April 2013

KAPPA THERAPEUTICS CONFERENCE

Program Book

CAMBRIDGE, MA, APRIL 24-27, 2013

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Program Committee

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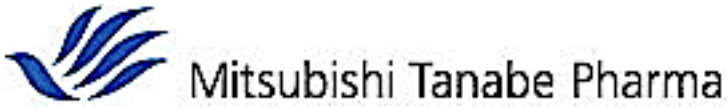


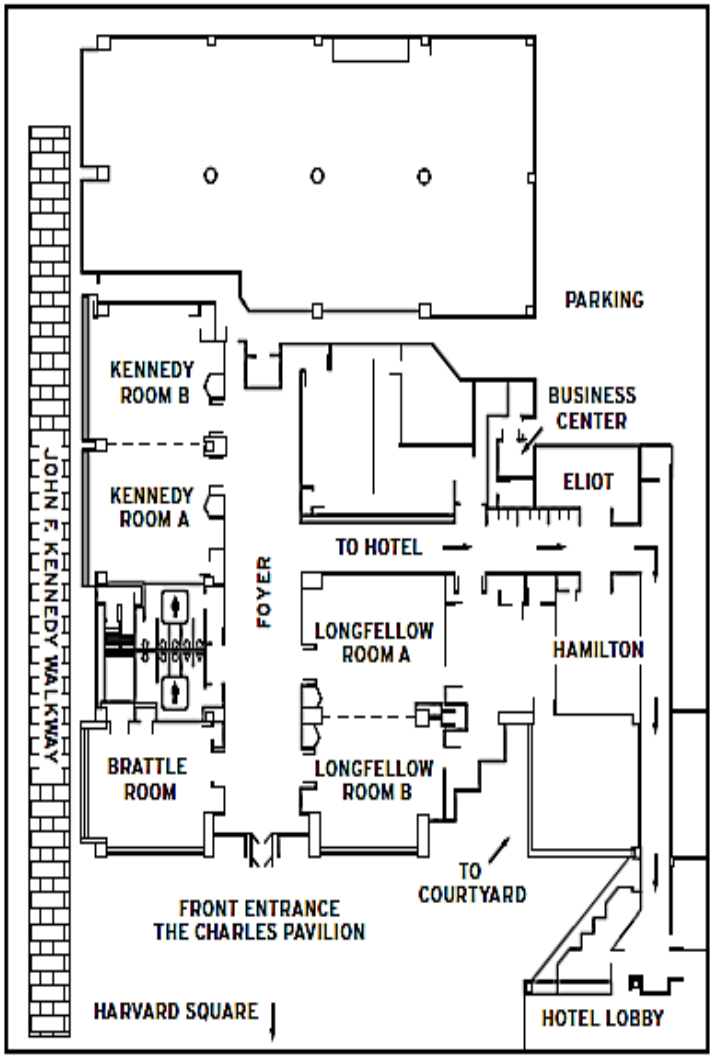
Kappa Therapeutics 2013 Conference Schedule

Time	Wednesday, April 24 Event/Location	Thursday, April 25 Event/Location[2]	Friday, April 26 Event/Location[3]	Saturday, April 27 Event/Location[4]
7:00 - 8:00		**Registration** (Pre-Assembly; 7:00 - 5:00) ***Breakfast*** *(Regattabar; 7:00 - 8:00)*	**Registration** (Pre-Assembly; 7:00 - 5:00) ***Breakfast*** *(Regattabar; 7:00 - 8:00)*	***Breakfast*** *(Regattabar; 7:30 - 8:30)*
8:00 - 10:00		**Welcome from organizers** (Ballroom; 8:00 - 8:30) **Oral Session 1: Clinical Studies** (Ballroom; 8:30 - 12:00)	**Oral Session 3: Systems** (Ballroom; 8:00 - 12:10)	**Oral Session 5: Pain & Other Indications** (Ballroom; 8:30 - 10:10)
10:00 - 10:30		***Morning Coffee Break/Discussion*** *(Pre-Assembly; 9:50 - 10:20)*	***Morning Coffee Break/Discussion*** *(Pre-Assembly; 10:00 - 10:30)*	***Morning Coffee Break/Discussion*** *(Pre-Assembly; 10:10 - 10:30)*
10:30 - 11:00		**Workshop/Data Blitz #1: How do we proceed from here?** (Ballroom; 10:20 - 11:20)	**Oral Session 3 continued** (Ballroom; 10:30 - 12:10)	**Oral Session 6: Future Developments** (Ballroom; 10:30 - 12:10)
11:30 - 12:00		**Oral Session 1 continued: Imaging** (Ballroom; 11:20 - 12:00)		
12:00 - 2:00		***Lunch*** *(Regattabar; 12:00 - 2:00)*	***Lunch*** *(Ballroom, Lowell-Wadsworth; 12:10 - 2:00)*	***Lunch*** *(Regattabar; 12:10 - 1:40)*
2:00 - 3:30		**Oral Session 2: Addiction** (Ballroom; 2:00 - 5:00)	**Oral Session 4: Stress & Dysphoria** (Ballroom; 2:00 - 5:00)	**Oral Session 7: Mechanisms & New Tools** (Ballroom; 1:40 - 4:00)
3:30 - 4:30			**Workshop/Data Blitz #2: How can we distinguish circuits underlying behaviors?** (Ballroom; 3:40 - 5:00)	**Workshop/Data Blitz #3: Functional Selectivity Opportunities** (Ballroom; 4:00 - 4:30)
4:30 - 5:00		**Discussion of dynorphin-dependent addiction mechanisms: George Koob moderator** (Ballroom; 4:40 - 5:00)		**Closing remarks** **Shippenberg Award/Travel Awards** (Ballroom; 4:30 - 5:00)
5:00 - 6:00			**Poster Session/Wine & Cheese** (Rogers Stratton; 5:00 - 7:00)	
6:00 - 7:00	**Registration** (Pre-Assembly; 6:00 - 9:00)	**Student & Postdoc Mixer** (Noir Bar, hotel lobby; 6:00 - 7:00)		
7:00 - 9:00	***Reception*** *(Regattabar; 7:00 - 9:00)*	**Dinner** (no host, local restaurants)	**Dinner** (no host, local restaurants)	

Hotel Accommodations

The Charles Hotel
1 Bennett Street
Cambridge, MA 02138
(617) 864- 1200

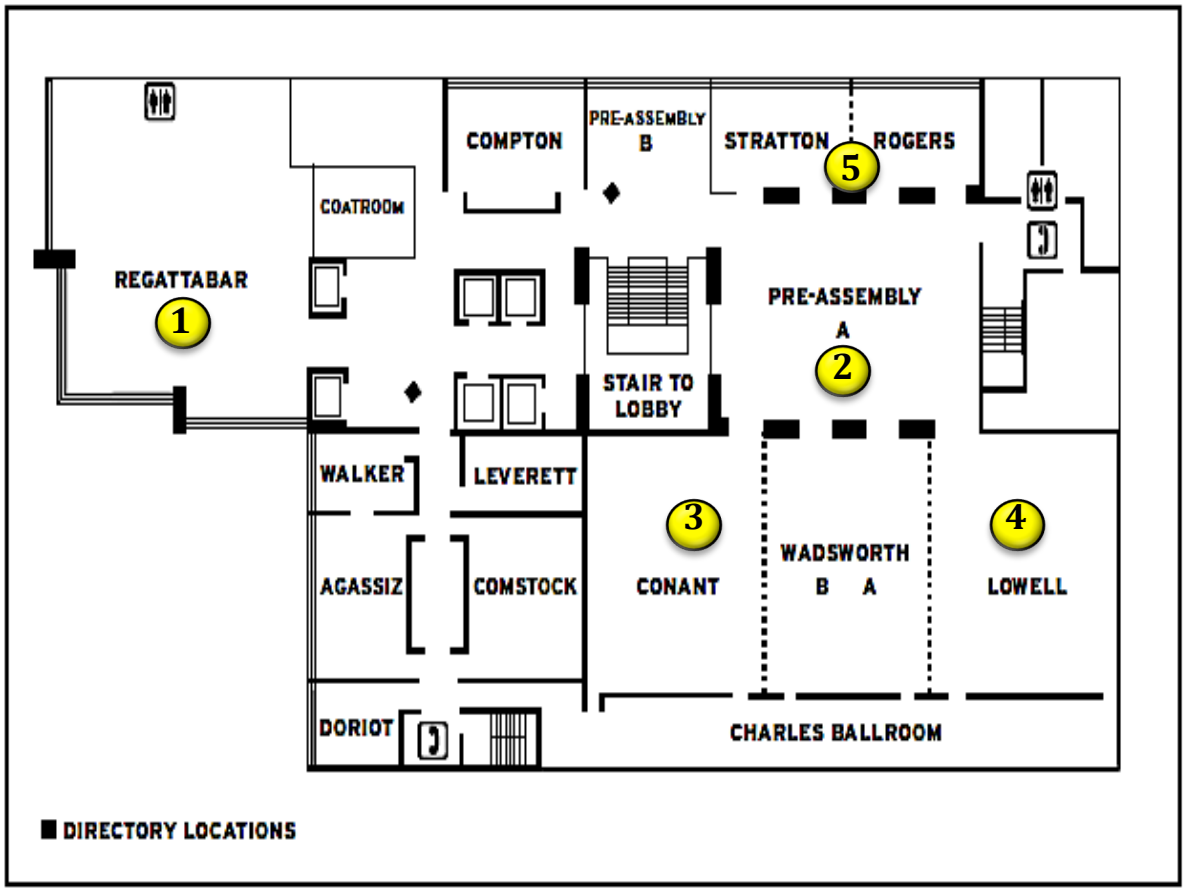
FLOORPLAN: *The Charles Pavilion (Lobby Level)*



SERVICES / FACILITIES:

- COMPLETE CONFERENCE SERVICE DEPARTMENT
- ON SITE AUDIO-VISUAL CONSULTANTS
- MULTI-LINGUAL STAFF
- BOSE SOUND SYSTEM
- COMPLIMENTARY WIRELESS INTERNET ACCESS
- INDOOR PARKING FOR UP TO 700 CARS

FLOORPLAN: *Ballrooms, Banquets & Meeting Rooms (Third Level)*



1. **Reception, breakfast (Thu, Fri, Sat), lunch (Thu, Sat)**
2. **Registration (Wed, Thu, Fri), coffee breaks (Thu, Fri, Sat)**
3. **General session (Thu, Fri, Sat)**
4. **Lunch (Fri)**
5. **Poster session (Fri), breakout room (Thu, Fri, Sat)**

General Information

The second conference on the therapeutic potential of kappa opioids in treating pain and addiction.

Conference Venue
Charles Hotel - Cambridge, MA

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 Pre-Assembly A (outside of General Sessions in Ballroom) and will be open Wednesday, April 24 through Friday, April 26.

Wednesday April 24	6:00pm – 9:00pm
Thursday April 25	7:00am – 5:00pm
Friday April 26	7:00am – 5:00pm

Meals
Breakfast and lunch will be provided at the Charles Hotel for all registrants.

Social Program

Wednesday April 24	Opening reception (Regattabar at the Charles Hotel; 7:00 – 9:00pm)
Thursday April 25	Student and Postdoc mixer (Noir Bar at the Charles Hotel; 6:00 – 7:00pm – No Host)
Friday April 26	Wine and cheese Poster Session (Rogers Stratton room at the Charles Hotel; 5:00 – 7:00pm)

Instructions for Presenters

Posters
Poster boards are 4 feet x 4 feet.

Pushpins will be provided.

Posters must be hung during lunch on Friday April 26.

Your poster number is listed in the Program (see pages 18 – 20, this book)

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.

Kappa Therapeutics 2013 Program

WEDNESDAY April 24, 2013

6:00 – 9:00 PM
Registration (Pre-Assembly, outside Ballroom)

7:00 – 9:00 PM
Opening reception (Regattabar)

THURSDAY April 25, 2013

7:00 - 8:00 AM
Continental Breakfast / Registration (Regattabar/Pre-Assembly)

8:00 AM
Welcome (Ballroom)
Charles Chavkin, Program Chair

Oral Session 1: Clinical Studies (Ballroom)
Charles Chavkin, Session Chair

8:10 AM
Chavkin C, Carlezon WA Jr, Chartoff EH, Graham DL.
The dynorphin/kappa opioid receptor system as a therapeutic target for the treatment of pain, addiction and stress-disorders.

8:30 AM
Ehrich E.
New clinical research in opioid modulation indicates novel utility in treating resistant depression.

8:50 AM
Walters BB, Buda JJ, Carroll I, Gay EA, Gilchrist KH, Gilmour BP, Grego S, Himel HD, Navarro HA, Kosten TR, Leshin SJ, Pitruzzello AM, Rehder KS, Swearingen D, Wang-Smith L.
Standard preclinical studies did not accurately predict an apparent clinical toxicity of JDTic.

9:10 AM
Rorick-Kehn L, Witcher J, Wong C, Gonzales C, Hart J, McKinzie J, Statnick M, Need A, Suico J, McKinzie D, Tauscher-Wisniewski S, Tauscher J, Mitch C, Lowe S.
The translation of rat to human pupillometry studies to determine the functional selectivity of the kappa opioid receptor antagonist LY2456302.

9:30 AM
Butelman ER, Ray B, Ho A, Brownstein AJ, Yuferov V, Kreek MJ.
Differences in KOP-r responsivity in normal volunteers vs drug-free former cocaine-dependent (DFFCD) volunteers: neuroendocrine biomarker effects of nalmefene.

9:50 – 10:20 AM
Discussion / Morning Coffee Break (Pre-Assembly)

10:20 – 11:20 AM
Workshop/Data Blitz Session 1*: How do we proceed from here?
Ivy Carroll and Mary Jeanne Kreek, Session Chairs (Ballroom)

10:20 AM
Brief Opening comments: Ivy Carroll & Mary Jeanne Kreek (5-10 min each)

*This workshop session is open to all participants; if you want to show 1 slide, please get your PowerPoint file to the projectionist before the end of the break.

11:20 AM
Huang HY, Zheng M, Nabulsi N, Naganawa M, Martinez D, Neumeister A, Morris ED, Carson RE.
Imaging the kappa opioid receptor in human with agonist and antagonist PET radiotracers.

11:40 AM
Martinez D, Slifstein M, Carson R, Huang H
Imaging kappa receptors in cocaine abuse.

12:00 - 2:00 PM
Buffet Lunch (Regattabar)

Oral Session 2: Kappa and Addiction (Ballroom)
George Koob, Session Chair

2:00 PM
Koob GF
Role for dynophin-kappa system in the dark side of drug dependence.

2:20 PM
Kreek MJ
The Role of Kappa Dynorphin Receptors in the Natural History of the Addictive Diseases: The Role of Stress Responsivity.

2:40 PM
Cashman JR & Marc AR
Potent inhibition of alcohol self-administration in alcohol-preferring rats.

3:00 PM
Schlosburg JE, Vendruscolo LF, Park PE, Whitfield TW, Koob GF
Dynorphin/kappa receptor system in the nucleus accumbens is critical in the escalation of heroin intake.

3:20 PM
Whitfield Jr TW, Wee S, Schlosburg J, Vendruscolo L, Edwards S, Gould A, Grant Y, Crawford E, Koob GF
Kappa receptor activation in the nucleus accumbens shell subregion underlies compulsive methamphetamine intake.

3:40 PM
Groblewski PA, Zietz C, Willuhn I, Phillips PEM, Chavkin C.
A behavioral economic analysis of the kappa opioid receptor-mediated effects of stress on addiction-like behaviors.

4:00 PM
Anderson SAR, Michaelides M, Ren Y, Thanos P, Wang GJ, Neumaier J, Keller E, Volkow N, Hurd Y.
Prodynorphin in the periamygdaloid cortex is associated with addiction and negative affect neurocircuitry.

4:20 PM
Al-Hasani R, Foshage AM, McCall JG, Bruchas MR.
Locus coeruleus kappa opioid receptors regulate the magnitude of cocaine reinstatement via a noradrenergic mechanism.

4:40 - 5:00 PM
Discussion of Dynorphin-dependent Addiction Mechanisms (Ballroom)
George Koob, Moderator

6:00 PM
Student and Postdoc Mixer (no host; Hotel Lobby Bar: Noir)

7:00 PM
Dinner (no host; local Restaurants, walk or short cab ride)

FRIDAY April 26, 2013

7:00 - 8:00 AM
Continental Breakfast / Registration (Reggattabar)
Oral Session 3: Systems (Ballroom)
Brendan Walker, Session Chair

8:00 AM
Kash T.
Kappa opioid regulation of amygdala circuits.

8:20 AM
Hurd YL, Anderson SA, Michaelides M, Jacobs MM, Yiannoulos G, Wilson S, Keller E, Neumaier J, Liu X, Jutras-Aswad D.
Ventral striatal prodynorphin, novelty-Seeking and reward choice.

8:40 AM
Muschamp JW, Hollander JA, Thompson JL, Onvani S, Hassinger LC, Kamenecka TM, Borgland SL, Kenny PJ, Carlezon WA Jr.
Role of hypocretin (orexin)-dynorphin neurons in motivated behavior.

9:00 AM
Walker BM.
Dissociable effects of kappa-opioid receptor activation on impulsive phenotypes.

9:20 AM
Zhou Y, Colombo G, Gessa GL, Kreek MJ.
Voluntary alcohol drinking alters corticotrophin-releasing factor and preprodynorphin mRNA levels in the central amygdale of Sardinian alcohol-preferring rats.

9:40 AM
Aragona BJ, Resendez SL.
A functional role for kappa receptors within reward/motivation circuitry: implications for the regulation of social bonding.

10:00 - 10:30 AM
Discussion / Morning Coffee Break (Pre-Assembly)

10:30 AM
Schwarzer C, Kastenberger I, Herzog H.
Anxiogenic effects of oestrogen involve the dynorphin – kappa opioid receptor system.

10:50 AM
Negus SS.
Effects of kappa opioids on pain-depressed behavior in rats.

11:10 AM
Margolis, Elyssa
Does KOR location matter? State-dependent changes in circuit function.

11:30 AM
Wagner JJ, Keralapurath MM.
Effects of intermittent, minor stressors on long-term potentiation in dorsal and ventral hippocampus.

11:50 AM
Tejeda HA, Counotte DS, Chefer V, Backman C, Shippenberg T, O'Donnell P.
Kappa opioid receptor regulation of mesocortical and limbic inputs to the prefrontal cortex.

12:10 - 2:00 PM
Buffet Lunch (Ballroom: Lowell-Wadsworth)
Oral Session 4: Stress and Dysphoria (Ballroom)
Elena Chartoff, Session Chair

2:00 PM
Carlezon WA Jr, Van't Veer A, Knoll AT, Cohen BM
Disruption of kappa-opioid receptor function produces anti-stress effects.

2:20 PM
Polter AM, Graziane NM, Bishop RA, Briand LA, Pierce RC, Kauer JA.
Kappa opioid receptors regulate stress-induced cocaine-seeking and synaptic plasticity.

2:40 PM
McCall JG, Al-Hasani R , Blessan S, Hong DY, Foshage A, Krashes MJ, Lowell BB, Kash TL, Bruchas MR.
Optogenetic and anatomical examination of striatal dynorphinergic neurons in aversion and reward.

3:00 PM
Ehrich JM, Messinger DI, Zietz C, Levin JR, Blaszka DJ, Song AJ, Evans SB, Bruchas MR, Phillips PEM, Chavkin C.
The aversive properties of kappa opioid receptor agonists include p38 MAPK activation in dopaminergic neurons of the ventral tegmental area.

3:20 PM
Chartoff EH.
Sex differences in sensitivity to kappa agonists.

3:40 - 5:00 PM
Workshop / Data Blitz Session 2*: (Ballroom)
How can we distinguish circuits underlying behaviors?
Bruce Cohen, Session Chair

*This workshop session is open to all participants; if you want to show 1 slide, please get your PowerPoint file to the projectionist before the end of the break.

5:00 – 7:00 PM
Poster session / wine and cheese (Rogers Stratton)

7:00 PM
Dinner (no host; local Restaurants, walk or short cab ride)

SATURDAY April 27, 2013

7:30 - 8:30 AM
Continental Breakfast (Regattabar)
Oral session 5: Pain and other indications (Ballroom)
Alan Cowan, Session Chair

8:30 AM
Soeberdt M, Knie U, Abels C.
Anti-inflammatory effects of kappa-opioid receptor agonist WOL071-007 in a murine model of oxazolone-induced contact dermatitis after topical application.

8:50 AM
Ross SE, Kardon AP, Polgar E, Todd AJ, Hachisuka J.
Dynorphin is a spinal neuromodulator that mediates the inhibition of itch.

9:10 AM
Zjawiony JK, Polelpally PR, Roth BL, do-Rego JC, Salaga M, Sobczak M, Fichna J.
New salvinorin A-derived orally available agent PR-38 reduces abdominal pain in mice.

9:30 AM
Liu R, Matsunaga F
Salvinorin A: a potent cerebral vascular dilator and potential neuroprotectant.

9:50 AM
Yamamizu K, Furuta S, Hamada Y, Yamashita A, Kuzumaki N, Narita M, Doi K, Katayama S, Nagase H, Yamashita JK, Narita.
Roles of kappa opioids in angiogenesis during development and tumor formation.

10:10 - 10:30 AM
Discussion / Morning Coffee Break (Pre-Assembly)
Oral Session 6: Future Developments (Ballroom)
Michael Bruchas, Session Chair

10:30 AM
Carroll FI, Kormos CM, Jin C, Cueva JP, Runyon SP, Thomas JB, Brieaddy LW, Mascarella SW, Gilmour BP, Navarro HA.
N-{4-[(3-Hydroxyphenyl)-3-methylpiperazin-1-yl]methyl-2-methylpropyl}-4-phenoxybenzamides are potent and selective kappa opioid receptor antagonists.

10:50 AM
Aube J, Stauson SR, Streicher JM, Frankowski KJ, Zhou L, Yoo E, Lovell K, Phillips A, Prisinzano TE, Bohn LM.
Designing new small molecule agonist and antagonist chemotypes as tools for probing kappa opioid receptor function.

11:10 AM
Aldrich JV, Senadheera SN, Eans S, Ganno ML, Mizrachi E, McLaughlin JP.
Diverse pharmacological profiles of novel peptide kappa opioid receptor ligands

11:30 AM
Banghart MR, Williams JT, Sabatini BL
Photoactivatable opioid receptor ligands for spatiotemporally precise manipulations of opioid signaling.

11:50 AM
Vukojevic V, Ming Y, Rogacki M, Terenius.
Quantitative live cell study of kappa-opioid receptor interactions in the plasma membrane by methods with single-molecule sensitivity.

12:10 – 1:40 PM
Buffet Lunch (Regattabar)
Oral Session 7: Mechanisms & New Tools (Ballroom)
Bill Carlezon, Jr., Session Chair

1:40 PM
Rives M-L, Provasi D, Portoghese PS, Filizola M, Javitch JA
Deciphering molecular mechanisms underlying functional selectivity at the kappa opioid receptor.

2:00 PM
Cowan A, Merrill C, Tsuda K, Takei K, Palumbo J
Nalfurafine: Basic pharmacology and clinical trial for treatment of uremic pruritus in subjects with end-stage renal disease receiving hemodialysis.

2:20 PM
Schattauer SS, Chavkin C.
Nalfurafine is an agonist at the kappa opioid receptor with low efficacy for p38 MAPK and high efficiency for ERK 1/2 activation.

2:40 PM
White KL, Vardy E, Roth BL.
Utilizing functionally selective ligands to probe specific signaling pathways of the kappa opioid receptor.

3:00 PM
Filizola M
Towards the Rational Design of Safer Analgesics Targeting the Kappa Opioid Receptor.

3:40 PM
Vardy E, Westkaemper RB, Mosier PD, Roth BL.
Molecular determinants for kappa-opioid receptor activation by Salvinorin A.

4:00 PM
Workshop / Data Blitz Session 3*: (Ballroom)
Functional Selectivity Opportunities
Bryan Roth & Bill Carlezon, Session Co-Chairs

*This workshop session is open to all participants; if you want to show 1 slide, please get your PowerPoint file to the projectionist before the end of the break.

4:30 PM
Closing Remarks / Presentation of Shippenberg/Travel Awards

Poster List

(Friday, 5:00 – 7:00; Rogers Stratton)

1. Al-Hasani R, Sheahan T, Foshage AM, Story GM, Bruchas MR
A novel role for kappa opioid receptors in the modulation of cold sensation

2. Becker HC, Lopez MF, Snyder LL, McCann RL
Kappa opiate receptor activity modulates altered forced swim behavior in ethanol dependent C57BL/6J mice

3. Butelman ER, Ray B, Ho A, Brownstein AJ, Yuferov V, and Kreek MJ
Differences in KOP-r responsivity in normal volunteers vs. drug-free former cocaine-dependent (DFFCD) volunteers: Neuroendocrine biomarker effects of nalmefene

4. Calipari ES, Yorgason JT, McCool BA, Weiner JL, Jones SR
Increases in rapid nucleus accumbens dopamine signaling and methylphenidate, but not cocaine, potency in socially isolated rats

5. Capik NA, McCall NM, Kendra L, and Kash T
Kappa Opioid Receptors Inhibit Glutamatergic Transmission to the Extended Amygdala In an Input-Specific Manner

6. Chartoff EH, Provencher BA, Sromek AW, Russell S, Knapp BI, Bidlack JM, Neumeyer JL
Comparison of the morphinans butorphan and MCL-420 with other high affinity kappa opioid receptor agonists

7. DiMattio KM, Chen C, Shi L, and Liu-Chen L-Y
K6.58(303) in the μ-opioid receptor (MOPR) is required for covalent binding of β-funaltrexamine (β-FNA)

8. Donahue R, Golden S, Russo S, Carlezon WA, Jr.
Social defeat-induced plasticity within brain kappa-opioid systems

9. Falcón E, Brookshire BR, Maier K, and Lucki I
Buprenorphine produces antidepressant-like effects in C57BL/6 mice

10. Huang P, Chen C, DiMattio KM, and Liu-Chen L-Y
Imaging of KOPR-tdTomato in a neuronal cell line

11. Lemos JC, Roth CA, Messigner DI, Gill HK, Phillips PEM, and Chavkin C
Repeated stress dysregulates kappa opioid receptor signaling in the dorsal raphe through a p38a MAPK dependent mechanism

12. Levin JR, Schattauer SS, Melief EJ, Groblewski PA, Chavkin C
JNK Dependent Mechanism of Opioid Receptor Inactivation

13. Li C, McCall NM and Kash TL
Kappa opioid effects on GABAergic transmission in dopaminergic cells of the ventral periaqueductal gray (vPAG)

14. Mosier PD, Vardy E, Polepally PR, Huben K, Setola V, Zjawiony JK, Westkaemper RB, Roth BL
Structural basis for the affinity and efficacy of Salvinorin A-based Michael acceptors at opioid receptors

15. Onvani S, Van't Veer A, Bechtholt AJ, Potter D, Wang Y, Liu-Chen L-Y, Schütz G, Chartoff EH, Rudolph U, Cohen BM, and Carlezon WA, Jr.
Ablation of kappa-opioid receptors from brain dopamine neurons has anxiolytic-like effects and enhances cocaine-induced plasticity

16. Pintar J. and Ansonoff M.
Ultrasonic vocalization patterning is altered in both neonatal and juvenile KOR-1 KO mice.

17. Polepally PR, Roth BL, White K, Vardy E, Zjawiony JK
Dicarboxylic ester – derived salvinorin A ligands to kappa opioid receptor

18. Provencher BA, Sromek AW, Russell S, Chartoff EH, Knapp BI, Bidlack JM, Neumeyer JL
Comparison of the pharmacological activity of (-)-butorphan (MCL-101) with its enantiomer (+)-butorphan (MCL-191)

19. Resendez SL, Keyes PC, Austin CJ, and Aragona BJ
Kappa-Opioid Receptors in the Nucleus Accumbens Shell are Important for Pair Bond-induced Attenuation of Drug Reward

20. Russell SE, Rachlin AB, Smith KL, Potter DN, Berry L, Zhao Z, Chartoff EH
Sex differences in sensitivity to the depressive-like effects of kappa opioid receptor activation in rats

21. Sirohi S, Walker BM
Chronic alcohol exposure induces escalated alcohol self-administration and increased kappa-opioid receptor signaling in the rat medial prefrontal cortex during acute withdrawal

22. Soeberdt M, Knie U, Abels C
Anti-inflammatory effects of kappa-opioid receptor agonist WOL071-007 in a murine model of oxazolone-induced contact dermatitis after topical application

23. Sparrow AM, Potter DN, Chartoff EH
The role of extracellular signal-regulated kinase (ERK) on reward states following kappa opioid receptor activation

24. Tejeda HA, Counotte DS, Chefer V, Backman C, Shippenberg T and O'Donnell P
Kappa-opioid receptor regulation of mesocortical and limbic inputs to the prefrontal cortex

25. Tuesta LM, Fowler CD, Lee BR, Kenny PJ
Brain Glucagon-Like Peptide-1 Regulates the Reinforcing Properties of Nicotine

26. Van't Veer A, Carroll FI, Carlezon WA, Jr.
Disruption of kappa-opioid receptor function attenuates corticotropin-releasing factor (CRF)-effects on startle

27. Yuferov V, Ho A, Morgello S, Kreek MJ
Increase of OPRK1 mRNA levels in the anterior cingulate in postmortem brain of HIV-infected subjects

Program Abstracts

Locus coeruleus kappa opioid receptors regulate the magnitude of cocaine reinstatement via a noradrenergic mechanism

Ream Al-Hasani [1,2], Audra M. Foshage [1,4], Jordan G. McCall [1,2,3], Michael R. Bruchas [1,2,4]

[1]Dept. of Anesthesiology, [2]Dept. of Anatomy & Neurobiology, [3]Program in Neuroscience, [4]Pain Center, Washington University in St. Louis, St. Louis MO, USA

Previous reports have demonstrated that stress causes dynorphin release, activating kappa opioid receptors (KOR) in monoamine circuits resulting in dysphoria-like behavioral responses, potentiation of cocaine conditioned place preference (CPP) and reinstatement of drug seeking. Activation of noradrenergic (NA) receptor systems have also been implicated in similar behaviors. Recent data suggest that dynorphin containing neuronal projections terminate within the locus coeruleus (LC), the primary source of norepinephrine in the forebrain. Together, these reports suggest a possible link between the noradrenergic and dynorphin/kappa opioid circuits. The anatomical and behavioral implications of this putative interaction have not been demonstrated. CPP was used to measure U50,488 (KOR agonist)-mediated reinstatement of cocaine seeking. We initially investigated the necessity of KOR in LC in U50-induced reinstatement of cocaine place preference by injecting NorBNI (KOR antagonist) into the LC.

The magnitude of KOR-dependent reinstatement is reduced following injection of the KOR antagonist, NorBNI into the LC where kappa-induced pERK is also absent, confirming localized KOR-inactivation. To determine the necessity of KOR activity in the LC we virally rescued KOR in the LC of KOR knockout mice. We found that KOR in the LC partially modulates U50-induced reinstatement. Consequently we assessed the role of NA circuits in KOR-dependent stress induced reinstatement of CPP in the presence and absence of the alpha2-agonist clonidine and beta-adrenergic receptor antagonist propranolol in male wild-type C57BL/6 mice. We found that injection of propranolol or clonidine prior to injection of U50,488 significantly potentiated reinstatement of CPP. We also show that the injection of propranolol prior to U50,488 is not sufficient to promote potentiated reinstatement in KO mice with lenti-viral rescue of KOR in the LC only. Together, these findings suggest that there may be an interaction between dynorphin/KOR circuitry and NA systems and that beta-adrenergic may act to tonically-inhibit KOR-dependent reinstatement.

Support: Work supported by NIDA, R00DA025182 (MRB), NIH Common Fund, NINDS R01NS081707 (MRB).
Conflict of interest: The authors declare no conflict of interest.

A novel role for kappa opioid receptors in the modulation of cold sensation

Ream Al-Hasani [1,2], Tayler Sheahan[3], Audra M. Foshage [1,4], Gina M. Story [1,4], Michael R. Bruchas [1,2,4]

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During the past decade, a subset of the transient receptor potential (TRP) family of ion channels was shown to be involved in temperature sensation. Recent studies have demonstrated that the TRP channel, TRPA1 facilitates the perception of noxious cold and is activated by temperatures ≤15°. It is expressed in peripheral projections and cell bodies of sensory neurons in the dorsal root ganglia (DRG), where expression of kappa opioid receptors (KOR) has also been reported. KOR agonists induce antinociceptive effects in the cold-water tail flick nociceptive assay, however the mechanism remains unclear. We investigated the role of KOR in cold sensation using the cold plate assay. Wild-type mice were injected with the KOR agonist U50,488 (5mg/kg i.p.) and placed on the cold plate (2-5°C) 30 min post-injection for 5 min, jumps were recorded as a nocifensive response. Mice injected with U50 on the cold plate showed a significant potentiation in the number of jumps compared to room temperature. This was confirmed using KOR knockout mice and mice injected with KOR antagonist, NorBNI, which both showed a significantly reduced nocifensive response. To ascertain the involvement of TRP channels in KOR mediation of cold sensation TRPA1 knockout mice were injected with U50 and placed on the cold plate. No significant change in the number of jumps was observed between TRPA1 KO mice injected with U50, suggesting that TRPA1 channels are necessary in the modulation of nocifensive response induced following activation of KORs. To examine an interaction between KOR and TRPA1, we measured DRG calcium responses in the presence and absence of KOR agonist and TRPA1 ligands. Together these observations suggest KOR can paradoxically potentiate noxious cold sensation and may interact with TRPA1. These studies have the potential of revealing a novel role of KOR in the modulation of noxious cold sensation.

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

Diverse pharmacological profiles of novel peptide kappa opioid receptor ligands

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The novel macrocyclic tetrapeptide natural product CJ-15,208 (cyclo[Phe-D-Pro-Phe- Trp]) was reported to be a kappa opioid receptor (KOR) antagonist *in vitro* (Saito *et al.*, *J. Antibiot.* 2002, *55*, 847), but exhibited mixed KOR and mu opioid receptor (MOR) antinociception as well as KOR antagonist activity *in vivo* (Ross *et al.*, *Br. J. Pharmacol.* 2012, *165*, 1097). We expected that the cyclic structure of this peptide would confer resistance to proteolytic degradation, and hypothesized that its hydrophobicity would facilitate penetration of biological barriers. Consistent with these expectations, CJ-15,208 is active after oral administration (Aldrich *et al.*, *J. Nat. Prod.* 2013, in press). Therefore, we are exploring the structure-activity relationships (SAR) of this lead peptide. The analogs were evaluated in mice with the 55oC warm-water tail withdrawal assay for antinociception and opioid antagonist activity following central (i.c.v.) and oral administration. In one series of analogs currently under investigation, several distinct opioid profiles have been identified, including different patterns of opioid receptor involvement in the antinociception. Interestingly, unlike the parent peptide some of the analogs do not exhibit KOR antagonist activity. Similar to the parent peptide, all of the analogs exhibited antinociception after oral administration, but with varying potencies. Analogs underwent additional evaluation in the rotorod assay for possible locomotor effects and sedation. At doses producing maximal antinociception, only one of the macrocyclic peptides tested to date exhibited evidence of hypolocomotion/sedation in this assay. Selected analogs are undergoing further evaluation to evaluate the hypothesis that mixed activity involving KOR and MOR results in improved liability profiles. The oral activity of these novel peptides makes them promising lead compounds for potential therapeutic development.

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Conflict of interest: Drs. Aldrich and McLaughlin have filed patent applications on the cyclic tetrapeptides.

Prodynorphin in the periamygdaloid cortex is associated with addiction and negative affect neurocircuitry

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Negative affect plays a significant role in conferring vulnerability to drug addiction. Rodent models of addiction and depression suggest prodynorphin (Pdyn) in the amygdala as a potential candidate substrate, but direct evidence in humans is limited. To obtain insights into the PDYN system in the human amygdala in relation to addiction and negative affective state, gene expression levels of PDYN were studied in the post-mortem human amygdala of heroin abusers and subjects with major depressive disorder (MDD). A common disturbance seen in multiple abuse populations and in MDD subjects, was reduced PDYN mRNA expression in the periamygdaloid cortex (PAC) subnucleus. A similar reduction was also evident in a rodent model of heroin addiction where the Pdyn mRNA alteration was recapitulated at a time point relevant to the negative symptoms characteristic of opiate withdrawal. Given that the PAC is a subregion of the amygdala that is virtually unstudied, and that the functional relevance of Pdyn neurons in this region is unknown, we developed a novel functional *in vivo* imaging technology to explore the functional relevance of PAC PDyn neurons. This strategy combined Designer Receptors Exclusively Activated by Designer Drugs (DREADD) with microPET imaging and was termed 'DREAMM'. Inhibition of the activity of PAC Pdyn-expressing neurons led to a pronounced activation of the extended amygdala, a key substrate in the extrahypothalamic brain stress system. Furthermore, selective inhibition of PAC Pdyn-expressing neurons in rats induced stress- and depression-related physiological and behavioral changes. Altogether, this translational approach has identified disturbance of PAC Pdyn as a neurobiological locus functionally linked to the extended amygdala, which underlies negative affect and addiction vulnerability.

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

A functional role for kappa receptors within reward/motivational circuitry: implications for the regulation of social bonding

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It is extremely well established that kappa-opioid receptors mediate aversion and quite possibly the anhedonic aspects of stressful encounters. Yet, kappa receptors are found (with substantial species differences within reward and motivational brain circuitry including the nucleus accumbens shell. This raises the question as to why such receptors would be robustly exist within such brain regions. Previous studies have suggested that these receptors play little to no role in the sudden off set traditionally studied motivated behaviors such as feeding. Here, we utilize the socially monogamous prairie vole to demonstrate that this species has a robust population of kappa-opioid receptors within the nucleus accumbens shell and that these receptors mediate the mate guarding behavior that is essential for the maintenance of the pair bond formed between mates. Moreover, we demonstrate that this receptor system interacts with the D1-like dopamine receptor system to achieve this behavior. Specifically, increased dopamine transmission provides a general enhancement of the agonistic motivation the organism needs to aggressively protect their bonded mate, whereas the kappa receptor system within the nucleus accumbens shell allows the bonded male to tag novel conspecifics (including novel females) with negative valance and therefore allowing it to perceive this individual as a treat to their mate, family, and indeed their entire social organization. Thus, together, the D1 and kappa systems within the nucleus accumbens shell provide a critical hub for pair bond maintenance. Additionally, we are collecting on going neurochemical data (both *in vitro* and *in vivo*) that provide mechanistic demonstrating how these neurochemical interactions occur and, interestingly, how they play an intriguing and important role in the neuroprotection against maladaptive take over of this brain system by strong artificial rewards (drugs of abuse) that provide robust but false fitness signals.

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Designing new small molecule agonist and antagonist chemotypes as tools for probing kappa opioid receptor function

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The kappa opioid receptor (KOR) has been shown to play a critical role in numerous physiological responses arising from stress, pain, anhedonia and cravings. This has aroused interest in the KOR as a therapeutic target for, *inter alia*, depression, addiction and antinociception. Recently, researchers in the field have begun to associate specific physiological responses with discrete biochemical signaling pathways, hinting at the potential to selectively elicit a desired physiological response without the complication of undesirable side effects. In depth investigations of these signaling pathways will depend on the development of new molecular tools possessing selective functional activity. Toward this end, we have developed five new small molecule ligand classes for KOR modulation. These new classes of KOR modulators are structurally unrelated to known KOR ligands and as such have potential as suitable molecular tools. Through an iterative SAR campaign we have optimized these chemotypes for potency, selectivity, and physical properties. Compounds were first evaluated in the canonical assays for KOR function (e.g. radioligand binding, arrestin recruitment and GTPγS activation) and the most promising candidates further probed as potential tools for exploring the relationship of functional bias to physiological function. Representative compounds highlighted here demonstrate in vivo efficacy and penetration of the blood brain barrier at therapeutically relevant concentrations.

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Conflict of interest: Jeffrey Aubé and Kevin Frankowski have a U.S. patent application (US 20100256142 A1; assignee: University of Kansas) related to the use of the isoquinolinone chemotype as KOR agonists.

Photoactivatable opioid receptor ligands for spatiotemporally precise manipulations of opioid signaling

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Conventional methods for the delivery of opioid agonists and antagonists in brain tissue are limited by diffusion in terms of both speed and localization. Photoactivatable or "caged" molecules can be released with millisecond and micron scale precision, and thus provide a means for robust delivery of specified quantities of ligand with a high degree of spatiotemporal control. To facilitate studies into opioid signaling, we have developed photoactivatable analogues of the opioid receptor peptide agonists dynorphin (1-8) and [Leu]5-enkephalin, and a photoactivatable analogue of the broad spectrum antagonist naloxone. The caged agonists were optimized pharmacologically by adding ultraviolet light-sensitive nitrobenzyl caging groups at different sites on the enkephalin peptide. The incorporation of various caging groups at these sites produced analogues that respond to a range of visible wavelengths and two-photon excitation. These findings were extended to produce a blue light-sensitive caged naloxone. The combination of caged agonist and antagonist was used to measure the kinetics of both activation and deactivation of mu opioid receptors in brain slices using brief flashes of visible light, allowing functional studies into the underlying mechanisms. These reagents enable efficient, quantitative characterization of opioid actions in brain slices and should be applicable *in vivo*. In particular, photorelease of naloxone may uncover functions of endogenous opioid signaling in behaving animals. Furthermore, the general pharmacological principles uncovered can likely be applied to render many opioid ligands light-sensitive.

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Kappa opiate receptor activity modulates altered forced swim behavior in ethanol dependent C57BL/6J mice

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We have shown using a mouse model of ethanol dependence that repeated cycles of chronic intermittent ethanol (CIE) exposure produces escalation of drinking and altered behavioral response to forced swim stress. Specifically, CIE-exposed mice exhibit reduced immobility in the forced swim test at 3 or 7 days following withdrawal. The present study was aimed at examining the effects of a kappa opiate receptor (KOR) agonist on forced swim behavior in CIE exposed compared to control mice. CIE-exposed mice received a single cycle of ethanol vapor exposure in inhalation chambers (16 hr/day for 4 days) while controls were similarly handled but exposed to air in control chambers. At 72 hr following withdrawal, all mice were tested in a 10-min forced swim procedure. Separate CIE-exposed and control groups were injected 10 min prior to the stress test with either vehicle or the KOR agonist U-50488 (*trans*-(+)-3,4-Dichloro-*N*methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide hydrochloride) (25 mg/kg, IP) (N=6/group). Results indicated that, as expected, CIE-exposed mice that received vehicle exhibited reduced immobility (greater amount of struggling) during the forced swim test compared to vehicle-treated controls. The KOR agonist U-50488 attenuated this effect in CIE-exposed mice (increasing immobility) while producing the opposite effect (reducing immobility) in controls. Thus, U-50488 differentially altered forced swim behavior in mice with a history of chronic ethanol exposure compared to ethanol-naïve controls. Follow-up studies are planned to further examine this effect with additional doses of the agonist as well as evaluation of the effects of the KOR antagonist nor-BNI (nor-binaltorphimine). These preliminary results suggest that changes in KOR activity may mediate altered stress response in ethanol dependent mice.

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Conflict of Interest: Dr. Becker has conducted research and consulting activities for Eli Lilly and Company, but these activities are not related to this project. Dr. Lopez, Ms. Snyder, and Ms. McCann declare no potential conflict of interest.

Differences in KOP-r responsivity in normal volunteers vs. drug-free former cocaine-dependent (DFFCD) volunteers: Neuroendocrine biomarker effects of nalmefene

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The KOP-r/dynorphin system is involved in the modulation of dopaminergic function, which prominently underlies the initial rewarding and addictive effects of cocaine. Functional differences in the endogenous KOP-r / dynorphin system may predispose individual vulnerability to cocaine addiction. Also, the KOP-r /dynorphin system undergoes plasticity in animal models of cocaine addiction, and in cocaine-exposed patients, as determined from post-mortem studies. The goal of these studies was to determine whether there were differences in the responsivity of the KOP-r system in normal adult volunteers (22 males, 24 females), versus DFFCD volunteers (9 males, 5 females) who had abstained from cocaine for at least 6 months. KOP-r responsivity was measured with a neuroendocrine biomarker response (blood prolactin levels) to administration of i.v. nalmefene (0 [saline vehicle], 3 or 10 mg). Nalmefene is a KOP-r partial agonist in addition to a MOP-r antagonist (Bart et al., 2005; *Neuropsychopharmacology* 30:2254-2262).

A three-way analysis of variance (ANOVA), Drug History Group X Gender X Nalmefene Condition, with repeated measures on the last factor showed that there was a significant main effect of nalmefene condition: each dose of nalmefene led to increased area under the curve (AUC) from 0-90 min compared to the saline condition, $p < 0.0002$. The DFCCD volunteers had lower AUCs overall than normal volunteers, although this main effect just missed significance, $F(2,56) = 3.89$, $p = 0.053$. Examining only the males yielded a significant effect of Nalmefene Condition, $p < 0.005$, and of Drug History; DFFCD volunteers had significantly lower AUCs than Normal Volunteers, $p < 0.05$. These studies thus detected a decreased KOP-r responsivity in DFFCD males compared to controls. This decreased sensitivity could be a pre-existing factor in DFFCD volunteers prior to cocaine exposure, or more likely may indicate a long-term functional adaptation occurring after chronic cocaine exposure and abstinence.

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Increases in rapid nucleus accumbens dopamine signaling and methylphenidate, but not cocaine, potency in socially isolated rats

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Social isolation (SI) during development is a model of early life stress that results in neurobiological alterations that lead to predisposition for anxiety- and addiction-like behaviors. Striatal dopamine circuitry is a key mediator of behavioral changes that occur due to long-term stressors. In the SI/GH model, increased striatal dopamine overflow to psychostimulants has been observed in SI versus group-housed (GH) animals, therefore, we aimed to assess the potency at the dopamine transporter (DAT) of two structurally dissimilar dopamine uptake inhibitors, cocaine and methylphenidate (MPH).

Long-Evans rats were either GH (4/cage) or SI (1/cage) from postnatal day (PD) 28-77 and neurochemical changes were assessed between PD 93-116 using fast scan cyclic voltammetry in brain slices. Voltammetric detection of dopamine allows for the assessment of uptake kinetics as well as shifts in drug potency at the DAT.

SI animals, relative to GH, had increased dopamine uptake rates, which, as confirmed by western analysis, are mediated by increased DAT levels. Surprisingly, the ability of MPH, but not cocaine, to inhibit dopamine uptake is significantly greater in SI animals relative to GH. To investigate whether increases in DAT levels may drive the MPH potency shifts, we assessed both MPH and cocaine potency in transgenic mice with 4 additional copies (6 total) of the DAT gene. This genetic manipulation results in elevated DAT levels at the cell surface. Similar to SI, MPH, but not cocaine potency was increased in DAT overexpressing mice as measured with both voltammetry and locomotor assays.

The differential effects of MPH and cocaine could be due to structural differences. MPH is structurally similar to amphetamine which is more behaviorally potent in DAT overexpressing mice and SI animals. Further, these data indicate that cocaine potency at the DAT is likely not driving the increased cocaine-induced dopamine overflow observed previously in SI animals.

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Kappa opioid receptors inhibit glutamatergic transmission to the extended amygdala in an input-specific manner

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The Bed Nucleus of the Stria Terminalis (BNST) is a component of the extended amygdala, and plays a key role in both stress and addiction. While the BNST receives multiple glutamatergic inputs, and expresses both dynorphin and Kappa Opioid Receptor (KOR), to date there have been no studies examining the impact of KOR signaling on glutamatergic transmission. In this study, we used a combination of electrophysiological and optogenetic techniques to demonstrate that KORs modulate glutamatergic transmission in the BNST in an input specific fashion. Using whole-cell patch clamp electrophysiology in male mice, KOR activation inhibited electrically evoked excitatory post synaptic current (eEPSCs). We next sought to determine the synaptic locus for this inhibition and examined the impact of KOR agonists on miniature synaptic transmission. We found that application of KOR agonists caused a decrease in frequency, but not amplitude of miniature EPSCs, suggesting the locus of action for this inhibition was presynaptic. We next explored the signaling involved in this inhibition. While a previous study found that ERK signaling was required for KOR inhibition of GABA release in the BNST, we found that KOR inhibition of glutamate release was p38-map kinase dependent. We then dissected the circuitry involved in KOR inhibition in the BNST. We targeted the prefrontal cortex (PFC) and basolateral amygdala (BLA) with AAV5-CamKiia-ChR2-eYFP to demonstrate pathway specific KOR inhibition. KORs inhibited light evoked EPSCs at BLA but not PFC inputs.

In combination with previous studies conducted by our lab, these results suggest that KORs inhibit GABA and glutamate transmission in the BNST via different signaling pathways. Ongoing studies are exploring how exposure to stress or drugs of abuse can selectively alter specific pathways and their modulation by KOR signaling.

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Disruption of kappa-opioid receptor function produces anti-stress effects

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Accumulating evidence indicates that brain kappa-opioid receptors (KORs) and dynorphin, the endogenous ligand that binds at these receptors, are involved in regulating states of motivation and emotion. These findings have stimulated interest in the development of KOR-targeted ligands as therapeutic agents. As one example, it has been suggested that KOR antagonists might have a wide range of indications, including the treatment of depressive-, anxiety-, and addictive disorders, as well as conditions characterized by co-morbidity of these disorders (e.g., PTSD). A general ability to reduce the impact of stress may explain how KOR antagonists can have efficacy in such a variety of animal models that would appear to represent different disease states. This presentation will provide a review of evidence—both published and unpublished—that disruption of KOR function attenuates prominent effects of stress. Behavioral and molecular endpoints will be described; data will include studies that characterize the effects of KOR antagonists and KOR ablation on the effects of stress itself, as well as on the effects of exogenously-delivered corticotropin-releasing factor (CRF), a brain peptide that mediates key effects of stress. Collectively, available data suggest that KOR disruption produces anti-stress effects and under some conditions can prevent the development of stress-induced adaptations. As such, continued evaluation of KOR antagonists as potential therapeutic agents for the treatment and even prevention of stress-related psychiatric illness is warranted.

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Conflict of interest: Dr. Carlezon and McLean Hospital are co-owners of a patent on the use of KOR antagonists to treat depressive disorders, and Dr. Carroll and RTI are co-owners of a patent on the KOR selective antagonist JDTic.

N-{4-[(3-Hydroxyphenyl)-3-methylpiperazin-1-yl]methyl-2-methylpropyl}-4-phenoxybenzamides are potent and selective kappa opioid receptor antagonists

F. Ivy Carroll, Chad M. Kormos, Chunyang Jin, Juan Pablo Cueva, Scott P. Runyon, James B. Thomas, Lawrence E. Brieaddy, S. Wayne Mascarella, Brian P. Gilmour, Hernán A. Navarro

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In 1978, Zimmerman and co-workers reported the discovery of a structurally unique series of opioid receptor pure antagonists based on N-substituted analogs of 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (LY272922). Further development of the LY272922 class of antagonist from our laboratory led to the potent and selective kappa opioid receptor antagonist JDTic. JDTic displayed robust activity in rodent models of depression, anxiety, stress-induced cocaine relapse and nicotine withdrawal. Preclinical studies led to phase 1 clinical studies of JDTic. These clinical studies were terminated due to adverse events. (The results from clinical studies will be presented at this symposium.) Since KOR antagonists may have potential as pharmacotherapies for treatment of psychiatric disorders and substance abuse, especially those produced or exacerbated by stress, there is continuing interest in the discovery and development of new κ opioid receptor antagonists. In addition, there is need for additional κ opioid receptor antagonists to further characterize the recently reported structure of the human κ opioid receptor. We recently reported that N-substituted 3-methyl-4-(3 hydroxyphenyl)piperazines (1) were a new class of opioid receptor antagonists. In this study we first report the syntheses of two piperazine JDTic-like analogs. Evaluation of the two compounds in an in vitro [35S]GTPγS binding assay showed that neither compound showed the high potency and κ opioid receptor selectivity of JDTic. In addition, a library of compounds based on the structure of 1 was synthesized and tested for their ability to inhibit [35S]GTPγS binding stimulated by the selective κ opioid agonist U69,593. These studies led to *N*-[(1*S*)-1-{[(3*S*)-4-(3-hydroxyphenyl)-3-methylpiperazin-1-yl]methyl}-2-methylpropyl]-4-phenoxybenzamide (2), a compound that showed good κ opioid receptor antagonist properties. A structure [35S]GTPγS assay efficacy study based on 2 provided 28 novel *N*-{4-[(3-hydroxyphenyl)-3-methylpiperazin-1-yl]methyl-2-methylpropyl}-4-phenoxybenzamide analogs. Evaluation of these 28 compounds in the [35S]GTPγS binding assay showed that several of the analogs were potent and selective κ opioid receptor antagonists.

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Potent Inhibition of Alcohol Self-Administration in Alcohol-Preferring Rats

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A substituted aryl amide derivative of 6-naltrexamine, previously shown to be a potent kappa opioid receptor antagonist, was used to characterize the physiochemical properties and efficacy to decrease alcohol self-administration in alcohol-preferring rats (P-rats). Pharmacokinetic studies showed that the lead compound had acceptable bioavailabililty. Safety studies showed that the lead was not hepatotoxic at doses 200-fold greater than an efficacious dose. The effect of the lead or separately, naltrexone on the hepatotoxicity of thiobenzamide was investigated, and, compared to naltrexone, the lead compound was observed to be a hepato-protectant. Based on the physiochemical properties of the lead compound, it was examined in rodent animal drinking models. To test the lead compound *in vivo*, we utilized two models of alcohol intake in the rat: oral operant self-administration in dependent and non-dependent P rats and "binge drinking" in both P rats and outbred Sprague-Dawley rats. Results from testing the lead compound in dependent and non-dependent P rats revealed that P rats showed a significant decrease in alcohol intake at doses of 0.00625, and 0.0125 mg/kg in both vapor conditions. In the "binge drinking" animal model, similar results were observed with both P rats and Sprague-Dawley rats showing significant reductions in ethanol intake at 0.00625 and 0.0125 mg/kg. These results suggest that the lead compound may be an effective treatment for alcoholism.

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Sex differences in sensitivity to kappa opioid receptor agonists

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There is growing evidence for pronounced sex differences in behavioral responses to stress and drugs of abuse: females are generally more sensitive to both the reinforcing and aversive effects of drugs and stress-induced relapse. Given the role of kappa opioid receptors (KORs) in mediating stress and dysphoria, we sought to determine whether there are sex differences in the effects of KOR activation on reward function. We measured the effects of the KOR agonist U-50488 on brain stimulation reward in male and female rats using intracranial self-stimulation (ICSS). We found that female rats were significantly less sensitive than males to the depressive-like effects of U-50488, with no difference in U-50488 brain levels. The effects of U-50488 on ICSS were independent of estrous cycle stage or testosterone levels, suggesting that sex differences in KOR activation are not dependent on activational effects of gonadal hormones. To identify neural and molecular substrates within the extended amygdala that are sexually dimorphic for KOR function, we measured the effects of U-50488 in male and female rats on c-Fos and on expression of genes implicated in negative affective states. We found that U-50488 induced more c-Fos expression in the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) and in the bed nucleus of the stria terminalis (BNST) in females compared to males. Using doublelabel immunohistochemistry, we found that the majority of c-Fos neurons in the PVN were corticotropin releasing factor (CRF)-positive and oxytocin-negative. In the BNST, the majority of c-Fos-positive neurons did not co-localized with CRF. Using quantitative real-time PCR (qRT-PCR), we found higher basal levels of dynorphin mRNA in the female PVN, BNST, and basolateral amygdala, suggesting that dynorphin tone may be higher in females and may occlude the effects of exogenously administered KOR agonists. Taken together, these data raise the possibility that the role of KORs in mood regulation is mechanistically different in males and females and underscores the importance of understanding KOR function in both sexes to develop evidence-based therapeutics.

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

Comparison of the morphinans butorphan and MCL-420 with other high affinity kappa opioid receptor agonists

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(-)-Butorphan and MCL-420 are potent kappa and mu opioid receptor (KOR and MOR, respectively) partial agonists that have been shown to attenuate drug taking behavior in animal models. Similarly, selective KOR agonists such as U-69,593 and salvinorin A, have also been shown to attenuate behavioral effects of cocaine. A major goal of several research efforts is to develop a therapeutic that suppresses the psychostimulant effects of cocaine without having aversive or rewarding properties on its own. Selective KOR agonists are not ideal for this purpose because they can have profoundly aversive effects in humans and laboratory animals. We hypothesize that mixed KOR and MOR partial agonists may have greater therapeutic potential than KOR agonists alone. The pharmacological and behavioral properties of butorphan, MCL-420, U-69,593 and salvinorin A will be compared and discussed.

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Conflict of Interest: The authors have no conflict of interest to disclose.

Nalfurafine: Basic pharmacology and clinical trial for treatment of uremic pruritus in subjects with end-stage renal disease receiving hemodialysis

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Nalfurafine was first described 15 years ago as a potent, centrally active kappa opioid agonist with an epoxymorphinan, rather than the usual arylacetamide, structure. Its preclinical pharmacological profile was typically kappa opioid – antinociceptive, sedative, diuretic and with a lower potential for physical dependence, respiratory depression and slowing of gastrointestinal transit, relative to morphine. Potent, dose-related anti-scratch activity was revealed in mouse assays using compound 48/80, norbinaltorphimine, and GNTI as pruritogens. Tolerance did not develop to the anti-scratch activity of nalfurafine (0.02 mg/kg, s.c.) against a standard dose of GNTI (0.3 mg/kg, s.c.) when injected daily in mice for 10 consecutive days. Additionally, nalfurafine inhibited c-fos expression induced by GNTI and compound 48/80 in the dorsal horn of the mouse spinal cord, suggesting inhibitory activity at the spinal level. On the basis of these preclinical studies, nalfurafine held promise as a potentially useful, first in class, antipruritic in human conditions involving itch. In 2009, nalfurafine received Japanese approval for the treatment of uremic pruritus (UP). UP is a chronic pruritic disorder of systemic origin which occurs in 70% of patients receiving chronic hemodialysis and is associated with increased morbidity, mortality, and impaired quality of life (QoL). Despite medical need, there is no approved treatment in North America. Nalfurafine is currently being investigated in the US and Canada as a once daily oral therapy for UP in chronic hemodialysis patients. 360 subjects from 90 centers will be randomized equally into 1 of 4 double-blind treatments (2.5, 5, or 10μg nalfurafine or placebo) in this Phase 2 study, which features an 8-week active treatment phase. Efficacy, safety, QoL, and pharmacokinetics will be evaluated.

Conflict of interest: Dr. Cowan consults for Mitsubishi Tanabe Pharma. Drs. Merrill, Tsuda, Takei and Palumbo are employees of Mitsubishi Tanabe Pharma.

K6.58(303) in the μ-opioid receptor (MOPR) is required for covalent binding of β-funaltrexamine (β-FNA)

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β-FNA is an irreversible antagonist at the MOPR and a reversible agonist at the κ-opioid receptor (KOPR). We previously reported that Lys5.39(233) in the MOPR formed a covalent bond with β-FNA (Chen C *et al*., *JBC* 271:21422, 1996), which was confirmed in the crystal structure of β-FNA-bound MOPR (Manglik A *et al*., *Nature* 485:321, 2012). However, this residue is conserved across the opioid receptors and, thus, does not explain the differential activities of β-FNA at μ and κ receptors. Based on comparative molecular docking using the crystal structures of MOPR and KOPR, we hypothesize that divergent residues at position 6.58 (K303 in MOPR and E297 in KOPR) play distinct roles in positioning the methyl acetate group of β-FNA, resulting in the differential binding of β-FNA at these two receptors. To test this hypothesis, we generated K303E MOPR and E297K KOPR mutants and stably expressed the wildtype and mutant KOPR and MOPR in Neuro2A cells at comparable levels. Saturation binding with [^{3}H]diprenorphine (DIP) demonstrated that the mutations did not affect [^{3}H]DIP affinity. Inhibition of [^{3}H]DIP by unlabeled β-FNA showed that the mutations did not appreciably affect β-FNA affinity. The K303E mutation in the MOPR abolished covalent binding of [^{3}H]β-FNA to the receptor; however, the E297K mutation in the KOPR did not enable irreversible binding of [^{3}H]β-FNA. Efficacy of β-FNA at the two receptors, as determined by [^{35}S]GTPγS binding, was unaffected by the mutations. Thus, K6.58(303) in the MOPR is crucial for β-FNA irreversible binding, but in the KOPR, more than E297K mutation is needed. We will continue to search for other mutations in the KOPR potentially allowing β-FNA irreversible binding.

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Social defeat-induced plasticity within brain kappa-opioid systems

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Kappa-opioid receptors (KORs) and their endogenous ligand dynorphin are expressed in brain areas implicated in stress responsiveness, and have been shown to play an important role in depressive-like and anxiogenic-like effects in animal models of stress. The present studies were designed to elucidate the role of KOR systems in mediating the molecular and behavioral effects of acute and chronic social defeat stress (SDS). SDS is a robust model of stress-related illness in mice that exploits the ethological relevance of territorial aggression. It reliably produces depressive-like behaviors, including social avoidance, increased anxiety, and anhedonia (reduced sensitivity to reward) as assessed by the intracranial self-stimulation (ICSS) test. Wild-type mice subjected to acute SDS showed significant increases in prodynorphin mRNA expression and nominal increases in KOR mRNA expression within the nucleus accumbens. In contrast, following a chronic (10-day) SDS regimen, prodynorphin mRNA expression was significantly decreased regardless of whether the mice showed a "stresssusceptible" or "stress-resilient" phenotype in social interaction tests. Susceptible mice treated with daily vehicle injections for 35 days continued to show significant decreases in prodynorphin mRNA, whereas mice treated daily with imipramine (20 mg/kg, IP) showed a reversal of both the downregulation of prodynorphin and the social avoidance phenotype. We also examined the effects of SDS in mutant mice in which KORs are ablated selectively in dopamine-containing neurons. Preliminary data indicate that these mice show signs of resilience to SDS, as indicated by delays in the onset of SDS-induced increases in ICSS thresholds (a sign of anhedonia) across the 10-day regimen. Ongoing studies are investigating the effects of SDS in the mutant mice using other tests that quantify depressive- and anxiety-like behaviors, including social avoidance. Our findings raise the possibility that endogenous KOR systems are involved in mediating the effects of acute stress, but may be subject to counter-adaptations following chronic stress.

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Conflict of interest: Dr. Carlezon and McLean Hospital are co-owners of a patent on the use of KOR antagonists to treat depressive disorders.

New Clinical Research in Opioid Modulation Indicates Novel Utility in Treating Resistant Depression

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The endogenous opioid system is thought to play a key role in the regulation of mood. Indeed, the "opium cure" was a pharmacologic mainstay of depression therapy prior to the advent of tricyclic and monoamine oxidase inhibitor anti-depressants in the 1950's. The precise mechanism of endogenous opioids in mood regulation, however, is uncertain. The contemporary use of opioids for depression is limited by abuse potential, presumably a result of mu opioid agonism. ALKS 5461 consists of buprenorphine (BUP), a partial mu agonist, combined with ALKS 33, a counter-acting mu antagonist, co-formulated for sublingual administration. The ALKS 33 component was designed to be highly potent and sublingually bioavailable with the latter two properties being essential for sublingual co-formulation. Initial clinical evaluation included a remifentanyl challenge study to establish mu opioid blockade by ALKS 33, and a pharmacokinetic and pharmacodynamic interaction study of BUP and ALKS 33 to ascertain appropriate ratios of the two ALKS 5461 components. Subsequently a pilot assessment of safety and efficacy of ALKS 5461 in treatment resistant depression (TRD) was conducted.

(1) Remifentanyl challenge study in N=20 non-addicted opioid-experienced volunteers. Serial remifentanyl or saline challenges were performed pre & up to 7 days post dose of 10 and 20mg doses of ALKS 33. Mu agonist effects were assessed by physiologic and subjective VAS assessments. (2) Double-blind two period randomized crossover interaction study assessing single doses of 0, 1, 4, 8 and 16mg of ALKS 33 co-administered with 8mg buprenorphine in N=16 volunteers. PK, physiologic and subjective assessments were obtained. (3) Double-blind, placebo-controlled pilot study in N=32 patients with TRD randomized to two cohorts. The ALKS 5461 8:1 (BUP:ALKS 33) ratio cohort was treated with escalating doses of 2:0.25 mg and 4:0.5 mg for 7 days. The 1:1 ratio cohort received escalating doses of 4:4 mg and 8:8 mg. Efficacy was measured using the 17-item Hamilton Rating Scale for Depression (HAM-D-17) and the Montgomery-Åsberg Depression Rating Scale (MADRS). Visual analog scales were used to assess drug liking, subjective drug effects and standard AE assessments.

The remifentanyl challenge study demonstrated that ALKS 33 is a potent mu antagonist with complete blockade of mu agonist effects lasting >24 hours following a single dose. The BUP – ALKS 33 interaction study demonstrated that a 8:1 ratio was associated with partial blockade of subjective and physiologic mu agonist effects of BUP whereas complete blockade was observed with a 1:1 ratio of the two agents. In the pilot study in patients with TRD, changes from baseline to day 7 in HAM-D-17 scores were -1.0 (4.2), -5.0 (6.1), and -6.7 (3.4), [mean (SD), placebo, 8:1 ratio, and 1:1 ratio respectively; p=0.032 for 1:1 ratio

vs. placebo]; changes in MADRS scores were -3.5 (5.8), -8.5 (7.4), and -11.4 (6.6), respectively (p=0.054 for 1:1
ratio vs. placebo). Patients receiving the 8:1 ratio experienced greater subjective scores of "Feeling High" and "Feeling Sedated" compared to the 1:1 ratio. The most common AEs were dizziness, nausea, vomiting, and sedation, which occurred most frequently in patients receiving the 8:1 ratio.

In patients with TRD, ALKS 5461 showed evidence of clinically important efficacy vs. placebo with rapid onset at both dose ratios. Greater efficacy was observed with the 1:1 ratio, i.e. with complete mu blockade. More favorable safety and subjective drug effect profiles were also observed for the 1:1 ratio as compared with the 8:1 partial mu blockade ratio in the pilot study. ALKS 5461 may represent a novel treatment of TRD with a rapid onset of effect.

Unique Data. We describe new clinical findings with opioid modulation in patients with major depression. We provide an overview of clinical findings, including new data from the MADRS and HAM-D scales from a phase 1/2 study of ALKS 5461, a novel combination of an opioid agonist and antagonist, which showed evidence of efficacy in a pilot study of TRD. This novel approach to treatment may overcome the limitations previously associated with unblocked mu agonist therapy and clarify underlying mechanisms of opioids in the regulation of mood.

Conflict of interest: All authors are employees of, and shareholders in, Alkermes, Incorporated.

The aversive properties of kappa opioid receptor agonists include p38α MAPK activation in dopaminergic neurons of the ventral tegmental area

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Although it has been demonstrated that activation of p38 mitogen-activated protein kinase (MAPK) in serotonergic neurons of the dorsal raphe nucleus (DRN) can mediate aversive properties of kappa opioid receptor (KOR) signaling, the aversive effects of KOR activation in other circuits remains poorly understood. Dopamine-deficient mice still show the aversive effects of the KOR agonist U50,488 (Land 2009), but injection of the KOR antagonist norBNI into the ventral tegmental area (VTA) did block U50,488-induced conditioned place aversion in wild-type mice. Lentiviral re-expression of KOR in the VTA of KOR(-/-) mice also restored U50,488-induced aversion, similar to the restoration observed after injection in DRN. Consistent with the requirement for p38a activation by KOR previously reported (Bruchas 2011), lentiviral expression of a mutated KOR in VTA which cannot activate p38 MAPK failed to reinstate the aversion. Conditional knockout of the isoform p38a MAPK in dopaminergic neurons blocked the aversive properties of U50,488. Conditional knockout mice performed equivalently to control littermates in the rotarod test, a dopamine-dependent model of aversive learning. They also showed equivalent conditioned place preference for cocaine to WT littermates. KOR activation inhibited dopamine release detected by *in vivo* fast scan cyclic voltammetry, consistent with prior reports using microdialysis. Dopamine release was electrically stimulated—both directly via the medial forebrain bundle and indirectly via the pedunculopontine tegmental nucleus and subsequent release of glutamate in the VTA—and recorded in the nucleus accumbens. Although U50,488 failed to inhibit dopamine release in KOR(-/-) mice, equivalent inhibition of dopamine release was observed in WT and conditional p38a MAPK knockout mice. These findings suggest that p38 MAPK activation in dopaminergic neurons is necessary for the aversive properties of KOR activation, and further that inhibition of dopamine release alone is not sufficient to mediate aversion.

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Buprenorphine produces antidepressant-like effects in C57BL/6 mice

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Major Depressive Disorder is the most prevalent form of mental illness in the US and current antidepressant medications are not effective on approximately 40% of patients. Buprenorphine (BPN) is currently used in the treatment of opiate dependence and chronic pain, with actions as a partial mu-opioid receptor agonist and kappa-opioid receptor (KOR) antagonist. Previous studies have shown that KOR antagonists produce antidepressant and anxiolytic-like effects in rodent models of depression. Here we examine the behavioral effects of BPN in mouse behavioral screening tests for antidepressant and anxiolytic effects. When C57BL/6J mice were tested 30 min after BPN treatment (0.065, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg i.p.) in the forced swim test (FST) and tail suspension test (TST), BPN produced significant reductions in immobility. However, these responses were due to hyperactivity induced by the drug at this timepoint. BPN (0.25 mg/kg) was able to significantly reduce immobility in the FST, but not in the TST, when tested 24 h after treatment. No significant differences in locomotor activity were observed at this timepoint. Nor-BNI (10 mg/kg i.p.), a long-lasting KOR antagonist, also produced a significant reduction in immobility in the FST, with no effects in locomotor activity, 24 h after treatment. A single injection of BPN (0.25 mg/kg) produced an anxiolytic-like effect in the novelty-induced hypophagia (NIH) test 24 h after administration. Interestingly, chronic (6 d) treatment of BPN (0.25 mg/kg) produced a sustained reduction of immobility in the FST and no difference in locomotor activity 24 h post-treatment. Additionally, chronic BPN treatment produced a sustained reduction in the latency to approach food in the NIH test. These studies support the use of BPN as a novel and rapid antidepressant for treatment-resistant depression, since C57BL/6 mice are resistant to the antidepressant and anxiolytic effects of fluoxetine.

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Towards the Rational Design of Safer Analgesics Targeting the Kappa Opioid Receptor

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The discovery of small molecule chemotypes with favorable drug-like properties at the kappa opioid (KOP) receptor has traditionally been hindered by the lack of high-resolution structural information of the receptor. The recent availability of crystal structures for all opioid receptor subtypes, coupled with new insights into ligand bias and receptor oligomerization, offer an unprecedented opportunity to discover more efficacious molecules acting at the KOP receptor. I will present an overview of intriguing, published and unpublished results we have recently obtained using a variety of computational biology approaches, ranging from virtual screening, chemoinformatics, and molecular dynamics simulations, towards the rational design of safer analgesics with lesser side effects targeting the KOP receptor. Some of these results have already been validated experimentally.

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Conflict of interest: A provisional patent including MF as an inventor was recently filed by Columbia University based on collaborative research along with Kansas University and Mount Sinai.

A behavioral economic analysis of the kappa opioid receptor-mediated effects of stress on addiction-like behaviors

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The involvement of kappa opioid receptor signaling in the effects of stress on drug-motivated behavior was examined utilizing a recently described behavioral economic approach to assess both the consummatory and appetitive characteristics of cocaine self-administration in two strains of rats (Oleson et al., 2011). Pre-treatment of male Wistar rats with norBNI (10 mg/kg, IP) reduced immobility and increased swimming behavior during the repeated 15-min forced swim (FSS) tests—an effect that was even more pronounced in the stress-prone Wistar Kyoto (WKY) strain. Exposure to FSS prior to extended access (4h) to cocaine self-administration resulted in a modest increase in escalation of drug intake by the Wistar rats over the four-day period. Following stress and extended cocaine access, rats were tested with the Oleson-Roberts threshold procedure that consisted of a series of descending cocaine doses under a fixed response requirement (FR1) in a single self-administration session. Wistar rats with a history of stress exposure exhibited an increased Pmax (the price that elicits maximum responding) suggesting that they were willing to pay a higher unit price for cocaine (i.e., increased motivation to obtain drug). Additionally, stressed Wistar rats self-administered more cocaine when costs were highest despite showing normal levels of consumption under low price constraints. Interestingly, norBNI pretreatment eliminated the stress-induced increase in Pmax but did not reduce the stress-induced increase in responding evident at higher costs in Wistar rats. Although unstressed control WKY rats showed an elevated baseline Pmax when compared to Wistar rats, stressed WKY rats showed no change in Pmax and no effects of norBNI pretreatment. These data show that repeated stress caused strain-dependent changes in drug motivated behavior and reward sensitivity (i.e., horizontal and vertical shifts in the cocaine demand curves, respectively) and that these dissociable shifts differentially rely on the kappa
opioid receptor system.

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Conflict of interest: The authors declare no conflict of interest

Imaging of KOPR-tdTomato in a neuronal cell line

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Agonist-induced GPCR trafficking and signaling may vary among cell types. Previous studies on KOPR trafficking were mostly conducted in CHO or HEK cells using antibody labeling against epitope tagged KOPR. Enhanced green fluorescent protein (eGFP) has been fused to several GPCRs, including KOPR, without significantly affecting receptor properties. DOPR-eGFP knockin mice have been generated and proven to be useful for correlating receptor trafficking with behavioral responses. Our objective is to use a KOPR-fluorescent protein fusion in a neuronal cell line for live cell imaging. We chose to use the red fluorescent protein tdTomato (tdT), in addition to eGFP, because of their high photostability and the much higher brightness of tdT. We have generated cDNA constructs of mKOPR-tdT and mKOPR-eGFP by fusing tdT or eGFP to the C-terminus of the mouse KOPR. When transiently transfected into Neuro2A cells (a mouse neuroblastoma cell line) with the same amount of DNA, mKOPR-eGFP and mKOPR-tdT were expressed to similar levels (~10 pmole/mg protein), and mKOPR-tdT was much brighter. mKOPR-eGFP and mKOPR-tdT had a Kd value of ~0.4 nM for [3H]diprenorphine as determined by saturation binding assays, similar to FLAG-mKOPR. Both mKOPR-tdT and mKOPR-eGFP were expressed on plasma membranes and were internalized to a perinuclear compartment following 30-min stimulation of cells with 1 mM U50,488H, similar to FLAG-mKOPR. Five-min stimulation with 1 mM U50,488H enhanced phosphorylation of p44/42 significantly in cells transfected with mKOPR-tdT, mKOPR-eGFP or FLAG-mKOPR. These results suggest that mKOPR-tdT is likely to retain the biological activities of the wildtype KOPR. We are establishing clonal Neuro2A cell lines stably expressing the three KOPR constructs, and we will conduct more functional comparisons and live cell imaging.

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Imaging the kappa opioid receptor in humans with agonist and antagonist PET radiotracers

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Multiple lines of evidence have indicated that the opioid system, especially the kappa opioid receptor (KOR), is critically involved in the biology of addictions. It is well established that the addictive potential of cocaine, other psychostimulants, and almost all drugs of abuse, derives from their direct impact on the reward circuitry, by stimulating dopamine (DA) release. The involvement of the KOR is thought to be through its ability to modulate DA function. Activation of the KOR by dynorphin or administration of KOR agonists inhibits psychostimulant-induced DA release, and attenuation of DA release has been shown to inhibit both the psychomotor effects and reinforcing behaviors of psychostimulants. Repeated administration of drugs of abuse leads to the dysregulation of the dynorphin/KOR modulatory system. PET imaging with KOR selective radiotracers is a valuable tool to probe the involvement of KOR and its potential dysregulation in addictive disorders. As such, there has been a longstanding interest to develop PET radiotracers for in vivo investigation of KOR. However, this effort, until recently, has been hampered by the lack of appropriate KOR selective antagonists, or the difficulties in using KOR agonist tracers for clinical applications due to the stringent requirement to control the injected mass in order to avoid the dysphoric agonist effects even at micro-dosing. Lately we have successfully advanced two PET radiotracers to clinical research applications, through the use of innovative chemistry methodologies, and by taking advantage of the recent availability of selective KOR antagonists as suitable candidates for PET tracer development. In this paper we present our work in the development and validation of the KORselective agonist [11C]GR103545 and antagonist [11C]LY2795050 as PET imaging agents. We will also discuss the current application of these radiotracers in imaging studies to investigate KOR function in cocaine abuse, alcoholism, depression, and post-traumatic stress disorder.

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Ventral Striatal Prodynorphin, Novelty-Seeking and Reward Choice

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Genetic factors impact behavioral traits relevant to addiction vulnerability and dysregulation of discrete neurobiological circuits are predicted to contribute to such individual differences. We explored whether individual genetic differences of the prodynorphin (*PDYN*) gene, which is enriched in striatonigral neurons, is associated with gene expression as well as behavioral traits of positive reward sensitivity as the striatonigral pathway is a key neuronal circuit implicated in positive 'Go' behavioral choice and action. The findings revealed that 3'UTR single nucleotide polymorphisms (SNPs) rs910080 and rs2235749 are significantly associated with striatal *PDYN* mRNA expression in the postmortem human brain. Carriers of the rs910080A allele (comparable to the rs2235749G allele) had elevated mRNA expression in the nucleus accumbens (NAc) shell and caudate nucleus. To interrogate a possible behavioral relevance of these SNPs, healthy adult subjects were assessed for Novelty Seeking, Reward Dependence and Impulsive Sensation-Seeking temperamental traits, as well as positive and negative reward learning performance in relation to the *PDYN* variants. There was a selective association of the *PDYN* SNPs with novelty-seeking trait and a strong genotype-dose association with positive reinforcement behavior. A translational animal approach was further conducted to assess the direct relevance of NAc Pdyn expression and striatonigral pathway to novelty and reinforcement behavior. Using a viral mediated strategy to knockdown *Pdyn* mRNA expression directly influenced novelty seeking and self-administration of a natural food reward. Overall, this translational study suggests a contribution of ventral striatal *PDYN* circuitry to novelty seeking and positive reinforcement traits

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Kappa opioid regulation of amygdala circuits

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Extensive behavioral studies have shown that Kappa Opioid Receptor (KOR) signaling is involved in a variety of stress induced alterations in function, including anxiety, relapse and dysphoria. Despite these studies, little is known about the mechanisms by which KOR can influence neuronal circuitry underlying these behaviors. Our efforts have focused on understanding how KOR can regulate neuronal function in the extended amygdala. The extended amygdala is a series of extensively interconnected structures, composed of the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST), that act as a critical regulators of emotional state via projections to hypothalamic and brainstem areas that mediate the specific stress-related behavioral outcomes. Interestingly, it was shown that a stressful stimulus leads to activation of KOR signaling in the BNST, but not the CeA. In order to develop a mechanistic understanding of how KOR can modulate neural circuitry, we have used a multidisciplinary approach to both characterize the impact of KOR signaling on defined synaptic inputs in to the BNST, and to probe the impact of repeated stress and drug exposure on these systems. We found that KOR activation inhibits both GABA and Glutamate release in the BNST, however the signaling mechanisms mediating these distinct forms of modulation are different. KOR inhibition of GABA release is mediated by ERK signaling, whereas KOR inhibition of glutamate release is mediated by p38 signaling. Preliminary evidence suggests that select KOR agonists can differentially modulate neurotransmitter release. This raises the possibility that biased KOR agonists can be used to differently modulate function in this circuit and ultimately behavior. Ongoing studies are probing both the source of dynorphin to the BNST and the behavioral significance of these different forms of modulation.

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Role for dynorphin-kappa system in the dark side of drug dependence

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Drug addiction has been defined as a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting intake, and (3) emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability), reflecting a motivational withdrawal syndrome, when access to the drug is prevented (defined here as dependence). The compulsivity associated with drugs of abuse has been hypothesized to derive from several mechanisms, one of which involves increased drug seeking that results from negative reinforcement. Here, the emergence of a negative emotional state with the development of dependence provides the aversive stimulus to drive drug seeking to remove the negative emotional state (negative reinforcement). Animal models of excessive drug taking associated with extended access have been developed where animals increase drug seeking with extended access/exposure. Multiple mechanisms can be envisioned to explain the role of kappa systems in the compulsivity associated with addiction, including interactions with the dopamine system (a within-system neuroadaptation where dynorphin drives decreases in dopamine function) and interactions with other brain stress systems (between-system neuroadaptation where dynorphin activates or is activated by corticotropin-releasing factor). Results from our laboratory show that blockade of kappa opioid receptors can block the escalation in intake of cocaine, methamphetamine, heroin, and nicotine. Evidence to date suggests that one site for these actions is the shell of the nucleus accumbens, but other data suggest a role for the central nucleus of the amygdala. One viable hypothesis under test is that nucleus accumbens dynorphin mediates the dysphoric-like effects of drug withdrawal, and the central nucleusof the amygdala mediates the anxiety-like effects of drug withdrawal. Further evidence suggests that once the kappa system is engaged, the neuroadaptations persist and are resistant to kappa antagonists. Thus, different brain areas may be responsible for these differential effects of activation of the dynorphin/kappa opioid system in the transition to addiction.

The Role of Kappa Opioid Receptor/Dynorphin System in the Natural History of the Addictive Diseases: The Role of Stress Responsivity

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Kappa opioid receptor and dynorphin gene variants have been reported (by our Laboratory and others) to be associated with opiate, cocaine and cocaine-alcohol addition, as well as alcoholism alone. By careful observations of persons with specific addictive diseases in a clinical research setting, we have been able to develop rodent models, both investigator-administered and self-administered, of the addiction-like cycles. In the self-administration models, we use a 10-18 hour extended access in rats and now have been able to extend our mouse self-administration from 2 hour to 4 hour access. Whether we study the effects of opiates, i.e. heroin, morphine or the most commonly misused prescription opiate, oxycodone, or cocaine or alcohol, we find that the kappa opioid receptor/dynorphin system is altered in a countermodulatory mode following chronic intermittent or binge drug administration; dynorphin gene expression is increased. It is of interest that a similar increase in dynorphin gene expression occurs in the setting of a single stressor, such as a forced swim test, further documenting that drugs of abuse may serve as modest stressors. We have shown that this stress-induced change is the result both of transacting factors previously described, as well as epigenetic factors. We have proposed that since there is excessive dynorphin tone in early abstinence, treatment with a kappa opioid antagonist could be effective at that time point in the addiction-like cycle to decrease depressive symptoms. However, we have also hypothesized that, in the setting re-exposure or relapse to a use of a specific drug of abuse, a kappa partial agonist would probably be most effective by modulating the dopaminergic surges and thus the rewards of the drug, but at the same time, would not enhance the possibility of reward as a kappa antagonist might do. At this time, there are no pure kappa partial agonists approved which can be introduced into humans. However, our translational studies have shown that nalmefene, primarily a mu-opioid receptor antagonist (recently approved for treatment of alcoholism in Europe), is also a kappa partial agonist, and as such causes a dose-dependent modest elevation of serum prolactin levels in healthy humans (a marker of dopaminergic tone).

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Repeated stress dysregulates kappa opioid receptor signaling in the dorsal raphe through a p38α MAPK dependent mechanism

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Repeated stress releases dynorphins and causes subsequent activation of kappa opioid receptors (KORs) in limbic brain regions. The serotonergic dorsal raphe nucleus (DRN) has previously been found to be an important site of action for the dysphoric effects of dynorphin-kappa opioid receptor system activation during stress-evoked behaviors, and KOR-induced activation of p38α MAP kinase in serotonergic neurons was found to be a critical mediator of the aversive properties of stress. Yet, how dynorphins and KORs functionally regulate the excitability of serotonergic DRN neurons both in adaptive and pathological stress states is poorly understood. Here we report that acute KOR activation by the selective agonist U69,593 inhibits serotonergic neuronal excitability within the DRN through both pre-synaptic inhibition of excitatory synaptic transmission and post-synaptic activation of G-protein gated inwardly rectifying potassium channels (GIRK) electrophysiologically recorded in brain slices. Repeated swim stress sessions of C57Bl/6 mice significantly reduced KOR mediated GIRK currents recorded in serotonergic neurons in DRN post-synaptically, without significantly affecting pre-synaptic KOR-mediated regulation of excitatory transmission. This effect was blocked by genetic excision of p38α MAPK selectively from serotonergic neurons. An increase in phospho-immunoreactivity suggests that this functional dysregulation may be a consequence of tyrosine phosphorylation of GIRK (KIR3.1) channels. These data elucidate a mechanism for stress-induced dysregulation of the excitability of neurons in the DRN and identify a functional target of stress-induced p38a MAPK activation that may underlie some of the negative effects of pathological stress exposure.

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JNK Dependent Mechanism of Opioid Receptor Inactivation

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c-Jun N-Terminal Kinase (JNK) was shown to mediate mu and kappa opioid receptor desensitization or inactivation, respectively. Acute analgesic tolerance to morphine requiresJNK2 (not JNK1 or JNK3), and long-lasting kappa antagonists, including norBNI, require JNK1 activation. In contrast, acute desensitization of mu and kappa receptors by fentanyl and U50,488, respectively, requires G-protein receptor kinase 3 (GRK3) activation (Melief et al., 2010, 2011). In the present study, we confirmed that the JNK-dependent receptor inactivation effects of norBNI did not require GRK3, similar to morphine. We found that one week after norBNI administration (10mg/kg i.p.), U50,488 (15mg/kp, i.p.) analgesia was blocked in both WT and GRK3-/- mice. Furthermore, morphine-induced increase in phospho-JNK-ir in mouse spinal cord did not require GRK3 expression. Morphine (10mg/kg, s.c.) specifically increased phospho-JNK2-ir in mouse spinal cord, supporting our previous analgesic tolerance findings. JNK activation by 10μM morphine in MOR-expressing HEK293 cells and 10μM norBNI in KORexpressing HEK293 cells both persist for 2hr. To further characterize the role of JNK in the long duration of norBNI, KOR-expressing HEK293 cells were treated with 10μM naloxone or 1μM and 10μM norBNI overnight and stimulated immediately with 10μM U50,488 (T0) or stimulated 24hr after washout (T24). NorBNI treatment blocked U50,488 activation of ERK1/2 at both T0 and T24, whereas naloxone treatment blocked phospho-ERK1/2-ir at T0 only. Pretreatment with the JNK inhibitor SP600125 (100nM) prevented norBNI block of U50,488 stimulated phospho-ERK1/2-ir at T24, but not T0. Furthermore, 10 min treatment with norBNI blocked U50,488 stimulated phospho-ERK1/2-ir at T0, but not after norBNI washout. This suggests that, similar to *in vivo*, the long lasting properties of norBNI are JNK dependent. Together, these data suggest that long lasting norBNI antagonism does not require GRK3/β-arrestin and provide a foundation for future work necessary to elucidate the role of JNK in MOR and KOR regulation.

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Kappa opioid effects on GABAergic transmission in dopaminergic cells of the ventral periaqueductal gray (vPAG)

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Numerous studies have pointed to the important role that endogenous opioid peptides play in regulating drug addiction, stress, anxiety, sleep, and pain-related behaviors, through the modulation of dopamine (DA) neuron activity. Most of this effort has focused on the VTA (ventral tegmental area). However, a population of DA neurons in the ventral periaqueductal gray (vPAG) has been shown critical for opiate-mediated behavior. The goal of this study is to characterize the influence of kappa opioids on GABAergic activity of vPAG DA neurons using a transgenic tyrosine hydroxylase reporter mouse line. We performed slice whole-cell recordings on vPAG dopamine neurons and found that KOR agonists reduced both spontaneous and miniature GABAergic transmission in vPAG dopamine neurons, suggesting a presynaptic modulation. Current studies also investigated the signaling mechanism that underlies kappa opioid receptor inhibition of GABA transmission. In addition, we utilized optogenetics and Designer Receptors Exclusively Activated by Designer Drugs (DREADD) to assess the projection and behavioral effects of vPAG DA neuron-activation. Our behavioral studies showed that activation of vPAG DA neurons produced anti-nociception in a Von Frey hyperalgesia assay, consistent with the important role in opiate-mediated analgesia. Surprisingly, we also found that vPAG dopamine neurons highly co-localize with neurons expressing vGlut2 in the vPAG. This finding provided additional insight on how the vPAG dopamine projections modulate regions critical in regulating negative emotional disorders, as well as drug addiction.

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Salvinorin A: a potent cerebral vascular dilator and potential neuroprotectant

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In this presentation, we provide an overview of our findings related to salvinorin A in two animal species (piglet and mouse). Using the piglet model, we demonstrated that salvinorin A (SA) potently induces cerebral vascular dilatation, an important property necessary for protection against hypoxia/ischemia (HI) injury, via an endothelial nitric oxide synthase (eNOS) mediated mechanism in a dose dependent manner. SA administration before HI upregulates the ERK/MAPK (extracellular signal-regulated kinase/mitogen-activated protein kinase) pathway and preserves cerebral vascular autoregulation, an important component for neurovascular integrity. SA administration after HI prevents ERK upregulation induced by HI and preserves cerebral vascular autoregulation. Using a mouse hypoxia model, we found that SA administration before hypoxia insult reduces mortality significantly and prevents hypoxia-induced neurological outcomes. These findings indicate that SA could be a potential novel medication for preventing catastrophic lifelong neurological disabilities. Further studies are needed. SA is the only known naturally occurring non-opioid KOR agonist that has been consumed by humans for centuries with known safety profiles. In a recent human study, no persisting adverse effects related to SA were observed. Unlike opioid KOR agonists, SA is highly selective for KOR, and produces no frank dysphoric effects. Because of its rapid onset when delivered either via oral mucosa or inhalation. It could be a unique medication that can be delivered quickly in acute settings especially where IV access is unavailable for neurological events like cerebral ischemic stroke and sudden cardiac arrest in out-of-hospital settings. There are 3 SA related clinical trials (http://clinicaltrials.gov/ct2/results?term=salvinorin+A) in healthy subjects for its potential clinical usage. These clinical trials could potentially provide useful data to fast track the development of SA for the proposed unique and innovative clinical implication.

Source: McCabe, Department of Anesthesiology and Critical Care at the Perelman School of Medicine at the University of Pennsylvania, NIH K08 (PI Renyu Liu).

Conflict of interest statement: Dr. Liu has a patent pending related to salvinorin A: Salvinorin compositions and uses thereof - PCT Patent 2012/006178. Felipe Matsunaga declares no potential conflict of interest.

Does KOR location matter? State dependent changes in circuit function

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In the mesolimbic circuit, KOR activation produces behavioral correlates of aversion. Systemic injections of KOR agonists produce conditioned place aversion (CPA) and decrease dopamine levels in the nucleus accumbens (NAc). Although local KOR activation in the ventral tegmental area (VTA) also causes CPA, it does not decrease dopamine levels in the NAc. Consistent with these findings, in *ex vivo* electrophysiology studies of retrogradely labeled VTA dopamine neurons we demonstrated that the selective KOR agonist U69593 hyperpolarized dopamine neurons projecting to the medial prefrontal cortex and the amygdala, but not to the NAc. The dopamine levels in the NAc instead appear to be controlled by KORs on the terminals of the dopamine neurons. In addition to this selective post-synaptic inhibition, KOR agonists in the VTA inhibit glutamatergic and GABAergic inputs to most VTA neurons. This raises the possibility that in addition to suppressing circuit-specific dopaminergic output neurons, KOR agonists can presynaptically inhibit active inputs to VTA neurons. Depending upon the distribution of the presynaptic receptors, this dual synaptic control could add both precision and flexibility to the operation of local circuits that control subsets of VTA dopamine output neurons. Together these findings suggest how endogenous peptides might produce state dependent changes in VTA circuit dynamics.

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Imaging kappa receptors in cocaine abuse

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One of the most difficult aspects of treating cocaine dependence is the propensity for relapse to cocaine use after a period of abstinence. While previous research has focused on positive reinforcement and relapse, recent studies have begun to explore the neurobiology of negative reinforcement. Drug use in setting of stress provides negative reinforcement by relieving the stress. Preclinical studies show that kappa receptor activation mediates stress-induced cocaine-seeking behavior, suggesting that that kappa receptor activation plays a crucial role in negative reinforcement.

Previous postmortem studies in cocaine dependence have shown that the kappa receptor and dynorphin are upregulated in this disorder. However, studies investigating the behavioral significance of this change have been lacking due to the inability to image this receptor in vivo. We are using the newly developed [^{11}C]GR103545, a kappa receptor agonist PET radiotracer, to investigate whether there are differences in kappa receptor in cocaine abusers compared to healthy controls. Within the cocaine abusing subjects, we use a laboratory model of stress-induced cocaine seeking behavior to explore the correlation between the neurobiology and negative reinforcement, based on the well-documented preclinical phenomena in which binge dosing of cocaine significantly increases dynorphin levels. In this study, the cocaine users participate in binge cocaine self-administration sessions. The sessions are performed over three days with up to 600 mg per day of cocaine available for self-administration. The hypothesis is that kappa receptor availability, as measured with [^{11}C]GR103545 PET, will be reduced following the cocaine binge. Data collection is ongoing, and preliminary results from this study will be presented.

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Optogenetic and anatomical examination of striatal dynorphinergic neurons in aversion and reward

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The adverse effects of stress are well documented, yet many of the underlying mechanisms remain unclear and controversial. The dynorphin/kappa opioid system is implicated in the mediation of stress and resultant vulnerability to drug abuse. It is thought that stress causes dynorphin release activating kappa-opioid receptors (KOR) within both dopaminergic and serotonergic nuclei as well as their striatal targets. Consequently, much attention has focused on these systems in the modulation of KOR-mediated responses. Despite our current knowledge of central dynorphinergic cell body populations, a clear description of the axonal projections of these neurons is unknown. To address this we crossed the Cre-dependent tdTomato (Ai9) reporter mouse to a mouse that expresses Cre recombinase under the same promoter as dynorphin (Dyn-Cre) so only dynorphinergic cells express tdTomato. This allows complete visualization of dynorphinergic circuitry throughout the brain. We show robust dynorphin expression in cell bodies throughout the brainstem and forebrain. Clear visualization of intact projections throughout the brain and dynorphinergic projections can be seen from and within the cortex, striatum, amydala, and numerous monoaminergic nuclei. Dynorphinergic neurons within the striatum are particularly interesting for the study of stress and drug abuse. Prior studies have shown that KOR agonists inhibit dopamine and serotonin release in the nucleus accumbens (NAc), which ultimately regulates aversive behaviors. Therefore, we investigated whether specific modulation of dynorphinergic neuronal firing in the NAc is sufficient to induce aversive behaviors. We virally targeted channelrhodopsin-2 to striatal dynorphinergic neurons and optogenetically activated neuronal populations in both the dorsal and ventral striatum. This activation significantly increased c-Fos immunoreactivity, a marker for neuronal excitation, in dynorphinergic neurons. Furthermore, this activation drives conditioned and real-time aversive behavior. Understanding the mechanisms by which the dynorphin/kappa opioid system regulate negative affective behaviors will provide valuable insight into potential treatments for drug abuse.

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Structural basis for the affinity and efficacy of Salvinorin A-based Michael acceptors at opioid receptors

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The neoclerodane diterpenoid Salvinorin A (SalA) is found in the leaves of *Salvia divinorum* and is the most potent naturally-occurring known hallucinogen, exhibiting its effects exclusively through activation of the kappa-opioid receptor (KOR). Despite having very high affinity for the KOR, salA lacks the basic functionality found in other high-affinity KOR ligands that serves as an "anchor" in the orthosteric binding site. Thus, to further elucidate the binding mode(s) of SalA and its derivatives, a series of analogs containing sulfhydryl-reactive Michael acceptor functional groups was synthesized and the affinity and efficacy were assessed at the KOR, delta-opioid receptor (DOR) and mu-opioid receptor (MOR). We have previously described the pharmacological properties of many of these compounds, several of which exhibit high affinity for the KOR and some of which exhibit interesting pharmacological profiles from full KOR agonist to partial DOR or MOR agonist to antagonist. Recently, the crystal structures of the opioid receptors were solved, providing unprecedented insight into structural features of these receptors. Here, we present putative binding modes for the SalA-based Michael acceptors within the context of the recently-solved opioid receptor structures guided by information gained from radioligand binding and functional assays. Based on the proposed binding modes and knowledge of GPCR activation mechanisms, we postulate a mechanism for KOR activation by these compounds.

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Role of hypocretin (orexin)-dynorphin neurons in motivated behavior

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Hypocretin (orexin) and dynorphin are neuropeptides with opposing actions on motivated behavior. Orexin is implicated in maintaining arousal and in the rewarding effects of food, sex, and drugs of abuse. Conversely, dynorphin is implicated in the dysphoric effects of stress that often accompany or precipitate depressive-like states. We show that, despite their opposing actions on mood and behavior, orexin and dynorphin are packaged in the same synaptic vesicles within the hypothalamus, the sole source of orexin in the mammalian CNS. Pharmacologic or genetic disruption of orexin function attenuates the rewarding effects of rewarding lateral hypothalamic (LH) electrical stimulation, eliminates cocaine-induced impulsivity, and markedly reduces cocaine self-administration. These behavioral deficits are reversed by concomitant disruption of dynorphin signaling. We also demonstrate that concomitant orexin and dynorphin exert opposing effects on in vitro excitability of ventral tegmental area (VTA) dopamine (DA) neurons, a prominent target of orexin-containing neurons, and that intra-VTA orexin antagonism produces deficits in cocaine intake that are reversed by dynorphin blockade. Our findings identify a novel cellular mechanism that promotes balance in the function of midbrain DA systems, disruption of which could contribute to disorders characterized by dysregulation of motivated behavior.

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Effects of kappa opioids on pain-depressed behavior in rats

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Kappa opioid receptor agonists produce robust antinociception in most preclinical pain assays, but they are not effective analgesics in humans due to limitations in efficacy and/or dose-limiting side effects that include psychotomimesis. This study compared effects of centrally penetrating and peripherally restricted kappa agonists with effects of reference compounds in preclinical assays of pain-stimulated and pain-depressed behavior in rats. Intraperitoneal injection of 1.8% lactic acid (1.0 ml/kg) served as a noxious stimulus to stimulate an abdominal stretching response (a conventional pain-stimulated behavior) and to depress intracranial self-stimulation of the medial forebrain bundle (ICSS; a novel assay of pain-depressed behavior). Effects of the nonsteroidal anti-inflammatory drug and clinically effective analgesic ketoprofen (0.01-1.0 mg/kg) were compared to effects of the selective and centrally penetrating kappa agoinst salvinorin A (0.1-3.2 mg/kg) and of two peripherally restricted kappa agonists [the tetrapeptide D-Phe-D-Phe-D-Ile-D-Arg-NH2 (a.k.a. ffir; 0.1-10 mg/kg); the nonpeptidic peripherally restricted kappa agonist ICI204448 (3.2-32 mg/kg)]. Only ketoprofen produced antinociception in assays of both pain-stimulated and pain-depressed behavior, blocking acid-induced stimulation of stretching and depression of ICSS at a dose that did not alter ICSS in the absence of pain. Salvinorin A decreased acid-stimulated stretching, but exacerbated acid-induced depression of ICSS and decreased control ICSS in the absence of pain. ffir and ICI204448 significantly decreased stretching, but doses that blocked acid-stimulated stretching had little (ICI204448) or no (ffir) effect on acid-induced depression of ICSS, and higher doses tended to exacerbate acid-induced depression of ICSS. The poor efficacy of kappa agonist effects in the assay of pain-depressed ICSS corresponds to the poor clinical efficacy of kappa agonists to treat pain and to shared effects of kappa agonists and pain on mesolimbic dopamine release. Preclinical assays of pain-depressed behavior may provide a useful experimental tool for further assessment of kappa opioids as candidate analgesics.

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Ablation of kappa-opioid receptors from brain dopamine neurons has anxiolytic-like effects and enhances cocaine-induced plasticity

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Brain kappa-opioid receptors (KORs) are implicated in states of motivation and emotion. Activation of KORs negatively regulates mesolimbic dopamine (DA) neurons, and KOR agonists produce depressive-like behavioral effects. To further evaluate how KOR function affects behavior, we developed mutant mice in which exon 3 of the KOR gene (*Oprk1*) was flanked with Cre-lox recombination (loxP) sites. By breeding these mice with lines that express Crerecombinase (Cre) in early embryogenesis (EIIa-Cre) or only in DA neurons (dopamine transporter [DAT]-Cre), we developed constitutive KOR knockouts (KOR-/-) and conditional knockouts that lack KORs in DA-containing neurons (DAT-KORlox/lox). Autoradiography demonstrated complete ablation of KOR binding in the KOR-/- mutants, and reduced binding in the DAT-KORlox/lox mutants. Quantitative reverse transcription polymerase chain reaction (qPCR) studies confirmed that KOR mRNA is undetectable in the constitutive mutants and reduced in the midbrain DA systems of the conditional mutants. Behavioral characterization demonstrated that these mutant lines do not differ from controls in metrics including hearing, vision, weight, and locomotor activity. Whereas KOR-/- mice appeared normal in the open field and light/dark box tests, DAT-KORlox/lox mice showed reduced anxiety-like behavior, an effect that is broadly consistent with previously reported effects of KOR antagonists. Sensitization to the locomotor-stimulating effects of cocaine appeared normal in KOR-/- mutants, but was exaggerated in DAT-KORlox/lox mutants. Increased sensitivity to cocaine in the DAT KORlox/lox mutants is consistent with a role for KORs in negative regulation of DA function, whereas the lack of differences in the KOR-/- mutants suggests compensatory adaptations after constitutive receptor ablation. These mouse lines may be useful in future studies of KOR function.

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Ultrasonic vocalization patterning is altered in both neonatal and juvenile KOR-1 KO mice

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The study of mouse ultrasonic vocalizations (USVs) has recently emerged as an important index of social interactions occurring not only in wild-type mice but also in mutant strains exhibiting altered behavioral traits potentially related to human psychiatric disease. USVs emitted by neonatal rodents are thought to guide pup retrieval and influence maternal behavior while adult vocalizations appear to be involved in several aspects of social interaction. In addition quantitative and qualitative differences in the patterns of USVs in both pups and adults of different background strains have been correlated with differences in social behavior. Thus, examination of ultrasonic vocalizations in both neonatal and juvenile mice appears to be a valid measure of both the magnitude and quality of social interactions. We have continued to determine whether neonatal 129S6 KOR-1 KO pups as well as juvenile KOR-1 KO mice show qualitative or quantitative differences from WT in ultrasonic vocalizations. We have quantitated both total USVs as well as the relative prevalence of ten waveforms that have been identified and characterized in both neonatal and adult mice. Following maternal separation of neonates, our data to date indicate that both the number and types of ultrasonic vocalization calls differs significantly in KOR-1 KO pups compared to wild type following maternal separation. Specifically, while overall USV number is decreased in KOR-1 KO mice, two relatively rare individual waveforms of the ten analyzed ("upward" and "downward"), show significant and opposing quantitative alterations in the KOR-1 KO. In addition, we have begun to determine whether diminished call frequency that characterized KOR-1 neonates might also extend to social interactions occurring in juvenile KOR-1 KO mice. We so far have quantified the timing and call number that occurs during interactions of 6-week old male-female WT and KOR-1 KO mice. The initiation of vocalization during KOR-1 male-female interaction was initiated significantly later than in WT mice; moreover, following the initiation of vocalization, call number was significantly reduced compared to 129S6 WT mice. These data continue to indicate that KOR-1 can regulate behaviors that characterize social interaction in mice.

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Dicarboxylic ester – derived salvinorin A ligands to kappa opioid receptor

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Salvinorin A, the active ingredient of the hallucinogenic plant *Salvia divinorum* is the most potent known naturally occurring hallucinogen. It is a highly selective κ-opioid receptor agonist. To understand the ligand-receptor interactions, a new series of dicarboxylic ester type of salvinorin A derivatives were synthesized and evaluated for their binding affinity at κ, δ, and μ-opioid receptors. Most of the analogues have shown high affinity to κ-opioid receptor. Methylmalonyl derivative has shown the highest binding affinity (*K*i = 2.0 nM), the ethylmalonyl ligand, methylsuccinyl analog and methylfumaryl derivative have shown significant affinity for κ-receptor (*K*i = 21, 36 and 39.1 nM).

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Kappa opioid receptors regulate stress-induced cocaine-seeking and synaptic plasticity

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Dopaminergic neurons in the ventral tegmental area (VTA) of the brain are important targets of drugs of abuse and stress. Our lab previously identified a long-term potentiation of GABAergic synapses on these neurons (LTPGABA). Nitric oxide release by the dopaminergic neuron activates guanylate cyclase in the presynaptic terminal of GABAergic inputs, resulting in accumulation of cGMP, activation of PKG, and increased release of GABA. Multiple drugs of abuse and acute stress block or inhibit LTPGABA (Nugent et al, Nature, 2007; Niehaus et al, European Journal of Neuroscience 2010), suggesting that this is a common mechanism that may play a role in addiction and stress-related diseases. Our recent work shows that the block of LTPGABA by stress is prevented by pretreatment with the kappa opioid receptor (KOR) antagonist NorBNI. Intra-VTA infusion of NorBNI prior to stress prevented reinstatement of cocaine self-administration after cold-water swim stress. These data indicate that LTPGABA may be a valid therapeutic target for treatment of stress-induced relapse.

We now want to ascertain how long the effects of stress on LTPGABA last. Here, we report that LTPGABA remains blocked for at least five days after an acute stressor but returns to normal levels by ten days after stress. Thus, stress causes long-lasting, but not permanent, alterations in plasticity of GABAergic synapses in the VTA. Our data suggest that loss of LTPGABA may be a mechanism by which acute stress can cause lasting changes in behavior. Future studies will investigate the mechanism by which the block of LTPGABA is maintained for several days after stress. These studies will focus on the KOR system as a potential mediator of the lasting effects of stress on plasticity and drug seeking behavior and may provide novel targets for reversing stress-induced neuroadaptations.

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Comparison of the pharmacological activity of (-)-butorphan (MCL-101) with its enantiomer (+)-butorphan (MCL-191)

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(-)-Butorphan (MCL-101) is a highly selective kappa agonist derived from levorphanol. MCL-101 has been shown to suppress cocaine self-administration in non-human primates and thus it is being further studied as a potential therapy to treat cocaine abuse. Its enantiomer, (+)-butorphan (MCL-191) has been synthesized from dextromethorphan, a well known antitussive and NMDA receptor antagonist. Both MCL-101 and MCL-191 are orally active as their HCL salts. A comparison of binding affinity, [35S]GTPγS, locomotor activity and intracranial self-stimulation (ICSS) of these two enantiomers will be discussed.

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Kappa-opioid receptors in the nucleus accumbens shell are important for pair bond-induced attenuation of drug reward

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A critical step to decreasing the prevalence of addiction is to understand how environmental conditions that protect against the reinforcing properties of abused drugs tune reward circuitry to decrease drug reward. One such condition is the presence of positive social relationships. Importantly, the neurobiology of such relationships can be studied in the socially monogamous prairie vole — a species that once pair bonded, no longer finds psychostimulants rewarding. As we have previously demonstrated that activation of kappa-opioid receptors (KORs) mediates pair bond maintenance, we investigated this receptors contribution to the neuroprotective benefits of pair bonding. Using a conditioned place preference (CPP) paradigm, we first considered the effect of previously untested social experiences on amphetamine reward. This investigation established that pair bonding, but not other social experiences, attenuated the rewarding properties of amphetamine. We next assessed the role of KORs in altering drug reward in pair bonded voles. We first show that peripheral blockade of KORs (norBNI; 50mg/kg) resulted in the development of an amphetamine CPP indicating that this system mediates the protective effects of pair bonding. To determine the neural site of action of KOR-induced protection, we administered norBNI (500ng) into the nucleus accumbens (NAc) shell, a region that mediates both drug and social reward. This manipulation resulted in a significant amphetamine CPP in pair bonded voles. These results indicate that pair bonding blunts drug reward through activation of KORs within the NAc shell. To extend these findings, we will next compare KOR density between sexually naive and pair bonded voles to determine if pair bonded results in an upregulation of KORs. Finally, we will use fast-scan cyclic-voltammetry to determine if dopamine release in response to amphetamine exposure is blunted in the NAc of pair bonded voles and if this blunting is due to an increase in activation of KORs.

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Deciphering molecular mechanisms underlying functional selectivity at the kappa opioid receptor (KOR)

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Kappa opioid receptor (KOR) agonists do not activate the reward pathway stimulated by morphine-like mu opioid receptor (MOR) agonists and thus have been considered to be promising non-addictive analgesics. However, KOR agonists produce other adverse effects, including dysphoria, diuresis and constipation. The therapeutic promise of KOR agonists has nonetheless recently been revived by studies showing that their dysphoric effects require arrestin recruitment, whereas their analgesic effects do not. The discovery of functionally selective drugs that are therapeutically effective without the adverse effects triggered by the arrestin pathway is thus an important goal. We have identified such an extreme G-protein biased KOR compound, 6'-guanidinonaltrindole (6'GNTI), a potent partial selective agonist at the KOR receptor for the G protein-activation pathway that does not recruit arrestin. Although this morphine derivative is a promising lead compound for non-addictive analgesics acting at the KOR receptor with reduced liability for dysphoria, its effective use as a drug is limited by its inability to cross the blood brain barrier. We have investigated the pharmacological properties of a number of other opioids in a search for additional functionally selective compounds. We have identified several other compounds that are G protein biased at KOR and are currently investigating the molecular determinants of this signaling bias.

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The translation of rat to human pupillometry studies to determine the functional selectivity of the kappa opioid receptor antagonist LY2456302

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LY2456302 is a functionally selective kappa opioid receptor antagonist in the efficacy dose range established in preclinical models; however, mu opioid receptor activity may be observed at higher doses. The objective was to assess the kappa selectivity of LY2456302. *In vivo* receptor occupancy (RO) and human PET imaging were used to measure central kappa receptor occupancy in rats and healthy human volunteers, respectively. It is well-established that mu agonist-induced mydriasis in rats and miosis in humans can be blocked by mu-preferring opioid antagonists such as naltrexone. Therefore, changes in pupil diameter were measured in response to mu agonist challenge as a pharmacodynamic biomarker in rats and humans to determine the level of mu receptor antagonism at higher doses of LY2456302. In rats, mu, kappa, and delta RO was measured up to 300 mg/kg PO. In separate experiments, the ability of LY2456302 (3 – 300 mg/kg PO) or naloxone (3 mg/kg SC) to block morphine (10 mg/kg, IP)-induced mydriasis was assessed. Naloxone completely blocked morphine-induced mydriasis, while LY2456302 produced a dose-related, but modest, blockade at the highest doses of 100 and 300 mg/kg (56% and 87% mu RO, respectively). In humans, kappa RO was measured using the selective kappa receptor antagonist radiotracer 11C-LY2789788 (*Tauscher et al. in preparation*). Maximal kappa RO was observed at approximately 10 mg LY2456302. In human pupillometry experiments, the ability of LY2456302 (4, 10, 25, or 60 mg orally) or naltrexone (50 mg orally) to block fentanyl (2 μg/kg, IV)-induced miosis was assessed. Naltrexone completely blocked fentanyl-induced miosis, consistent with previous results. LY2456302 produced a dose- and exposure-response relationship with modest blockade of miosis at 25 and 60 mg doses, and minimal-to-no blockade at 4 and 10 mg. Data indicate that kappa receptor selectivity can be achieved at clinical doses of LY2456302 which exhibit maximal kappa RO.

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Dynorphin is a spinal neuromodulator that mediates the inhibition of itch

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One of most effective ways to relieve itch is through scratching. However, the neural circuits that mediate this inhibition of itch are unknown. We now provide genetic evidence that spinal dynorphin plays a key role in the inhibition of itch by counter-stimuli. Within the spinal cord, dynorphin is expressed in a specific population of inhibitory interneurons that are required for normal itch sensation—mice lacking these neurons have abnormally elevated itch and develop self-inflicted skin lesions. Furthermore, intrathecal delivery of kappa opioid receptor agonists and antagonists bidirectionally modulate itch sensitivity, while having no effect on pain behaviors. Finally, in electrophysiological experiments, we show that dynorphin-expressing spinal neurons are directly activated by primary afferent fibers that likely mediate the inhibition of itch by counter-stimuli. These studies suggest that spinal dynorphin functions to inhibit itch, raising the possibility that modulation of the spinal kappa opioid receptors may provide therapeutic benefit for pruritus.

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Sex differences in sensitivity to the depressive-like effects of kappa opioid receptor activation in rats

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The majority of studies on the aversive effects of kappa opioid receptor (KOR) activation have been done in males. However, there are pronounced sex differences in behavioral responses to stress and drugs of abuse: females are generally more sensitive to both the reinforcing and aversive effects of drugs and stress-induced relapse. To determine whether there are sex differences in the effects of KOR activation on reward function, we measured the effects of the KOR agonist U-50488 on brain stimulation reward in male and female rats using intracranial self-stimulation (ICSS). We found that female rats were significantly less sensitive than males to the depressive-like effects of U-50488, with no difference in the pharmacokinetic profile of U-50488 brain levels, and independent of activational changes in gonadal hormone levels. To identify neural substrates of sexually dimorphic for KOR function, we measured the effects of U-50488 on c-Fos expression in male and female rats. We found that U-50488 induced more c-Fos expression in the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) and in the bed nucleus of the stria terminalis (BNST) in females compared to males. The majority of c-Fos-positive neurons in the PVN were corticotropin releasing factor (CRF)-positive and oxytocin-negative. In the BNST, the majority of c-Fos-positive neurons did not co-localize with CRF. Using quantitative realtime PCR (qRT-PCR), we found higher basal levels of dynorphin mRNA in the female PVN, BNST, and basolateral amygdala, suggesting that dynorphin tone may be higher in females and may occlude the effects of exogenously administered KOR agonists. Taken together, these data raise the possibility that the role of KORs in mood regulation is mechanistically different in males and females and underscore the importance of understanding KOR function in both sexes to rationally design pharmacotherapeutics.

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Nalfurafine is an agonist at the kappa opioid receptor with low efficacy for p38 MAPK and high efficacy for ERK1/2 activation

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Previous studies have shown the aversive properties of kappa opioid receptor (KOR) activation to be mediated through GRK/arrestin-dependent p38 MAPK activation, suggesting that KOR agonists that activate G-protein signaling, but not p38, may have therapeutic potential as non-dysphoric opioid analgesics. We recently reported striking differences in the relative potencies and efficacies for ERK1/2 and p38 activation between rodent KOR (rKOR) and human KOR (hKOR), suggesting important ligand-directed signaling differences (Schattauer et al., 2012). While butorphanol and pentazocine had similar relative efficacy and potency for ERK1/2 activation by hKOR and rKOR, the pentazocine potency and butorphanol efficacy for p38 activation were significantly lower for rKOR than hKOR. Interestingly, clinical trials studying the analgesic efficacy of the KOR agonist nalfurafine (TRK-820) in treating pruritus did not report dysphoric side effects (Kumagai et al., 2010, 2012). While the lack of dysphoric effects could be a consequence of weak blood-brain barrier penetration, an alternative hypothesis is that nalfurafine does not effectively activate p38 MAPK. Consistent with the latter explanation, we found that alfurafine (100nM, 5 min) significantly ($p<0.01$) increased ERK1/2 phosphorylation in both rKOR and hKOR expressing HEK293 (80±13% and 84±11% of the increase stimulated by 1μM U50,488, respectively). In contrast, no significant increase in p38 phosphorylation was observed in nalfurafine (100nM, 30 min) treated hKOR HEK293 cells (8.7±19%). A nonsignificant ($p=0.06$) increase in p38 phosphorylation was observed in nalfurafine (100nM, 30 min) treated rKOR HEK293 (133±60%), suggesting nalfurafine may be more potent for p38 activation in rKOR than hKOR (opposite of pentazocine and butorphanol). A lower nalfurafine concentration (1nM) was also effective for rKOR stimulated ERK1/2 (76±19%) but not p38 (15±22%) activation. These results indicate that the KOR agonist nalfurafine preferentially activates ERK1/2 but not p38 MAPK, and this may contribute its lack of dysphoric effects reported in clinical trials.

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Dynorphin/kappa receptor system in the nucleus accumbens is critical in the escalation of heroin intake

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Abuse of opioid drugs, both illicit and prescription, is a persistent concern accounting for 31 million users across the globe each year. Current pharmacotherapy focuses on suppressing immediate withdrawal symptoms and harm reduction using long-acting opioids. However, the mechanisms leading to opiate dependence and potential therapeutic targets for preventing opiate dependence are still poorly understood. We have evidence that kappa opioid receptor (KOR) activation during chronic opioid intake and withdrawal contributes to the motivation to take increasing amounts of drug. We are now examining the potential neural substrates where KOR activity drives heroin-taking behavior.

Using a 12-hr long-access model of heroin self-administration (60 μg/kg/infusion), rats show escalation of heroin intake over several weeks. A single, systemic dose (30 mg/kg, s.c.) of the KOR antagonist norbinaltorphimine (nor-BNI) did not suppress initial heroin intake in long-access rats or in rats stably taking heroin on a one-hour short-access schedule, but the escalation curve of long-access heroin rats was significantly blunted. This was paralleled by reduced motivation to respond for heroin infusions in a progressive ratio test. These effects persisted for at least a month, and rats previously treated with nor-BNI demonstrated normal escalation of heroin intake and increased progressive ratio responding after a month-long drug washout period. These differences were also associated with significant decreases in heroin withdrawal-associated anxiety as measure by elevated plus maze open arm time. Interestingly, treatment with nor-BNI subsequent to escalation of intake had no effect on heroin intake or motivation.

Identical suppression of heroin escalation was observed upon bilateral local injection of nor-BNI (4 μg/side) within the shell of the nucleus accumbens, with reduce progressive ratio responding. This area was also enriched with prodynorphin among long-access heroin rats compared to naïve controls. Results suggest a dynamic activation of the dynorphin-kappa system in the transition to addiction.

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Anxiogenic effects of oestrogen involve the dynorphin - kappa opioid receptor system

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The influence of ovarian hormones on behaviour is well accepted and oestrogen replacement therapy has proven beneficial in several cases of menopausal mood disorders.
However, there are also adverse effects and a number of women respond negative to such a therapy. The neurochemical background of aversive actions of oestrogen on emotions is largely unclear. Female rodents were shown display anxiogenic like effects during the early pro-oestrus phase, which provides an interesting tool to investigate oestrogen induced aversive effects. We observed reduced exploratory drive in wild-type, but not prodynorphin deficient mice during the early pro-oestrus phase, suggesting an interplay of the oestrogen and dynorphin/kappa opioid receptor (KOR) systems. To overcome fast hormonal fluctuations in female rodents we investigated ovariectomized female and intact male mice. The short-term application of specific agonists for nuclear oestrogen receptors did not result in marked behavioural changes. In contrast, stimulation of a G-protein coupled oestrogen receptor (GPER1, the former orphan receptor GPR30) induced anxiogenic effects in ovariectomized female and male mice. The anxiogenic effects induced by the specific GPER1 agonist G-1 were comparable to those observed after low doses of the general oestrogen receptor agonist 17â-oestradiol in male mice, thereby reflecting the behavioural changes observed in intact female mice during early pro-oestrus. Noteworthy, anxiogenic effects of the GPER1 agonist were completely blocked by pre-treatment of mice with the KOR antagonist norBNI. In line with this, no anxiogenic effects of GPER1 activation were observed in prodynorphin deficient mice. Our results support the hypothesis of individual sex steroid sensitivity as a cause of adverse effects of oestrogen replacement therapy, identifying GPER1 as a potential candidate to induce anxiogenic effects through a mechanism involving the dynorphin / KOR system.

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Chronic alcohol exposure induces escalated alcohol self-administration and increased kappa-opioid receptor signaling in the rat medial prefrontal cortex during acute withdrawal

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Previously, it has been shown that the endogenous ligand for the kappa-opioid receptors (KOR), dynorphin (DYN), is upregulated in alcohol-dependent animals and is implicated in the excessive alcohol self-administration that accompanies alcohol withdrawal. However, the effect of chronic alcohol exposure on KOR function is largely unknown. The present study examined the effect of chronic alcohol vapor exposure on KOR-mediated G-protein signaling in the rat medial-prefrontal cortex (mPFC) using [35S] GTPγS assay. The assay was optimized using mPFC tissue of alcohol-naïve rats. The initial results indicated that DYN A stimulated GTPγS coupling in a concentration-dependent manner and this signaling was KOR antagonist (norBNI) reversible. Subsequently, male Wistar rats 1) were trained to self-administer 10% alcohol (w/v), 2) exposed to air or intermittent alcohol vapor for three months and 3) confirmed to display the characteristic escalation of self-administration when dependent for at least two-weeks prior to brain extraction during acute withdrawal (6-8 hrs into withdrawal). mPFC tissue were dissected and homogenate incubated with [35S]GTPγS in the presence of DYN A prior to liquid scintillation spectrophotometry. DYN A produced concentration-dependent increases in GTPγS coupling in the mPFC of non-dependent and alcohol-dependent rats. DYN A-stimulated GTPγS coupling was significantly elevated in the mPFC of alcohol-dependent group compared to non-dependent controls. Increased signaling via the KOR is consistent with recent evidence showing increased KORs mRNA in the comparable region of human alcoholics. However, this is the first functional demonstration of increased KOR signaling in the mPFC following chronic alcohol exposure. These changes have been observed in brain regions implicated in the cognitive control of addictive behavior. Therefore, an upregulated DYN/KOR system in this region may contribute to neurocognitive dysregulation commonly observed in alcoholics that promote excessive drinking. Most importantly, the present results have identified a novel therapeutic target for the treatment of alcohol dependence.

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Anti-inflammatory effects of kappa-opioid receptor agonist WOL071-007 in a murine model of oxazolone-induced contact dermatitis after topical application

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Efficacy of the KOR agonist WOL071-007 (KOR Ki = 0.25 nM, KOR GTPgammaS binding EC50 = 1.99 nM) was assessed in mice with oxazolone-induced hypersensitivity (7 challenges). For sensitization oxazolone (100 µL, 1.0% in acetone) was applied to their preshaved abdominal surface (day 0). On day 5, oxazolone (0.5%, 20 µL/ear) was applied to the right ear. On days 7-18, vehicle, WOL071-007 (0.02, 0.1, 0.5 and 2.5 mg), Nalfurafine (0.5 mg), hydrocortisone (0.6 mg) in 20 µl acetone/ethanol (1:1) each or 0.1% Tacrolimus® ointment (20 mg) were daily applied on the right ear 3 h before oxazolone challenge (0.5%, 20 µL/ear) on days 7-12 and dosing continued without oxazolone through day 18. Ear swelling was measured daily predose through day 19. Ear edema was calculated by subtracting thickness of right ear on day 0 from right ear on day of treatment. Topical administration of hydrocortisone and Tacrolimus® reduced oxazolone-induced ear swelling ranging from 42% to 73% and 13% to 51%, resp. The effect was significant ($p<0.05$) over a 12-day period (days 8-19) and on days 8 and 11-17, resp. WOL071-007 at 2.5 mg reduced significantly ($p<0.05$) ear swelling on days 9-14. Treatment with Nalfurafine or lower doses of WOL071-007 did show little or no effect. Histological analysis (H&E) of treated ears showed dose dependent effects of WOL071-007 on ear thickness, epidermal thickness and dermal infiltrate. Anti-inflammatory effects of the 0.1 and 0.5 mg doses were slightly weaker than the effects of 0.6 mg hydrocortisone. The 0.02 mg dose showed an effect comparable to Tacrolimus® ointment. Topical application of a selective and potent KOR agonist showed significant anti-inflammatory effects in a dermatitis model. Thus, KOR agonists should be further investigated as treatment for inflammatory skin diseases.

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The role of extracellular signal-regulated kinase (ERK) on reward states following kappa opioid receptor activation.

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Activation of kappa opioid receptors (KORs) within the mesocorticolimbic dopamine system suppresses dopamine release and can produce negative affective states. Intracellularly, KORs can activate the extracellular signal-regulated kinase (ERK) pathway, which has been implicated in motivated behavior. We hypothesized that ERK signaling in the nucleus accumbens (NAc), a dopaminergic target important for reward function, is necessary for KOR-mediated negative affective states. To test whether KORs expressed on dopamine neurons are necessary for KOR-mediated activation of ERK in the NAc, genetically engineered mice in which KORs were conditionally removed in dopaminergic neurons (DAT-KORlox/lox) were treated with the KOR agonist U50,488 (20 mg/kg, i.p.) and Western blot analysis was used to measure levels of activated (phosphorylated) ERK (pERK) and downstream substrates. We found that, U50,488-induced pERK was reduced in DAT-KORlox/lox mice. Furthermore, U50,488 failed to induce pERK in global KOR knockout mice. To test whether NAc ERK is necessary for the depressive-like effects of KOR activation, herpes simplex virus (HSV) vectors expressing a dominant negative form of ERK (HSV-dnERK) or GFP as a control were infused into the NAc of rats trained to lever press for intracranial self-stimulation (ICSS). HSV-dnERK in the NAc increased basal ICSS thresholds, indicative of a decrease in reward function (anhedonia). The ability of the KOR agonist salvinorin A (1.0 mg/kg, i.p.) to increase ICSS thresholds was blunted in rats treated with HSV-dnERK. Taken together, these findings suggest that ERK blockade in the NAc unmasks a negative affective state that subsequently decreases the net anhedonic effect of KOR activation. These findings are consistent with, and extend, a role for NAc ERK in mediating motivated behavior and begin to address the mechanism by which KORs contribute to binge cocaine-induced dysregulation of reward function.

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Kappa-opioid receptor regulation of mesocortical and limbic inputs to the prefrontal cortex

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Kappa-opioid receptors (KORs) are important for motivation and other medial prefrontal cortex (mPFC)-dependent behaviors. Although KORs are present in the mPFC, their role in regulating dopamine and glutamate transmission in this brain region are not known. Using *in vivo* microdialysis in rats and mice , we demonstrate that intra-mPFC administration of the selective KOR agonist U69,593 decreased local dopamine (DA) overflow, while reverse dialysis of the KOR antagonist nor-Binaltorphimine (nor-BNI) enhanced mPFC DA overflow. To determine whether KOR regulation of mPFC DA overflow was mediated by KOR on DA terminals, we utilized a Cre recombinase-driven mouse line lacking KOR in DA neurons. In these mice, basal DA release or uptake was unaltered relative to controls, but attenuation of mPFC DA overflow by local U69,593 was not observed, indicating KOR acts directly on mPFC DA terminals to locally inhibit DA levels. Extracellular amino acid levels were also affected by KORs, as U69,593 reduced glutamate and GABA levels driven by the glutamate reuptake blocker, l-transpyrrolidine-2,4-dicarboxylate. Whole-cell recordings from mPFC layer V pyramidal neurons revealed that U69,593 decreased the frequency, but not amplitude, of glutamatergic mini EPSPs. These findings demonstrate that mPFC KORs presynaptically inhibit glutamate transmission, however, it is not clear what glutamatergic afferents are modulated by KORs. To determine whether basolateral amygdala (BLA) afferents into the mPFC are modulated by KORs, we examined the effects of mPFC KOR activation on BLA-evoked synaptic responses and single unit activity using *in vivo* extracellular recordings. Pressure ejected U69,593 near the recording site inhibited BLA-evoked field PSPs and decreased the activity of most mPFC neurons. Collectively, these results suggest that mPFC KORs inhibit VTA and BLA inputs via presynaptic inhibition. This provides a framework whereby mPFC KOR systems can modulate local processing of motivational and affective information.

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Brain Glucagon-Like Peptide-1 Regulates the Reinforcing Properties of Nicotine

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Cigarette smoking is a principal cause of preventable death and disease in developed nations, with approximately $160 billion being spent yearly in the United States to cover direct health care costs from resulting diseases. Nicotine is the major reinforcing component in tobacco smoke that contributes to the development of the harmful smoking habit in humans. The reinforcing actions of nicotine are mediated through various subtypes of nicotinic acetylcholine receptors located within brain reward circuitries. Central glucagon-like peptide-1 (GLP-1) is a neuropeptide produced in the nucleus of the solitary tract (NTS), a brain stem structure abundant in nicotinic acetylcholine receptors. Whilst noradrenergic efferents from the NTS have been implicated in drug dependence processes, the potential contribution of GLP-1 output from the NTS in this process has not been investigated.

We found that GLP-1 receptor KO mice consume more nicotine when compared to wildtype littermates, across all doses tested. Conversely, GLP-1 neuron-specific stimulation via creinducible M3 DREADDs show that brain-derived GLP-1 reduces the reinforcing properties of nicotine. Our results suggest that GLP-1 transmission serves as a negative regulator of nicotine reinforcement. Recently, our laboratory reported that excitatory inputs from the medial habenula to the interpeduncular nucleus (IPN) also serve to negatively regulate nicotine intake. Intriguingly, GLP-1 receptors are densely expressed in IPN. Electrophysiological recordings coupled with optogenetic or electrically evoked activity revealed that GLP-1 receptors located on presynaptic terminals enhanced activity of IPN neurons. Further, stimulation of GLP-1 receptors in IPN via microinfusion of the GLP-1 receptor agonist, Exendin-4, reduces nicotine intake in rats through a cAMP-dependent mechanism.

These data identify a new brain circuit – IPN-projecting GLP-1 neurons – that negatively regulate nicotine intake. Moreover, these findings suggest that modulation of GLP-1 receptor transmission may be viable target for the development of novel therapeutics for smoking cessation.

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Disruption of kappa-opioid receptor function attenuates corticotropin-releasing factor (CRF)-effects on startle

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KOR antagonists reduce depressive- and anxiety-like behavior and block the effects of CRF, a key regulator of the stress response. Although there is strong evidence that activation of KORs expressed on midbrain dopamine (DA) neurons can produce depressive-like effects, their role in anxiety-like effects is not known. Considering the high comorbidity of depressive and anxiety disorders, we hypothesized that the KORs in DA neurons also contribute to stress-induced anxiety-like behavior. The present studies characterized interactions of KOR-CRF systems by determining if global or DA neuron-specific KOR disruption can affect increases in startle reactivity produced by central infusion of CRF. To test the effects of systemic KOR blockade, mice were administered injections of the selective and long-lasting KOR antagonist JDTic immediately following surgery to implant an intracerebroventricular cannula for delivery of CRF. JDTic attenuated CRF-induced increases in the acoustic startle response without affecting baseline startle reactivity. CRF-enhanced startle was also tested in constitutive KOR KO mice (KOR-/-) and conditional KOs in which KORs are selectively ablated in DA-containing neurons (DAT-KORlox/lox). CRF-enhanced startle was unaffected in KOR-/- mice and attenuated but not significantly blocked in DAT-KORlox/lox mice. To determine if the ability of JDTic to reduce CRF-enhanced startle could be due to off-target effects unrelated to KOR blockade, KOR-/- mice were pretreated with JDTic before a CRF-enhanced startle test. CRF significantly increased startle responding independent of JDTic treatment, suggesting that systemic JDTic effects were the result of on-target effects of the drug. Thus, compensation in KOR function during development in the KO lines may account for the dissociation between JDTic-treated and KO mice. This work confirms previous studies that indicate a role for KOR signaling in stress-sensitized behaviors and raise the possibility that KOR function in regions outside DA neurons play an important role in regulating CRF effects on startle.

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Molecular determinants for kappa-opioid receptor activation by Salvinorin A

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The kappa opioid receptor (KOR) is the only known molecular target for the potent hallucinogen salvinorin A. Unlike most of other known KOR agonists, salvinorin A does not contain a basic functional group. We used the crystal structure of human KOR bound to the inverse agonist JDTic, to study the binding site of salvinorin A. Docking experiments resulted in two top-ranking poses of salvinorin A in the binding pocket of KOR. Radioligand binding and functional assays were used to analyze mutations in the proposed binding pockets of the two predicted poses. Most of the tested mutations affected either salvinorin A binding or function or both. Tyrosines from TM2, 3 and 7 were modified to both alanines and phenylalanines. These mutant KORs revealed interesting patterns of binding and activation, providing evidence for the importance of the aromatic moiety or the hydroxyl group of the tyrosines for binding, activation or both. Strikingly, mutation of the conserved TM3 aspartate D138 to either alanine or asparagine enhanced the affinity of salvinorin A and further increased its potency as a kappa agonist. The results collected from binding and functional assays are presented in a structural context, and a potential mechanism of receptor activation is postulated.

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Quantitative live cell study of kappa-opioid receptor interactions in the plasma membrane by methods with single-molecule sensitivity

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Quantitative characterization of ligand-receptor interactions and cellular responses initiated by these interactions remains a daunting task. Due to the dynamic nature of the processes involved and the low abundance of interacting molecules, methods with high spatial and temporal resolution and single-molecule sensitivity need to be applied for such studies. To date, fluorescence imaging and correlation spectroscopy are the only techniques that enable quantitative studies of the kinetics of molecular interactions in living cells. Using live PC12 cells stably transformed to express the kappa opioid receptor fluorescently tagged with the enhanced Green Fluorescent Protein (eGFP-KOP), synthetic dynorphin A fluorescently labeled with tetramethylrhodamine (Dyn A-TAMRA) and fluorescence correlation and cross-correlation spectroscopy, we could measure the concentration of Dyn A-TAMRA in the bulk solution, in the immediate vicinity of the plasma membrane, where its concentration is locally increased due to electrostatic interactions with the negatively charged proteoglycans protruding from the plasma membrane, and characterize quantitatively the membrane-mediated peptide-receptor interactions. The apparent dissociation constant (KD) of the Dyn A-KOP complex was measured to be of the order 10-7 M. Furthermore, we report here results on the study of eGFP-KOP interactions with β-Arrestin 2 fluorescently labeled with the monomeric Red Fluorescent Protein (β-Arr 2-mRFP). These interactions were studied in live PC12 cells stably expressing eGFPKOP and transiently expressing β-Arr 2-mRFP, before and after stimulation with Dyn A. The aim of this presentation is to introduce fluorescence correlation spectroscopy and demonstrate how it can be used for quantitative characterization of KOP interactions with specific ligands in living cells. Mechanistic knowledge generated by such studies can provide invaluable information on the effect of specific drugs on KOP-mediated cellular signaling, but also on off-target effects.

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Effects of intermittent, minor stressors on long-term potentiation in dorsal and ventral hippocampus

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The dorsal hippocampus (dH) is involved in spatial mapping and cognitive abilities whereas the ventral hippocampus (vH) is involved in anxiety/fear and reward. Stress differentially affects synaptic plasticity in these sectors of hippocampus as demonstrated by enhanced long term potentiation (LTP) in vH compared to decreased LTP in dH. The ratio of vH:dH LTP is < 1 in naïve rats whereas this ratio is reversed (vH:dH LTP > 1) one hour following an acute stressful experience. The current study determines if the minor stressors associated with i.p. injections (saline) and exposure to environmental novelty (open field) in locomotor sensitization experiments can result in stress-like effects on vH & dH LTP. All rats (except naïve controls) were tested for initial locomotor activity (pre conditioning) and then conditioned with saline (i.p.) using a behavioral sensitization-type protocol (5 daily saline injections) before being challenged (post conditioning) 1 week later. Hippocampal slices were prepared 8-14 days after the challenge and LTP assessed using field potential recordings in the CA1 region of vH and dH. As compared with naïve rats kept only in their home cage environment (vH:dH LTP = 0.67), the vH:dH LTP ratio was significantly shifted in rats experiencing the behavioral sensitization-type protocol (vH:dH LTP = 1.69). Note that this dramatic shift in the synaptic plasticity of the ventral and dorsal sectors of the hippocampus is persisting 1-2 weeks. Thus subjecting rats to intermittent, minor procedures associated with common behavioral testing protocols can result in sufficient stress to modulate the balance of synaptic plasticity between vH & dH. Nor-binaltorphimine (10mg/kg) prevented the enhancement of LTP in the vH, but not the decrease of LTP in the dH (vH:dH LTP ratio of 1.09). This suggests a dissociation of vH and dH stress mechanisms, as kappa antagonist treatment prevents the former but not the latter.

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Dissociable effects of kappa-opioid receptor activation on impulsive phenotypes

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The kappa-opioid receptor (KOR) is the primary target for the endogenous opioid peptide dynorphin (DYN) and KORs reside within brain circuitry underlying the complex integration of information related to different behavioral domains such as motivation, decision-making and negative affect. Alterations in extended amygdala dynorphins (DYN) and kappa-opioid receptor (KOR) function following chronic alcohol exposure have been shown to mediate escalated alcohol self-administration during acute withdrawal. In addition to excessive alcohol consumption and increased negative affect, other symptoms of alcohol dependence include compromised impulse control. Given that DYNs and KOR expression are dysregulated within prefrontal brain circuitry associated with decision-making and impulsivity in alcohol-dependent humans and rodents, we hypothesized that KOR activation could contribute to impulsive phenotypes. To test this hypothesis, separate cohorts of male Wistar rats were trained in delay discounting (DD) or stop-signal reaction time (SSRT) tasks and once stable responding was observed, they received intracerebroventricular infusions of the KOR agonist U50,488 (0-50 ug) according to a within-subject dosing regimen. The results demonstrated a dissociable effect of U50,488 on impulsive phenotypes related to intolerance to delay or response inhibition. Furthermore, the pro-impulsive effects of KOR activation were rescued by pretreatment with the KOR antagonist nor-binaltorphimine (norBNI). Therefore, KOR activation was shown to induce an impulsive phenotype that was norBNI-sensitive. Dysregulation of impulsive behavior by increased DYN / KOR activity could serve to increase vulnerability for the initiation, or perpetuate existing patterns of excessive alcohol abuse and can enhance the probability of relapse in dependent individuals. Furthermore, KOR-mediated impulsivity has implications for numerous neuropsychiatric disorders.

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Standard preclinical studies did not accurately predict an apparent clinical toxicity of JDTic

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Healthy, adult, male subjects were to be enrolled as 3 cohorts in a double-blind, placebocontrolled study to evaluate the safety, tolerability, and pharmacokinetics (PK) of single, escalating, oral doses of JDTic (1, 3, or 10 mg). Pre- and postdose safety assessments included: orthostatic vital signs; 6 lead continuous telemetry monitoring (approximately 15 hours predose to 24 hours postdose); 12 lead ECGs; clinical chemistry, hematology, and urinalysis; psychomotor functioning (using the Wayne Saccadic Fixator [WSF]); and adverse events. Safety results were unremarkable with the notable exception that two subjects each experienced a single event of multiple beats of non-sustained ventricular tachycardia. Both subjects had received 1 mg JDTic, and their events were temporally similar with respect to dose time (and to a preclinical NSVT event in a monkey). Both subjects were asymptomatic and without sequelae. These events triggered a study-stopping rule. No apparent differences were observed between the 6 placebo and 6 JDTic subjects with respect to clinical chemistry, hematology, urinalysis, vital signs, WSF, or 12 lead ECG parameters. Plasma JDTic levels were below the lower level of quantitation (0.05 ng/mL) in all subjects.

The lowest concentration at which QTc prolongation occurred in monkeys was 430 ng/mL (920nM). Subsequent, preliminary *in vitro* experiments were performed on cultures of beating human cardiomyocytes derived from induced pluripotent stem cells. In studies using microelectrode arrays, JDTic reduced beat rate and conduction velocity at 300 nM, the lowest concentration studied. The usual single site of beat initiation clearly was disrupted after two hours' exposure to 1 μM JDTic (the only concentration evaluated in that paradigm); by 4 hours beat rate variability was significantly increased. Using impedance measurements, amplitude was reduced at 10 ng/mL, and the concentration at which >20% of the beats were arrhythmic was estimated to be between 1 and 10 ng/mL.

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Utilizing functionally selective ligands to probe specific signaling pathways of the k opioid receptor

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The κ opioid receptor (KOR)-dynorphin system has been implicated in the pathogenesis and pathophysiology of affective disorders, drug addiction, and psychotic disorders (Sheffler and Roth, 2003). Drugs targeting KOR as either antagonists or partial agonists have potential utility for a number of indications--especially as antidepressants and anxiolytics. Additionally, KOR agonists are gaining attention as potential antiaddiction medications, analgesics without a high abuse potential, and anti-epileptics. However, the adverse effects produced by KOR agonists, including aversion, sedation, dysphoria, and hallucinations, have limited their clinical use. I aim to determine if the therapeutic effects of KOR agonism are mediated by a separate KOR signaling pathway than the pathway causing the unwanted side effects (G-protein signaling vs. β-arrestin signaling). If so, there is a huge potential for functionally selective ligands as KORmediated analgesics devoid of dysphoric effects. I have identified several KOR-selective G-protein biased ligands and β-arrestin biased ligands *in vitro* by screening KOR ligands in the GloSensor cAMP biosensor assay (Promega) and the Tango assay. Additionally, I have identified β-arrestin dependent behaviors *in* vivo by comparing β-arrestin 2 KO mice with WT C57B/6 mice. We are further characterizing and confirming the role β- arrestin signaling in mediating behavior using our biased ligands *in vivo.* Understanding the roles G-protein and β-arrestin signaling play in mediating behaviors will improve KOR-based therapeutic potential.

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Kappa receptor activation in the nucleus accumbens shell subregion underlies compulsive methamphetamine intake

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Methamphetamine (METH) abuse is a chronically relapsing condition characterized by escalated drug intake and persistent drug craving. Given the known involvement of the kappa opioid receptor (KOR) system in psychostimulant abuse, we evaluated the selective KOR antagonist norbinaltorphimine's (NorBNI) ability to attenuate escalation of METH intake in an extended-access self-administration model. In this model, we demonstrated that systemic pretreatment with NorBNI (30mg/kg) attenuated escalation of intake during 6hr long-access (LgA) sessions. Furthermore, NorBNI decreased escalation of intake during the 1st hr of the SA session for the LgA, but not during the 1 hour of the short-access (ShA), condition. Similarly, NorBNI continued to decrease METH intake after abstinence, demonstrating that the behavioral effects of a single NorBNI injection can persist for at least 40 days. Rats were also tested under a progressive ratio (PR) schedule after escalation. NorBNI decreased the elevated PR breakpoints for rats in the LgA condition. A separate cohort of rats was given access to METH self-administration under ShA and LgA conditions, and were transcardially perfused 24hr following the final escalation session for the immunological detection of prodynorphin in the ventral striatum, specifically the nucleus accumbens (NAc) core and shell subregions. Rats with a history of ShA showed an increase in prodynorphin IR in both NAc core and shell subregions, however LgA animals showed a selective increase in the NAc shell. A separate cohort of animals was trained to self-administer METH and received intracranial pretreatment with NorBNI (4ug/0.5ul/side) directly into the NAc shell. LgA rats that received NorBNI showed significantly attenuated escalation of intake during 6hr sessions, decreased escalation of intake during the 1st hr of the SA session, and decreased PR responding for METH. These data indicate that the compulsive responding for METH in extended access to METH selfadministration depends on activation of the KOR system in the NAc shell.

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Roles of kappa opioids in angiogenesis during development and tumor formation

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Opioids are effective analgesics for the management of moderate to severe cancer pain. Here we show that K opioid receptor (KOR) agonists act as anti-angiogenic factors in development and tumors.(1) In development; we revealed that KOR agonists negatively regulate endothelial cell differentiation in embryonic stem cell-derived Flk1+ vascular progenitors by suppressing the expression of VEGF receptors, VEGFR2 and Neuropilin1. Furthermore, KOR-null or dynorphin (an endogenous KOR agonist)-null mice showed a significant increase in overall vascular formation at embryonic day-10.5 (Yamamizu, et al., Blood 2011). (2) In tumor; treatment with KOR agonists, U50,488H and TRK820, significantly inhibited human umbilical vein endothelial cell (HUVEC) migration and tube formation by suppressing VEGFR2 expression and VEGFR2 phosphorylation. In contrast, treatment with a K opioid receptor agonist, DAMGO, or a K opioid receptor agonist, SNC80, did not prevent angiogenesis in HUVECs. Lewis lung carcinoma (LLC) or B16 melanoma xenografted in KOR knockout mice showed increased proliferation and remarkably enhanced tumor angiogenesis compared with those xenografted in wild type mice. On the other hand, repeated intraperitoneal injection of TRK820 (1μg/kg, b.i.d.) significantly inhibited tumor growth by suppressing tumor angiogenesis. These findings indicate that KOR agonists play an important role in angiogenesis during development and tumor formation. This knowledge could lead to a novel strategy for cancer therapy.

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Increase of OPRK1 mRNA levels in the anterior cingulate in postmortem brain of HIV-infected subjects

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Earlier studies showed that chronic inflammation, glutamate neurotoxicity and synaptodendritic abnormalities in the frontal cortex may contribute to development of neuropsychological impairments in HIV/AIDS patients. In this study, we examined mRNA levels of opioid receptors OPRK1 and OPRM1 in the caudate and anterior cingulate in postmortem brain of HIV-positive and HIV-negative subjects. Postmortem tissues of HIV-infected (n=24) and control subjects (n=14) were obtained from the Manhattan HIV Brain Bank. Quantification of OPRK1 and OPRM1 mRNA was performed using RT-PCR. There were increased OPRK1 mRNA levels in the anterior cingulate ($p<0.005$) of HIV+ subjects compared to HIV negative subjects, but not in the caudate. In contrast, there was no difference in the levels of OPRM1 mRNA between HIV+ and HIV- subjects in either brain region. OPRK1 mRNA levels in the anterior cingulate in HIV+ subjects did not correlate with neurocognitive impairment. However, in this region, there was significant positive correlation of mRNA levels between OPRK1 and the scavenger receptor CD163, a marker of perivascular ramified microglia that are the primary targets of HIV, and that are implicated in neuroprotective pathways. Stimulation of KOR has been implicated in inhibition of dopamine and glutamate release, and in downregulation of HIV replication in cultured microglial cells. Correlation of OPRK1 and CD163 may suggest a mechanistic association in their putative roles to minimize HIV-induced brain pathologies.

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Voluntary alcohol drinking alters corticotropin-releasing factor and preprodynorphin mRNA levels in the central amygdala of Sardinian alcohol-preferring rats

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The stress-response corticotropin-releasing factor (CRF) and dynorphin systems are critically involved in alcohol drinking and "anxiety" behaviors. Selectively bred Sardinian alcohol-preferring (sP) rats display high inherent "anxiety"-related behaviors, in comparison with their alcohol-non preferring counterpart (sNP rats). The present study was undertaken to investigate: (1) if there were genetically determined differences in basal gene expression levels of CRF, CRF-R1, preprodynorphin (ppDyn) and kappa opioid receptor (KOP-r) between sP and sNP rats. Specifically, mRNA levels of the above genes were measured in the central amygdala (CeA), hypothalamus and other stress responsive and mesolimbic regions of alcohol-naive sP and sNP rats before alcohol exposure; and (2) if the above mRNA levels were altered by voluntary alcohol drinking in the alcohol-preferring rats, after sP rats exposed to the standard, homecage 2-bottle "alcohol vs water" choice regimen 24 hours/day for 17 days. Higher basal CRF mRNA levels were found only in CeA of alcohol-naive sP rats, compared with sNP rats; this level was decreased after alcohol consumption in sP rats. In contrast, ppDyn mRNA levels in CeA of sP rats were increased by alcohol consumption, but with no basal difference. Although higher basal ppDyn mRNA levels were found in hypothalamus of sP rats, compared with sNP rats, there was no alteration after alcohol drinking in sP rats. No difference for the above mRNA levels was observed in other regions, including nucleus accumbens shell or core, caudate-putamen, ventral tegmental area and medial/basolateral amygdala, between the two rat lines before or after alcohol consumption. Our results demonstrate the existence of genetically determined high basal CRF mRNA levels in CeA of sP rats. Alcohol consumption decreased CeA CRF mRNA levels with parallel increases in CeA ppDyn mRNA levels. Our results suggest that dynorphin, increased by alcohol, provides negative feedback mechanisms opposing high CRF activity in sP rats.

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New salvinorin A-derived orally available agent PR-38 reduces abdominal pain in mice

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Salvinorin A (SA) is known for its antinociceptive and antidiarrheal properties in animal models of abdominal pain and functional gastrointestinal (GI) disorders. Because of its short duration of action and strong hallucinogenic effects, SA never advanced to human clinical trials. Chemical modifications of SA, driven by close proximity of the side chain at C-2 to Cys315 in the binding pocket, led to discovery of PR-38, an analog with dual affinity to KOR (9.6 nM) and MOR (52 nM) as compared to SA (KOR 1.7 nM). Our studies showed that PR-38 significantly inhibits GI motility in physiological and pathophysiological conditions and produces a potent, analgesic effect in mice models of abdominal pain. Moreover, PR-38 showed a remarkable bioavailabilty being orally available and active after oral administration. It is worth noting that PR-38 does not produce any adverse effects in the central nervous system. PR-38 has a great potential to become a powerful drug candidate for the treatment of abdominal pain in irritable bowel syndrome (IBS) and other GI functional disorders.

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Conflict of interest: Application for provisional patent for PR-38 involving JKZ, PRP, BLR, J-CdR, and JF is pending.

The second conference on the therapeutic potential of kappa opioids in pain and addiction.

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