by either arrestin-dependent or JNK-dependent mechanisms based on ligand-directed signaling differences. Recently the cellular substrates responsible for JNK-mediated receptor desensitization were identified through a series of silac-proteomic experiments (Schattauer, submitted), which demonstrated that JNK activation recruited peroxiredoxin 6 (PRDX6) to generate reactive oxygen species and cause the depalmitoylation of opioid receptor associated Gαi. Depalmitoylated Gαi was found to tightly bind to the GPCR in a conformation that prevents agonist-stimulated GDP:GTP exchange. In the present study, we report that treatment with MJ33, a selective inhibitor of PRDX6 (Chatterjee 2011), blocked the long-lasting actions of nor-BNI. U50,486 inhibits the nociceptive response of mice in the warm water (52.5°C) tail withdrawal test; pretreatment with norBNI 1 week prior blocked the effects of U50,488. In contrast, mice pretreated with both MJ33 and norBNI 1 week prior to analgesia testing, showed a normal U50,488 induced increase in withdrawal latency. MJ33 pretreatment also blocked acute analgesic tolerance to morphine, but not fentanyl. Daily injection of morphine for 5 days also produced analgesic tolerance measured by the tail-flick assay, and this tolerance was blocked by MJ33 daily pretreatment. Future studies will examine the potential use of MJ33 as a pharmacological adjunct to expand the clinical utility of MOR agonists and KOR antagonists.

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Kappa and Mu Opioid receptor activation stimulates the production of reactive oxygen (ROS) via PRDX6 and JNK.

The molecular mechanisms responsible for kappa opioid receptor inactivation by norBNI treatment have been somewhat controversial. Although norBNI can be detected in brain at low levels weeks after administration, the duration of antagonism is not correlated with pharmacokinetic clearance rates. Instead, long acting KOR antagonists have been found to activate c-Jun N-terminal kinase (JNK) in both in vitro and in vivo assays and the duration of antagonist action of a broad range of KOR antagonists is well-correlated with in vivo JNK activation. JNK inhibition by SP600125 or JNK-IN-8 or JNK1 isoform gene deletion makes norBNI into a short acting competitive antagonist. These results suggest that JNK activation phosphorylates a component of the receptor signaling complex to block receptor functioning, but the effects of JNK on GPCR function have not been established. To understand how JNK activation inactivates KOR, we performed a proteomic screen of kappa receptor interacting proteins in transfected HEK cells whose association was affected by JNK. These studies identified Peroxiredoxin 6 (PRDX6) as an interactor whose association was specifically increased by norBNI-stimulated JNK. PRDX6 has not previously been implicated in GPCR regulation, thus how its recruitment affected KOR was not evident. However, it is known that PRDX6 is a dual-function enzyme that has separately regulated glutathione peroxidase and phospholipase A2 (PLA2) activity. Using cell fractionation combined with phospholipase enzyme activity assays, we identified a membrane-localized increased in phospholipase A2 activity, with was JNK and PRDX6 mediated. From these data we inferred norBNI-stimulated JNK promoted PRDX6 translocation from cytosol to the KOR signaling complex. The PLA2 activity of phospho-PRDX6 stimulated the production of reactive oxygen (ROS) that was detected by both the CellRox dye assay and the genetically encoded ROS sensor HyPerRed. NorBNI significantly increased CellRox staining in KOR transfected HEK293 cells, and this increase was blocked by pretreatment with SP610025 (JNK inhibitor), JNK-IN-8 (JNK inhibitor), MJ33 (PRDX6 inhibitor) or N-acetyl cysteine (NAC, anti-oxidant). ROS production was also blocked by siRNA knock down of PRDX6. Naloxone binds to KOR, but does not active JNK and did not increase CellRox staining. KOR agonists also transiently increase pJNK-ir, but this effect is terminated by arrestin recruitment. Incubation of KOR cells with U50,488 induced ROS, but with an inverted U-shaped dose response, indicating that inhibition of ROS stimulation occurs at higher concentrations. ROS activation was also evident after treatment with partial agonists at other Gi-coupled GPCRs. Morphine, but not fentanyl treatment of myc-MOR expressing HEK293 cells significantly increased CellRox staining. Quinpirole treatment of Dopamine D2 expressing HEK cells also significantly increased CellRox staining. These results identify a novel signaling pathway that links GPCR activation to ROS production via PRDX6.

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NorBNI inactivates Dopamine D2 receptors on VTA nerve terminals by stimulating ROS production through a JNK/PRDX6 mechanism.

Previous work from the lab has shown that nor-binaltorphimine (norBNI) disrupts KOR signaling by activating C-Jun N-Terminal Kinase (JNK). Subsequently we found that norBNI action on KOR results in JNK activation of PRDX6, which increases the production of reactive oxygen ROS in HEK293 cells. This results in an increased association between the G-protein and the receptor as well as blockade of receptor signaling. We hypothesized that this ROS mediated inactivation of the receptor was a general mechanism for G_i-coupled-GPCR inactivation. Consistent with this prediction, Quinpirole, a D2-agonist was also found to stimulate ROS in HEK293 cells expressing either the D2-Long or D2-Short isoform. The increase in ROS was not evident in untransfected HEK293 cells treated with quinpriole and was blocked by the PRDX6 inhibitor MJ33, providing evidence that this PRDX-6 mediated ROS production is generalizable. To assess if this mechanism also regulates D2 receptor function *in vivo*, mice were treated with quinpirole to produce hypolocomotion. A second quinpriole dose (2 hours later) produced significantly less hypolocomotion, and this acute tolerance was not evident in mice pretreated with MJ33. The local production of ROS could potentially cause heterologous desensitization, and to test this concept, HEK293 cells co-expressing KOR and D2 were generated. Quinpirole treatment increased phospho-ERK1/2 detected by subsequent western blot analysis. Consistent with cross-desensitization, pretreatment with norBNI significantly inhibited quinpirole stimulation of pERK1/2 in HEK cells expressing both KOR and D2, but not in cells expressing only D2 or pretreated with MJ33. To assess whether cross desensitization also occurs in vivo, D2 receptor function was measured by recording evoked dopamine release in striatal slices using fast scan cyclic voltammetry. Quinpirole inhibited evoked dopamine release in a dose-dependent manner. Pretreatment with norBNI significantly reduced quinpirole inhibition, and the norBNI effect was significantly inhibited by pretreatment with MJ33. These results demonstrate that D2 receptors are also regulated by the JNK/PRDX6 mechanism and that norBNI may produce heterologous cross-desensitization. Inhibition of D2 autoreceptor function might contribute to the behavioral effects of norBNI and might possibly contribute to the cardiotoxic effects of JDTic.

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Characterization of kappa opioid receptor (KOR)-expressing and dynorphin-containing neurons in the mouse brain

The circuitry underlying interactions between dynorphin-containing neurons and KOR-expressing neurons has been difficult to access due to technical limitations. The development of KOR-Cre (Cai et al., 2016) and prodynorphin-Cre (pDyn-Cre; Krashes et al., 2014) transgenic mouse lines has enabled a wide range of Cre-dependent tools to identify and manipulate KOR/dynorphin containing neurons. As these transgenic lines have not been widely used, our first set of studies aimed to characterize whether KOR-Cre and pDyn-Cre mouse lines recapitulated the pattern of expression previously observed by in situ hybridization (Allen Brain Mouse Brain Atlas). KOR-Cre and pDyn-Cre mice were crossed with a floxed tdTomato reporter mouse line and tdTomato expression was analyzed throughout the brain. As expected, dense KOR expression was observed in many regions involved in mood and reward, including the medial prefrontal cortex (mPFC), the basolateral amygdala (BLA), hippocampus, striatum, dorsal raphe nucleus (DRN), and ventral tegmental area (VTA), confirming that the KOR-Cre mouse line is a valuable tool for accessing KOR-expressing cells. KOR expression in tdTomato-containing neurons was determined by immunohistochemical (IHC) staining with a C-terminally directed anti-KOR antibody (KT2). The specificity of KT2 was validated by staining KOR transfected and untransfected HEK293 cells and comparing images of wildtype and KOR-/- mouse brain. In the ventral tegmental area of KOR-Cre mice, all tdTomato-containing neurons also co-expressed tyrosine hydroxylase (TH), indicating that KOR-expressing neurons in the VTA are primarily dopaminergic. This observation was then validated with a KT2 IHC stain in the VTA. Additionally, a population of neurons in the DRN co-expressed TH and tdTomato in KOR-Cre mice, identifying a novel population of KOR-expressing dopaminergic neurons. We then aimed to characterize the dynorphin-containing neurons projecting into VTA dopamine neurons. To this end, we injected a Cre-dependent retrograde canine adenovirus expressing a green fluorescent protein (CAV-DIO-ZsGreen) into the VTA of pDyn-Cre mice. Our analyses revealed multiple regions that project dynorphin afferents into the VTA, including glutamatergic neurons in the mPFC and serotonergic neurons in the DRN. Injecting DRN or PFC of pDyn-Cre mice with AAV-DIO-ChR2 to assess populations of dynorphin-containing neurons, we found additional dynorphin neuron projections from PFC to striatum, as well as DRN projections to multiple sites, including the lateral septum, and hypothalamic nuclei. Finally, we optogenetically stimulated the ChR2 expressing dynorphin neurons in the DRN and assessed phosphorylated KOR levels in the VTA using a recently re-generated phosphoselective KOR antibody (KORp). KORp was validated using mycKOR expressing HEK293 cultured cells treated with the KOR agonist U50,488. There was a robust increase in fluorescence and internalization of the staining in U50,488-treated mycKOR HEK293 cells. This increase in KORp fluorescence was also observed in fixed brain tissue slices from U50,488-treated mice within the BLA, striatum, DRN, and VTA compared to saline-treated mice, congruent with historical findings. Future studies will extend these circuitry analyses to other KOR/dynorphin-containing neurons. Together, these studies describe novel KOR-expressing neuron populations in the mouse brain and identify dynorphin-containing neurons projecting to the ventral tegmental area.

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Structure-Activity Relationship Exploration of a Bisamide Series of Kappa Opioid Receptor Agonists

Despite their potent antinociception and utility in the treatment of pruritus, Kappa opioid receptor (KOR) agonists are not used in the clinical setting due to adverse side effects such as dysphoria and sedation. Recently, there has been a resurgence of interest in the therapeutic utility of KOR agonists that selectively activate the G-protein pathway over β-arrestin recruitment.

Following the discovery of the bisamide chemotype of KOR agonists in a high-throughput screening campaign, utilization of the Ugi multicomponent reaction has enabled a comprehensive exploration of its chemical space. This route provides bisamide analogues in a single synthetic step with structural modifications at all four components. In total, over 140 bisamides have been synthesized and tested *in vitro* for further investigation into structure-activity relationships of this chemotype with the KOR. In the most recent analogue set, particular attention was given to finding a replacement for the thiophene or furan moiety, as it is a potential metabolic liability. A summary of the trends and the results from the most recent sets of analogues will be presented.

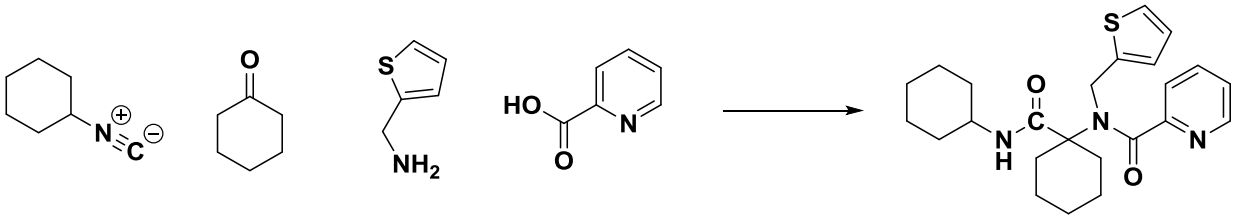

Figure 1. Representative example of the synthesis of a bisamide using the Ugi reaction

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ABSTRACT

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In silico design of novel probes for the atypical opioid receptor MRGPRX2

We aimed to discover small molecule probes for the MRGPRX2 orphan GPCR, a primate-exclusive receptor expressed in the dorsal root and trigeminal ganglia and mast cells. Briefly, we leveraged the results of an *in vitro* β-arrestin screen to develop structural models of MRGPRX2 and virtually screen against ~3.7 million small molecules to predict novel activating scaffolds. We validated our screening hits, predicted agonists, and the *in silico* MRGPRX2 model using mutagenesis and cell-based assays. We also found that opioid agonists from the *in vitro* screen and our selective ligand promoted intracellular calcium release and degranulation in the LAD2 human mast cells, where MRGPRX2 is endogenously expressed. siRNA knockdown of MRGPRX2 significantly reduced opioid-induced or selective probe-induced degranulation. We conclude that MRGPRX2 appears to be a unique atypical opioid receptor that mediates opioid-induced degranulation in LAD2 human mast cells. Our approach prompted the discovery of a pair of demonstrably selective probes for precisely interrogating MRGPRX2 function, which are now commercially available.

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Electrophysiological characterization of BTRX-335140, a novel selective kappa opioid receptor antagonist, in ventral tegmental area dopamine neurons in rat

Endogenous opioid drive on kappa opioid receptors (KOR) inhibits activity of dopamine (DA) neurons. As such, selective antagonists show great promise for treating a variety of neurobehavioral disorders. Here we used an acute midbrain slice and whole cell electrophysiology preparation to evaluate the potency, selectivity and reversibility of a novel KOR antagonist, BTRX-335140, in a specific group of ventral tegmental area (VTA) neurons. BTRX-335140 reduced U69593-induced outward currents in a concentration-dependent manner. The IC_{50} of 1.1 nM in DA neurons was consistent with the potency we measured using a recombinant cell line stably expressing rat KORs (IC_{50} = 3.2 nM). In contrast to BTRX-335140, we found PF-04455242 exhibited partial antagonist activity in this VTA preparation, with a maximum of 60% blockade of the U69593 effect. PF-04455242 also generated an outward current and a decrease in membrane resistance in a subset of neurons, consistent with a channel opening. BTRX-335140 had no effect on responses to a saturating dose of the mu opioid receptor agonist DAMGO in VTA neurons (93±10% of baseline response, n = 7) at a concentration that fully blocked the U69593 responses. Compared to the KOR antagonist nor-BNI, which as expected did not show any washout during slice experiments, responses to U69593 recovered to baseline within 10 minutes of washout of both 1 and 10 nM concentrations of BTRX-335140. Together these data provide electrophysiological evidence in neurons from a circuit critical to our behavioral disorders of interest that BTRX-335140 is a potent, selective, and short-acting KOR antagonist.

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Kappa opioid receptor activation in the amygdala mediates depressive-like behaviors following morphine abstinence through p38 MAPK.

The association between opiate withdrawal and depressive-like symptoms is well documented, however, the molecular mechanism underlying opiate withdrawal-induced depression remains unclear. In the present study, we found that following two weeks of morphine abstinence, significant depressive-like behaviors were induced in mice, which can be blocked by kappa antagonist norBNI or the deletion of oprk1, a gene encodes kappa opioid recetor. The depressive-like behaviors were accompanied with increased prodynorphin and dynorphin A expression in the amygdala. We further found that p38 MAPK was activated in the amygdala following morphine exposure and could be blocked by norBNI pretreatment. Microinjection of p38 MAPK inhibitor SB203580 significantly blocked morphine withdrawal-induced depressive like behaviors, demonstrating that p38 MAPK activation induced by kappa opioid receptor in the amygdala mediated depressive-like behaviors following morphine abstinence. Together, these results suggested that activation of p38 MAPK via kappa receptor activation induced depressive-like behaviors following morphine abstinence.

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Exploring the role of Sodium binding pocket in kappa opioid receptor activation

The presence of a highly conserved sodium binding pocket among Class A G protein-coupled receptors (GPCRs), along with well documented functional role of sodium ion among several GPCRs has led to an appealing hypothesis of sodium pocket mediated mechanism of GPCR activation. 'Sodium binding pocket' is conveniently located below the ligand orthosteric pocket to propagate conformational effects of the bound ligand. Furthermore, ligand functionality specific conformational changes have been observed in the region in X-ray crystal structures. To study the impact of sodium ion on the kappa opioid receptor (KOR) conformations, we simulated molecular dynamics runs for inactive and active conformational form of kappa opioid receptor, in presence and in absence of bound sodium ions and its cognate water network. Fast and persistent capture of sodium ion in inactive conformation of KOR, especially when compared to active form of KOR, indicated effectiveness of inactive-state sidechain conformations in retaining the sodium ion in the pocket. Therefore, inactive-state sidechain conformations of sodium pocket region were replicated in active state and the system was simulated under MD conditions for an extended period of time. Conformational analysis of various GPCR 'micro-switches' and transmembrane movement of the obtained trajectories showed conversion of active-state KOR conformation into inactive-state KOR model in a very limited set of MD runs. The results, perhaps indicates limited exploration of conformational space among the models. Therefore, to extend the exploration of relevant conformations, trajectory showing complete conversion of active to inactive-KOR conformation were used to create a large library of conformations via umbrella sampling. These extended libraries of KOR conformations would enable more detailed study of plausible receptor activation mechanism by invoking Markov state modelling and network analyses. Better understanding of the receptor conformational change resulting in functional effects of various co-factors is highly beneficial for the rational drug discovery process of functionally selective ligands and may further help to derive interactions especially important for the functionally 'biased' ligands, including bitopic ligands.

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Rational identification of functionally selective kappa-opioid receptor ligands

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Traditionally, kappa-opioid receptor (KOR) has been dismissed as ineffective analgesics in humans. Recent studies focusing on functionally selective KOR compounds have uncovered the potential therapeutic roles for KOR in the treatment of pain, affective disorders and addiction. In particular, activation of KOR that predominantly signal through G protein-mediated pathways, promise analgesia without the dysphoric or rewarding side-effects of current opioid medications that are often caused through arrestin-signaling.

We have identified several potent and efficacious KOR agonists and found that these compounds selectively activate KOR over ~330 GPCRs. Characterization of different downstream signaling pathways indicates that these compounds preferentially signal through G protein-mediated pathways and further small molecule optimization is aimed towards maximum G protein-bias. Secondly, to develop a molecular understanding of the different conformations of KOR activating distinct downstream pathways, we have also generated single chain nanobodies that recognize specific conformational states of the receptor. Using a proximity assay, we show that KOR agonists preferentially recruit the active-state recognizing nanobody to the receptor while rejecting the inactive-state recognizing nanobody. Using high-affinity ligands in combination with conformationally selective nanobodies we aim to determine the active-state structure of KOR, to provide a molecular basis for KOR activation and inform future drug optimization towards the design of pathway selective therapeutics with reduced side-effects.

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Structure based discovery of new antagonist and biased agonist chemotypes for Kappa Opioid Receptor

Kappa opioid receptor (KOR) antagonists are promising candidates for treating depression, anxiety and addiction, while G-protein-biased agonists of KOR show favorable addiction-free profile in chronic pain management. New ligand chemotypes with such functional profiles may be beneficial in avoiding side effects, associated with JDTic and some of the clinical candidates. The crystal structure of KOR in complex with JDTic provides a key template for virtual screening for new ligand chemotypes. We used the KOR structure, as well as ligand optimized structural models to screen for new KOR chemotypes among large libraries of lead-like and fragment like compounds. The prospective virtual screening campaign yielded 32% hit rate, identifying several novel fragment-like and lead-like chemotypes of KOR ligands. Furthermore, the first round of optimization for the top six chemotypes resulted in more than 11 new submicromolar KOR binders, with the best K_i = 90 nm and the best ligand efficiency LE = 0.53. Functional assessment of the top compounds shows submicromolar antagonist activity in KOR-expressing cells for at least two top binders. Moreover, one compound was identified as a biased KOR agonist, with submicromolar agonist activity of in G-protein pathway, and minimal β-arrestin mediated response. These results support virtual screening as an effective tool in discovery of new lead chemotypes for KOR with functional profiles that are beneficial for potential therapeutic applications.

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Chemogenetic inhibition of the lateral septum in male C57BL/6 mice induces social aggression and consequent social defeat behaviors in both male and female intruders.

Despite higher lifetime prevalence of depression and anxiety in women compared to men, and existing gender differences in etiology and response to treatment for neuropsychiatric disorders, a majority of basic research studies aimed at elucidating the neurobiological mechanisms underlying depression have been conducted using male animals. Exposure to repeated social defeat stress (SDS) produces a validated animal model of depression-like behaviors with ethological significance and face validity. Traditionally, this model exploits a strong innate territorial aggression in male rodents towards other males. Using this model, male C57BL/6 mice repeatedly subjected to bouts of SDS by a larger, dominant male mouse (aggressor) demonstrate dynorphin-mediated social defeat behaviors. While other species, such as the California mouse (*Peromyscus californicus*), exhibit female intrasexual territorial aggression, we confirm that female C57BL/6 mice (*Mus musuclus*) do not spontaneously exhibit robust aggression towards unfamiliar males or females. Because lesioning the lateral septum (LS) is known to engender "septal rage" in males, we tested whether LS inhibition using a DREADD-mediated approach induces social aggression by bilaterally targeting AAV-hSyn-hM4D$_i$-mCitrine to the LS (LS^{hM4Di}) of C57BL/6 male and female mice. After allowing two weeks for hM4D$_i$ expression to occur, singly housed LS^{hM4Di} males were screened for aggressive behavior with the resident-intruder (R-I) paradigm, in which an unfamiliar male mouse (intruder) was placed in the home cage of the LS^{hM4Di} male (resident). On test day, two R-I assays were conducted: one in the morning (t = 0) in which the LS^{hM4Di} resident male was saline-pretreated, and a second assay at t = 4 hours in which the same LS^{hM4Di} resident male was CNO-pretreated (3 mg/kg) to induce LS inactivation. Relative to saline-pretreatment, CNO-pretreatment significantly enhanced intrasexual aggression exhibited by LS^{hM4Di} males (exemplified by increased frequency and duration of attacks) towards intruders, effectively inducing social defeat of the intruder male (characterized by the presence of submissive postures, immobility, escape attempts, and defensive upright stances). Moreover, CNO-induced aggression was reversible: saline-pretreated LS^{hM4Di} males utilized in a third R-I assay at t = 24 hrs exhibited attenuated intrasexual aggression relative to aggression levels displayed following CNO-pretreatment. CNO-induced aggression was also reversible: following the initial screen for aggressive behavior, CNO-pretreated LS^{hM4Di} males were successfully utilized for numerous (>3) subsequent SDS trials conducted over the course of several weeks. In contrast to the robust aggression exhibited by CNO-pretreated LS^{hM4Di} males, CNO-pretreatment in LS^{hM4Di} female residents did not promote intrasexual aggression towards intruders. We were unable to evoke LS^{hM4Di} intrasexual aggression under myriad circumstances, including the utilization of both virgin and lactating female intruders, as well as resident LS^{hM4Di} mice which were both singly- and group-housed. Interestingly, LS^{hM4Di} males exhibited aggression (not sexual behavior) resembling that of male intrasexual aggression towards female intruders if the female intruder had previously been swabbed with urine collected from male mouse. Socially defeated female intruders exhibited social defeat behaviors mirroring those of socially defeated male intruders. These results confirm the feasibility of SDS as a model of depression in females, thus increasing the utility of this paradigm in defining neurobiological therapeutic targets for depression treatment.

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