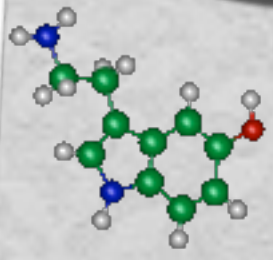
# Behavior, Biology, and Chemistry:
## Translational Research in Addiction

**March 5-6, 2011**

**La Quinta Inn & Suites**

**Medical Center**

**San Antonio, TX**



# Acknowledgements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University of Arkansas for Medical Sciences

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University of Texas at San Antonio (College of Sciences)

University of Texas Health Science Center at San Antonio (Center for Biomedical Research; Department of Pharmacology; Office of the Dean, Graduate School of Biomedical Sciences; Office of the Dean, School of Medicine; Office of the President)

University of Texas Medical Branch at Galveston (Department of Pharmacology & Toxicology and Center for Addiction Research)

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# *Program Overview*

## Friday March 4, 2011

| | |
|---|---|
| 4:00 pm - 7:00 pm | Registration |
| 7:00 pm - 10:00 pm | Opening Reception at Rio Rio on the San Antonio Riverwalk<br>Buses depart from La Quinta at 7:00 PM |

## Saturday March 5, 2011

| | |
|---|---|
| 7:00 am - 5:00 pm | Registration |
| 8:00 am - 8:15 am | Welcome and Opening Remarks |
| 8:15 am - 10:35 am | Plenary Symposium: "New concepts in mu-opioid pharmacology - implications for addiction and its management"<br>Speakers: Lakshmi Devi, John Traynor, Tom Prisinzano, Jennifer Whistler<br>(Chairs: John Traynor and Kelly Berg) |
| 10:35 am - 11:00 am | Coffee Break |
| 11:00 am - 12:00 am | Junior Investigator Oral Communications 1 (Chair: Emily Jutkiewicz) |
| **12:00 pm - 1:15 pm** | **Lunch** |
| 1:15 pm - 2:35 pm | Junior Investigator Oral Communications 2 (Chair: Brett Ginsburg) |
| 2:35 pm - 3:00 pm | Coffee Break |
| 3:00 pm - 4:00 pm | Special Lecture: Alexandros Makriyannis "The molecular basis of cannabinoid activity: biochemical studies on endocannabinoid targets" (Chair: Andrew Coop) |
| 4:00 pm - 4:30 pm | Poster Set-up |
| 4:30 pm - 7:00 pm | Poster Session |
| **7:00 pm - 9:00 pm** | **Dinner**<br>After Dinner Speaker: Klaus Miczek; "Social stress and escalation of alcohol and cocaine self administration: common neural circuitry?" (Chair: Wouter Koek) |
| 9:00 pm - 11:00 pm | Hospitality and Entertainment |

## Sunday March 6, 2011

| | |
|---|---|
| 8:00 am - 9:40 am | Open Oral Communications 1 (Chair: Laura O'Dell) |
| 9:40 am - 9:55 am | Coffee Break |
| 9:55 am - 11:15 am | Open Oral Communications 2 (Chair: Noelle C. Anastasio) |
| 11:15 am - 11:30 am | Coffee Break |
| 11:30 am - 12:30 pm | Special Lecture: Mary Jeanne Kreek; "Mu opioid receptor, SNPs, and altered stress responsivity" (Chair: Alan Frazer) |
| **12:30 pm - 1:30 pm** | **Lunch**<br>Presentation of travel awards and awards for oral and poster presentations<br>Closing remarks |

***See you at BBC 2012!***

# *Program Details*

## Friday March 4, 2011 (7:30 pm - 10:00 pm)

### Opening Reception

#### Rio RIo on the Riverwalk

| | |
|---|---|
| 7:00 pm | Buses depart from La Quinta |
| 7:30 pm - 10:00 pm | Reception at Rio Rio |
| 9:30 pm | First bus departs for La Quinta |
| 10:00 pm | Last bus departs for La Quinta |

Come and enjoy a fabulous evening on the beautiful San Antonio Riverwalk. Buses will depart from the La Quinta hotel at 7:00 pm to take you to Rio Rio, a Mexican restaurant on the Riverwalk. Buses will return to La Quinta at 9:30 pm and 10:00 pm. You will need your badge to board the bus and for dinner. TIckets for spouses and significant others can be purchased at the registration desk for $60.00.

## Saturday March 5, 2011

### Welcome and Opening Remarks (8:00 am - 8:15 am)

### Plenary Symposium (Chairs: John Traynor, Kelly Berg)

#### New concepts in mu-opioid pharmacology - implications for addiction and its management

Addiction to morphine and legal and illegal opioid drugs remains a serious problem, and prescription drug abuse and misuse is on the rise. In spite of much research over many years, fundamental questions remain concerning the mechanisms underlying opioid dependence and abuse and how these processes can be prevented or reversed. In addition, the search for the elusive non-addictive analgesic continues. In this symposium we will explore recent findings that shed light on the functioning of mu-opioid receptors; in particular, how mu-opioid receptors co-operate with other co-expressed receptors, roles for accessory proteins, the importance of receptor trafficking for opioid behavior, and the development of structurally unique ligands.

| | |
|---|---|
| 8:15 am - 8:50 am | ***Lakshmi Devi***; Mount Sinai School of Medicine<br>Opioid receptor interactions: implications in analgesia and addiction. |
| 8:50 am - 9:25 am | ***John Traynor***; University of Michigan<br>A 2-way street: opioid receptors and regulators of G protein signaling. |
| 9:25 am - 10:00 am | ***Tom Prisinzano***; University of Kansas<br>Mu-agonists from Salvinorin A. |
| 10:00 am - 10:35 am | ***Jennifer Whistler***; University of California, San Francisco<br>Morphine-induced mu opioid receptor trafficking enhances analgesia and reward yet prevents tolerance, dependence and compulsive drug use. |

### Coffee Break (10:35 am - 11:00 am)

## Saturday March 5, 2011 (continued)

### Junior Investigator Oral Communications 1 (Chair: Emily Jutkiewicz)

| | |
|---|---|
| 11:00 am -11:20 am | ***Courtney Keller***, Louisiana State University Health Sciences Center, Shreveport<br>Increased GluR1 phosphorylation associated with attenuation of cocaine cue-reactivity from combination of metyrapone and oxazepam. |
| 11:20 am- 11:40 am | ***Katherine Serafine***, American University<br>Assessment of the neurochemical mediation of cocaine-induced conditioned taste aversions. |
| 11:40 am - 12:00 pm | ***Michelle Baladi***, University of Texas Health Science Center at San Antonio<br>Eating high fat chow differentially affects sensitivity of adolescent male and female rats to cocaine-induced locomotor activity. |

### Lunch (12:00 pm - 1:15 pm)

### Junior Investigator Oral Communications 2 (Chair: Brett Ginsburg)

| | |
|---|---|
| 1:15 pm - 1:35 pm | ***J. Elliott Robinson***, University of North Carolina<br>Investigating the effect of neurokinin-1 receptor blockade on the potentiation of brain stimulation reward by morphine. |
| 1:35 pm - 1:55 pm | ***Joshua Gowin***, University of Texas Health Science Center at Houston<br>The Effects of Childhood Abuse on Adult Aggression: Cortisol as a Mechanism. |
| 1:55 pm - 2:15 pm | ***Oscar Torres***, University of Texas at El Paso<br>Developmental and sex differences in the expression of key molecular targets in a rat model of nicotine withdrawal. |
| 2:15 pm - 2:35 pm | ***Colin Cunningham***, University of Texas Health Science Center at San Antonio<br>Effects of nicotine, varenicline, and cytisine in separate groups of mice discriminating one of three doses of nicotine. |

### Coffee Break (2:35 pm - 3:00 pm)

### Special Lecture 3:00 pm - 4:00 pm (Chair: Andrew Coop)

**Alexandros Makriyannis** (Northeastern University)**: "The molecular basis of cannabinoid activity: biochemical studies on endocannabinoid targets"**

## Saturday March 5, 2011 (continued)

### Poster Set-up (4:00 pm - 4:30 pm)

### Poster Session (4:30 pm - 7:00 pm)

4:30 pm - 5:45 pm Odd numbered posters should be attended by their presenters.

5:45 pm - 6:30 pm Even numbered posters should be attended by their presenters.

Poster Awards: The Awards Committee will hear oral poster presentations (≈10 min) from undergraduate, graduate students, and post-doctoral fellows. One award will be made to the best undergraduate/graduate student poster presentation and one award will be made for the best post-doctoral fellow poster presentation.

Poster judging (for post-doctoral fellows and graduate students) will begin at 4:30 pm or 5:45 pm for odd and even numbered posters, respectively. Judges will begin with the lowest numbered posters and proceed to the higher numbered posters. Separate groups of judges will evaluate post-doctoral posters and graduate student posters.

If you do not wish to be included in the poster competition, please notify us at the registration table.

### Dinner (7:00 pm - 9:00 pm)

### After Dinner Lecture (Chair: Wouter Koek)

**Klaus Miczek** (Tufts University)**: "Social stress and escalation of alcohol and cocaine self administration: common neural circuitry?"**

### Hospitality and Entertainment (9:00 pm - 11:00 pm)

Come and enjoy the fun in the ballroom!

## Sunday March 6, 2011

### Oral Communications 1 (Chair: Laura O'Dell)

8:00 am - 8:20 am — ***Rosa De Almeida***, Universidade Federal do Rio Grande do Sul
Alcohol, flunitrazepam and aggressive behavior in mice and rats.

8:20 am - 8:40 am — ***Justin Anker***, University of Minnesota
Stimulant seeking in adolescent vs. adult rats.

8:40 am - 9:00 am — ***Emily Jutkiewicz*** , University of Michigan
The effects of delta-opioid receptor ligands on responding maintain by cocaine-paired stimuli.

9:00 am - 9:20 am — ***Balaji Krishnan***, University of Texas Medical Branch, Galveston
Serotonin 2C receptor (5-HT2CR) signaling in cocaine-associated conditioning results in a downstream activation of phospholipase D (PLD) and tracks with a distinct behavioral phenotype expressed in conditioned hyperactivity to cocaine.

9:20 am - 9:40 am — ***Amanda Sharpe***, University of Texas Health Science Center at San Antonio
Meal schedule influences food restriction-induced locomotor sensitization to methamphetamine.

### Coffee Break (9:40 am - 9:55 am)

### Oral Communications 2 (Chair: Noelle C. Anastasio)

9:55 am - 10:15 am — ***Amy M. Deveau***, The University of New England
In vitro characterization and in vivo blockade study of lead compound LAA-2-27b.

10:15 am - 10:35 am — ***Xiu Liu***, University of Mississippi Medical Center
Involvement of the opioid μ1 receptors in nicotine reinforcement in a rat model of nicotine self-administration.

10:35 am - 10:55 am — ***James Orfila***, University of Texas at El Paso
Cholinergic levels in the nucleus accumbens (NAcc) are enhanced in adolescent versus adult rats exposed to nicotine but are similar in both age groups following nicotine withdrawal.

10:55 am - 11:15 am — ***Shivani Ruparel***, University of Texas Health Science Center at San Antonio
Role of cytochrome P450 enzymes in oxidized linoleic acid metabolite-mediated inflammatory pain.

### Coffee Break (11:15 am - 11:30 am)

### Special Lecture 11:30 am - 12:30 pm (Chair: Alan Frazer)

**Mary Jeanne Kreek** (The Rockefeller University)**: "Mu opioid receptor, SNPs, and altered stress responsivity"**

### Lunch (12:30 pm - 1:30 pm)

Presentation of awards for travel, oral, and poster presentations

Closing Remarks and Adjournment

# Abstracts

## Oral Communications

### 1

**Increased GluR1 phosphorylation associated with attenuation of cocaine cue-reactivity from combination of metyrapone and oxazepam**

Keller, Courtney M., Salvatore, Michael F., Guerin, Glenn F., Goeders, Nicholas E.
Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA

Cocaine addiction results in long-term neuroadaptations that play an essential role in relapse. Altered glutamatergic signaling in several brain regions, including the nucleus accumbens and striatum, has been identified as an important mediator of relapse. Additionally, it has been demonstrated that re-exposure to an environment associated with cocaine leads to a decrease in GluR1, an AMPA receptor subunit. Therefore, this study was designed to determine if drugs that attenuate cocaine seeking (e.g., the combination of low doses of metyrapone and oxazepam) may also affect GluR1 function. Adult, male rats were implanted with indwelling jugular catheters and trained to self-administer cocaine (0.25 mg/kg/infusion) on an FR4 schedule. Each infusion of cocaine was paired with a tone and house light compound stimulus that became a secondary reinforcer. Once stable responding was obtained, rats were placed into abstinence for two weeks. One day following abstinence, the rats underwent a 15-minute cue-reactivity session. Rats were placed back in the experimental chamber, and lever presses resulted in the presentation of the secondary reinforcer, but no cocaine was delivered. Rats were pretreated with either the combination of metyrapone (50 mg/kg) and oxazepam (10 mg/kg) or vehicle 30 minutes before the cue-reactivity session. The striatum, nucleus accumbens, amygdala, and prefrontal cortex were dissected immediately after the session and analyzed for dopamine content, total GluR1, and phosphorylated GluR1. No significant differences were found in dopamine content or total GluR1 for any of the four brain regions. However, there was an increase in GluR1 phosphorylation at Ser831 in all four regions. Phosphorylation of GluR1 at Ser831 is thought to increase trafficking of the receptor to the membrane. Therefore, increased GluR1 subunit phosphorylation of the AMPA receptor may be a neurobiological mechanism by which the combination of metyrapone and oxazepam attenuates cocaine seeking behavior by increasing receptor number in the membrane, thereby restoring GluR1 levels.

### 2

**Assessment of the neurochemical mediation of cocaine-induced conditioned taste aversions.**

Serafine, KM[1]; Rice, KC[2]; Riley, AL[1].
1 Department of Psychology, American University, Washington, DC. 2Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD.

Although cocaine induces conditioned taste aversions (CTA), little is known about the mechanisms underlying this effect. It has been suggested that cocaine's actions as a nonselective monoamine transport inhibitor may be mediating this suppression. The present experiments assessed if preexposure to compounds selective for each of the monoamines (desipramine [DMI; NE], fluoxetine [FLU; 5-HT] and vanoxerine [GBR; DA]) affected cocaine-induced CTAs. Preexposure to cocaine was also assessed for its effects on each monoamine transporter inhibitor. Following one-way ANOVAs on the Final Aversion Test for each assessment, Fisher LSD post-hoc analysis revealed that DMI preexposure attenuated aversions induced by itself and by cocaine. FLU and GBR preexposure attenuated aversions induced by themselves but not by cocaine. Cocaine preexposure attenuated aversions by cocaine and FLU but not those induced by GBR or DMI (where aversions were potentiated). DA appears to have no role in cocaine-induced CTAs; some role for 5-HT and NE is suggested but their specific functions remain unknown.

### 3

**Eating high fat chow differentially affects sensitivity of adolescent male and female rats to cocaine-induced locomotor activity**

Baladi, Michelle G[1] and France, Charles P[1,2]
1Pharmacology and 2Psychiatry; University of Texas Health Science Center at San Antonio, TX

Many factors impact the behavioral effects of drugs acting on dopamine systems. For example, after just 1 week of eating high fat chow, male adolescent rats are more sensitive to the locomotor stimulating effects of cocaine as compared with male adolescent rats eating standard chow. This study examined whether eating high fat chow modifies sensitivity of female adolescent rats to the locomotor effects of cocaine (1.0-17.8 mg/kg). Sprague Dawley rats (PND 25) had free access to standard (5.7% fat) or high fat (34.3% fat) chow, or restricted access to high fat chow (body weight matched to rats eating standard chow). As with males, eating high fat chow further increased the locomotor effects of cocaine in females, but only after 4 weeks of eating the chow. These data suggest that eating high fat chow has a similar effect on sensitivity to cocaine in both male and female adolescent rats; however, the effect occurs more rapidly in males. Thus, adolescence might represent a critical period when eating high fat food influences sensitivity to, and possibly vulnerability to abuse, cocaine or other related drugs.

CPF is supported by Senior Scientist Award DA17918.

### 4

**Investigating the effect of neurokinin-1 receptor blockade on the potentiation of brain stimulation reward by morphine.**

Robinson, J. Elliott and Malanga, C. J.
University of North Carolina at Chapel Hill School of Medicine, Dept. of Neurology, Chapel Hill, NC 27599.

**Background:** Endogenous opioids signaling through the μ-opioid receptor (MOR) modulate dopamine release in the nucleus accumbens and mediate the rewarding properties of drugs of abuse. Several studies have suggested that the neurokinin 1 receptor (NK1) plays a role in opioid reward and receptor signaling. In this study, we used intracranial self-stimulation (ICSS), an operant behavioral method in which mice self-administer rewarding electrical current (brain stimulation rewards or BSRs) to study the reward-potentiating effects of morphine before and after neurokinin 1 blockade.
**Methods:** Adult male C57BL/6 mice were implanted with unipolar stimulating electrodes in the medial forebrain bundle and taught to self-administer BSRs. Using a curve-shift method, the BSR threshold (θ0) was determined before and after administration of morphine (1.0, 3.0, 10.0, 17 mg/kg i.p.) or saline. Animals were also pre-treated with the NK1 antagonist L-733,060 (10 or 17 mg/kg i.p.), the MOR antagonist naltrexone (1 or 3 mg/kg i.p.) or saline 15 minutes before morphine (10 mg/kg i.p.) or saline treatment.
**Results**: Morphine dose-dependently decreased BSR threshold across 2 hours of testing with maximal effect (50% reduction in threshold) seen 1.5 hours after administration. Naltrexone and L-733,060 pre-treatment studies are being performed and will be discussed.
**Conclusions:** Experimenter-administered morphine potentiated brain stimulation reward at all doses tested, which confirms previous findings in C57BL/6 mice. Forthcoming data using L-733,060 should elucidate the role of NK1 in morphine reward and provide insight into its utility as a therapeutic target for opioid and alcohol dependence.

# Oral Communications

## 5

**The Effects of Childhood Abuse on Adult Aggression: Cortisol as a Mechanism**

Gowin, Joshua L[2]; Lane, Scott D[1]; Moeller, F Gerard[1]; Swann, Alan C[1]

Early exposure to a stressful or trauma-inducing environment presents a significant risk factor to later developing anti-social behavior. One result of experiencing neglect or abuse as a child may be the maladaptation of the body's secretion of cortisol in response to a stressor. The dysregulation of the cortisol system may play a role in the development of aggression and anti-social behavior later in life. The relationship between childhood abuse/neglect and cortisol has not been widely studied in humans with regards to anti-social behavior and merits further investigation. In this ongoing study (current N=25, 18 male) we assessed abuse and neglect using the Childhood Trauma Questionnaire and measured aggression using a computer paradigm. We also administered acute doses of cortisol and placebo in a double-blind, counter-balanced and within-subject design. We also collected saliva samples from subjects to measure cortisol levels and measured heart rate, pupil size and blood pressure. Data will be analyzed using a linear regression model for main effects and interaction effects. We hypothesize that subjects with higher childhood neglect/abuse will be more aggressive in the computer task and that an altered cortisol system may mediate this relationship. Additionally, we hypothesize that cortisol administration will produce a main effect on aggressive responding.

1. Department of Psychiatry & Behavioral Sciences, School of Medicine
2. Program in Neuroscience, Graduate School of Biomedical Sciences
University of Texas Health Science Center -- Houston

## 6

**Developmental and sex differences in the expression of key molecular targets in a rat model of nicotine withdrawal.**

Torres, Oscar V., Natividad, Luis A., Byers, Donna M., and O'Dell, Laura E.
University of Texas at El Paso, Dept. of Biology and Psychology, El Paso, Texas 79968

Previous work in our laboratory has demonstrated developmental differences in the behavioral effects of nicotine withdrawal. However, little is known about the cellular mechanisms that mediate developmental and sex differences during nicotine withdrawal. This study characterized the expression of gene targets associated with withdrawal in adolescent and adult male and female rats. Animals were prepared with subcutaneous pumps that delivered either saline or a dose of nicotine (4.7 mg/kg/day in adolescents; 3.2 mg/kg/day in adults; base). After 14 days of nicotine exposure, the pumps were removed and the nucleus accumbens (NAcc) and amygdala were collected for RNA isolation. Four genes were selected based on their affinity for nicotine binding (α7, α4) and catecholamine innervation (dopamine D1 and D2 receptors). Differential expression of these targets was measured using quantitative PCR technology. Overall, the most robust changes in gene expression produced by nicotine withdrawal were observed in the NAcc. Specifically, the expression of genes associated with the nicotinic targets were up-regulated to a greater degree in adolescent relative to adult rats. In the amygdala, the expression of catecholamine innervation by D2 receptors was up-regulated to a greater extent in adolescent and adult females relative to their male counterparts. These data suggest that developmental and sex differences during nicotine withdrawal may be linked to differential responses in gene expression. Further, our finding may suggest that age and sex may be crucial factors to consider for different pharmacological treatments that aid in smoking cessation. (Supported by NIH-NCRR-RCMI G12-RR08124).

## 7

**Effects of nicotine, varenicline, and cytisine in separate groups of mice discriminating one of three doses of nicotine.**

Colin Seamus Cunningham, Lance Richard McMahon.
Pharmacology, UTHSCSA, San Antonio, TX

For some drug classes, agonist efficacy can be an important determinant of behavioral effects; this study examined the relationship between nicotine agonist efficacy and behavioral effects in C57BL/6J mice discriminating three training doses (0.56, 1, or 1.78 mg/kg base s.c.) of nicotine. To examine receptor mechanisms, antagonists that vary in nicotinic receptor subtype selectivity, mecamylamine (1 and 3.2 mg/kg) and dihydro-betaerythroidine (DHBE; 3.2 mg/kg), were combined with nicotine. As training dose increased, there was a decrease in the potency of nicotine to produce discriminative stimulus effects and to modify response rate. Mecamylamine produced rightward shifts in the nicotine dose-response curves; the magnitude of shift was inversely related to the training dose of nicotine. DHBE was a less effective antagonist of nicotine than mecamylamine. Varenicline and cytisine did not substitute for nicotine in all mice and the potency of both did not vary as a function of training dose. Partial substitution of varenicline and cytisine for nicotine is consistent with differences in agonist efficacy, and unlike nicotine, there was no orderly relationship between the effects of varenicline or cytisine and the training dose of nicotine. This suggests that differences in selectivity for subtypes of nicotine receptor might contribute to differences in behavioral effects. Funded by USPHS grant DA25267.

## 8

**Alcohol, flunitrazepam and aggressive behavior in mice and rats**

[1]Rosa Maria Martins de Almeida and [2]Klaus A. Miczek
1Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
2Departments of Psychology, Pharmacology, Neuroscience and Psychiatry, Tufts University, Medford and Boston, USA.

Rationale: Higher doses of benzodiazepines and alcohol induce sedation; however, in low to moderate doses these drugs can increase aggressive behaviour in men and rodents. Objectives: to assess firstly the effects of ethanol, secondly the effects of flunitrazepam, a so-called club drug, and thirdly the effects of flunitrazepam plus alcohol on aggression in mice and rats. Methods: Exhaustive behavioral records of confrontations between a male resident and a male intruder were obtained twice a week, using CF-1 mice and Wistar rats. The salient aggressive and non-aggressive elements in the resident´s reperetoire were analyzed. Initially, the effects of ethanol (1.0 g/kg), secondly flunitrazepam (0; 0.01; 0.1; and 0.3 mg/kg) were determined in all mice and rats; subsequently, flunitrazepam or vehicle, given intraperitoneally (0; 0.01; 0.1; and 0.3 mg/kg) was administered plus ethanol 1.0 g/kg or vehicle via gavage. Results: The most significant finding is the escalation of aggression after a moderate dose of ethanol, a low dose of flunitrazepam. The largest increase in aggressive behaviour occurred after combined flunitrazepam plus ethanol treatment in mice and rats. Conclusions: Ethanol can heighten aggressive behavior and flunitrazepam further increases this effect in male mice and rats.

# Oral Communications

## 9

**Stimulant seeking in adolescent vs. adult rats.**

Anker, Justin J and Carroll, Marilyn E
Department of Psychiatry, University of Minnesota, Minneapolis, MN USA

Adolescence is a period of increased vulnerability to the initiation and subsequent abuse of drugs. Adolescents are also more likely to binge on drugs of abuse and they may be especially vulnerable to factors that precipitate relapse such as the drug itself, drug-related cues, and stress. Impulsivity, an important factor in drug abuse vulnerability, is also greater during adolescence, and this may further increase drug abuse vulnerability. The purpose of this research was to compare adolescent and adult rats under several conditions to determine whether there are differences in their drug-seeking and drug-taking behavior. The first was a maintenance procedure with intermittent periods of signaled availability and nonavailability for iv cocaine. In the second procedure adolescent and adult rats were compared on the escalation of methamphetamine (METH) self-administration during long-access (6 h), and in the third procedure evaluated the reinstatement of cocaine seeking using a multi-component reinstatement procedure with drug-, cue-, and stress-related priming conditions. In the fourth study, adolescent and adult rats were tested on a delay discounting (DD) task for food that measured impulsive choice, a strong predictor of drug abuse vulnerability. Results indicated that reinforced and nonreinforced cocaine seeking under the maintenance procedure was greater in adolescent rats compared to adult rats and that adolescents escalated METH intake faster than adult rats. Adolescents also responded more following cocaine and yohimbine (stress-inducing) injections under the reinstatement procedure, while adults (vs. adolescents) showed greater reinstatement responding following presentations of drug-associated cues. Impulsivity for food under the DD task was also higher in adolescents compared to adults. These results demonstrate that adolescent (vs. adult) rats exhibit greater vulnerability across several measures of drug-seeking behavior, and they are more likely than adults to engage in impulsive choice, a major liability factor in drug abuse.

## 10

**The effects of delta-opioid receptor ligands on responding maintain by cocaine-paired stimuli**

Jutkiewicz, Emily M[1,2]; Meurice, Claire[1]; Traynor John R[1,2]
1Department of Pharmacology, University of Michigan; 2University of Michigan Substance Abuse Research Center, University of Michigan

Delta opioid agonists, such as SNC80, increase locomotor activity, produce conditioned place preference, enhance the activity of psychomotor stimulants, and generalize to the discriminative stimulus effects of cocaine. However, SNC80 fails to promote or maintain self-administration behavior and does not increase dopamine release as measured by microdialysis in the rat striatum. This unusual profile of behavior suggests that delta-opioid agonists have some stimulant-like effects produced potentially through a different mechanism of action. Delta-opioid agonists have been shown to stimulate glutamate release, which is a pathway involved in regulating the dopamine system and the actions of primary and conditioned reinforcers. Considering delta-opioid agonists do not have primary reinforcing effects, this study evaluated the effects of SNC80 and the delta-opioid antagonist naltrindole (NTI) on responding for the stimuli paired with cocaine in rat self-administration procedures. In rats with a history of cocaine self-administration, non-contingent SNC80 administration enhanced operant responding maintained by stimuli previously associated with cocaine, but did not alter responding in the absence of the cocaine-paired stimuli or on the inactive lever. NTI decreased responding for stimuli paired with cocaine in the absence of drug. Overall, these data suggest that the DOR system may modulate the conditioned reinforcing effects of cocaine-paired cues and possibly cocaine-seeking behavior. These studies were supported by the Small Grants Program from the University of Michigan Substance Abuse Research Center.

## 11

**Serotonin 2C receptor (5-HT2CR) signaling in cocaine-associated conditioning results in a downstream activation of phospholipase D (PLD) and tracks with a distinct behavioral phenotype expressed in conditioned hyperactivity to cocaine**

Krishnan Balaji[1,2],Anastasio Noelle C[1,2], Fox Robert G[1,2], Stutz Sonja J[1,2] & Cunningham Kathryn A[1,2]
1Center for Addiction Research, 2Pharm/Tox Dept., UTMB, Galveston, TX 77555

Environmental cues can become classically conditioned to cocaine exposure and contribute to craving and relapse in addicts. Signaling through 5-HT2CR within the corticolimbic circuit is implicated in the mechanisms underlying cocaine-associated conditioning events, including reward salience. We propose that 5-HT2CR signaling activates PLD in distinct brain regions such as the amygdala and the hippocampus and 5-HT2CR-PLD mediated signaling events track with cocaine-cue dependent behavior following withdrawal. Male rats received repeated pairings of a distinct test setting with saline or cocaine for 7 days. Paired and unpaired rats received cocaine (15 mg/kg, i.p.) in the activity monitor with motility assessment and home cage, respectively, and saline in the alternative setting; control rats received saline in both settings. A 30-min drug-free test for motility occurred 14 days after the last pairing; rats were sacrificed and brain tissue processed for Western blot analysis. Expression of conditioned hyperactivity was observed in paired rats (843±39 counts/30min) vs. unpaired (699±43) or control rats (604±36; $p<0.05$). As hypothesized, we observed a concomitant increase in the synaptosomal expression of both PLD (PLD1, PLD2) and 5-HT2CR in amygdala ($p<0.05$), while both were decreased in hippocampus ($p<0.05$) of paired vs. unpaired or control groups. The present findings suggest that the 5-HT2CR-PLD mediated signaling in the amygdala and the hippocampus contribute to the salience of the drug-association with the environment. Elucidating the differential attributes of 5-HT2CR-PLD mediated signaling will be an invaluable first step to identify potential therapeutic targets of cue-based neuroplasticity underlying craving and relapse.
Financial Support: DA020087, DA024157, DA006511, Jeane B. Kempner Scholarship, Center Addiction Research Pilot Funds

## 12

**Meal schedule influences food restriction-induced locomotor sensitization to methamphetamine.**

Amanda L. Sharpe, Joshua D. Klaus, Micahel J. Beckstead.
Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX.

Rationale: Traditional protocols for inducing sensitization to psychostimulants use an intermittent or “binge”-like drug administration, and binge-eating behavior is co-morbid with drug abuse in humans. Food restriction increases the reinforcing properties of many drugs of abuse and the self-administration that they support.

Objective: The present studies tested the hypothesis that (1) food restriction induces sensitization to the locomotor stimulation observed in response to methamphetamine, and (2) that a binge feeding schedule during food restriction results in increased sensitization compared to equally restricted mice fed in meals.

Methods: Male DBA/2J mice were fed ad libitum or were food restricted to either an 8 or 16% loss of body weight. Additionally the food restricted mice were divided into two groups that were fed in one meal (binge) or three equal sized meals. After steady body weight was obtained, mice were tested for locomotor activity following saline and methamphetamine injections.

Results: Both 16% food restricted groups exhibited sensitization to methamphetamine. Opposite of our hypothesis, the 8% meal but not the 8% binge food restricted group demonstrated locomotor sensitization. Serum corticosterone levels at the time of the locomotor sessions were significantly higher in the 8% meal group compared to the 8% binge and ad lib groups.

Conclusions: These results support a role for feeding schedule and plasma corticosterone levels in food restriction-induced enhancement of the effects of methamphetamine.

# Oral Communications

## 13

**In Vitro Characterization and In Vivo Blockade Study of Lead Compound LAA-2-27b**

**T**anya Lawrence[a,b], Elizabeth A. Andrews[a], Andrea L. Pelotte[a], Tanya Lawrence[a,b], Christina M. Dersch[c], Mario Ayestas[c], Denise Giuvelis[b], Edward J. Bilsky[b], Richard B. Rothman[c], Amy M. Deveau[a]
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In our program to discover novel agents to reverse opiate overdose and treat addiction, we have discovered lead compound LAA-2-27b. This compound possesses subnanomolar affinity at and subtype specificity for the mu opioid receptor (MOR). LAA-2-27b also functions as a neutral antagonist in opiate-conditioned cells. In this paper, the in vitro data and in vivo blockade study of LAA-2-27b will be discussed. In conclusion, the current research contributes to our understanding of the structure-activity profile for antagonist functional binding at the MOR.

## 14

**Involvement of the opioid μ1 receptors in nicotine reinforcement in a rat model of nicotine self-administration.**

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There has long been an interest in examining the involvement of opioid neurotransmission in nicotine rewarding process and addiction to nicotine. Over the past 3 decades, however, clinical effort to test the effectiveness of nonselective opioid antagonists (mainly naloxone and naltrexone) for smoking cessation has yielded equivocal results. In light of the fact that there are three distinctive types of receptors mediating actions of the endogenous opioid peptides, this study, using a rat model of nicotine self-administration, examined involvement of different opioid receptors in the reinforcement of nicotine by selective blockade of the μ1, the δ, and the κ opioid receptors. Male Sprague-Dawley rats were trained in daily 1 h sessions to intravenously self-administer nicotine (0.03 mg/kg/infusion, free base) on a fixed-ratio 5 schedule. After establishment of stable nicotine self-administration behavior, the effects of the opioid antagonists were tested. Separate groups of rats were used to test the effects of naloxanazine (selective for μ1 receptors, 0, 5, 15 mg/kg), naltrindole (selective for δ receptors, 0, 0.5, 5 mg/kg), and 5'-guanidinonaltrindole (GNTI, selective for κ receptors, 0, 0.25, 1 mg/kg). In each individual drug group, the 3 drug doses were tested by using a within-subject and Latin-Square design. The effects of these antagonists on food self-administering behavior were also examined in the same rats in each respective drug group after retrained for food self-administration. Pretreatment with naloxonazine, but not naltrindole or GNTI, significantly reduced responses on the active lever and correspondingly the number of nicotine infusions. None of these antagonists changed lever-pressing behavior for food reinforcement. These results indicate that activation of the opioid μ1, but not δ or κ, receptors is required for the reinforcement of nicotine and suggest that opioid neurotransmission via the μ1 receptors would be a promising target for the development of opioid ligands for smoking cessation. This study was supported by NIH grant DA-017288 from NIDA and startup fund from the University of Mississippi Medical Center.

## 15

**Cholinergic levels in the nucleus accumbens (NAcc) are enhanced in adolescent versus adult rats exposed to nicotine but are similar in both age groups following nicotine withdrawal**

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Previous work has shown that adolescent rats are less sensitive to the behavioral effects of nicotine withdrawal relative to adults (Natividad et al., 2010). However, the neurochemical mechanisms that mediate these developmental differences are presently unclear. Thus, the goal of this study was to compare acetylcholine (ACh) levels in the NAcc of adolescent and adult rats experiencing nicotine withdrawal. Adolescent (PND 28-30) and adult (PND 60-70) male Wistar rats were prepared with subcutaneous pumps that delivered an equivalent nicotine dose in these age groups (4.7 mg/kg/day for adolescents and 3.2 mg/kg/day for adults). Following 13 days of nicotine exposure, rats were implanted with microdialysis probes in the NAcc. The next day, dialysis samples were collected during baseline and following systemic administration of the nicotinic-receptor antagonist mecamylamine (1.5 mg/kg or 3.0 mg/kg) to precipitate withdrawal. In order to examine potential age differences in ACh metabolism, we also compared ACh levels following systemic administration of the ACh-esterase inhibitor, methanesulfonyl fluoride (MSF; 2.0 mg/kg). Dialysate levels of ACh were quantified using HPLC-EC methods. Our results revealed that during baseline, adolescent rats displayed enhanced levels of ACh relative to adults. However, we did not observe any age differences during precipitated withdrawal or following administration of the ACh-esterase inhibitor. Taken together, our results suggest that chronic nicotine administration enhances basal cholinergic transmission in adolescent rats, and this may confer enhanced vulnerability to the rewarding effects of nicotine during adolescence. Importantly, the baseline differences in ACh do not appear to be related to age-dependent differences in ACh metabolism since both age groups displayed similar changes following MSF administration. Lastly, our results suggest that developmental differences to the behavioral effects of nicotine withdrawal are not mediated via age differences in cholinergic transmission. Future neurochemical studies will elucidate the role of other potential mechanisms that mediate developmental differences to nicotine withdrawal.

## 16

**Role of Cytochrome P450 Enzymes in Oxidized Linoleic Acid Metabolite-Mediated Inflammatory Pain**

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Our group has recently demonstrated that oxidized linoleic acid metabolites (OLAMs) are released from injured tissues and activate TRPV1; a voltage gated ion channel that plays a pivotal role in inflammatory heat hyperalgesia and thermoregulation. However, the mechanism by which linoleic acid is converted to the OLAMs is unknown. Our recent results show the involvement of cytochrome P450 (CYPs) enzymes in the formation of these OLAMs. Importantly, treatment of animals with antibodies against the OLAMs or drugs that inhibit CYPs at peripheral sites results in anti-hyperalgesia in a rat model of inflammatory pain. We also extended the previous studies performed in rodents to a new evaluation of the OLAM system in humans with the use of dental pulp specimens removed from normal wisdom teeth and from teeth that were extracted due to pain. We observed that the formation of oxidized metabolites of [14C]-linoleic acid is increased three-fold when incubated with inflamed human tissue compared with normal dental pulp. Moreover, the addition of two CYP inhibitors, ketoconazole and voriconazole reduce OLAM production by 25% and 45%, respectively. Analysis of CYP expression in normal vs. inflamed human dental pulp shows minimal expression in normal specimens, whereas expression is dramatically increased and widespread within the inflamed samples, including a striking expression within inflammatory cells. Taken, together, these preclinical results demonstrate that OLAMs comprise a new family of physiologically relevant endogenous TRPV1 agonists and identify the OLAM system (OLAMs and the enzymes that produce OLAMs) as potential new targets for analgesic drug development.

# Poster Communications

## 1

**Behavioral and neurophysiological predictors of psychopathy and aggression**

J.L. Alcorn III, S.D. Lane, G.F. Moeller, A.C. Swann

Aggression is an enduring problematic social behavior often resulting in deleterious consequences upon criminal justice system and mental health institutions. Additionally, aggression is prominent in several psychiatric disorders (e.g., substance use disorders and personality disorders such as antisocial and borderline), and is a key feature in the clinical construct of psychopathy. Therefore, scientific investigations of aggression may elucidate the biobehavioral aspects of aggression and psychometric correlates of psychopathy, and suggest therapeutic interventions.

In the present study we collected subjects from a community sample with a variety of substance use, criminal, and psychiatric backgrounds in addition to healthy volunteers. We measured subject's aggressive responding using the Point Subtraction Aggression Paradigm (PSAP); a real time well validated measure of aggression. Subjects also completed the auditory oddball paradigm using electroencephalogram (EEG) to record the event related potential P300; a positive-going electrical potential elicited from relatively rare sensory stimulus (low tone) demanding attention that is intermingled with frequently occurring sensory stimuli (high tones). P300 abnormalities are suggested be markers of psychopathy and aggressive behavior problems. Lastly, subjects completed self report psychometric measures of aggression, impulsivity, and psychopathy.

We conducted principle components analyses (PCA) from: (i) neurophysiological data from EEG electrode recording sites; (ii) psychometric scores from self-report measures; and (iii) presence of a DSM-IV substance use and/or personality disorder. Preliminary regression analyses on the PCA factor scores indicate of that aggressive responding on the PSAP and two neurophysiological components derived from the ERP data are predictive of an antisocial/psychopathy factor (derived from a cluster of psychometric and diagnostic factors). These preliminary findings suggest that combinations of behavioral, neurophysiological, and psychometric measures of aggression and impulsivity may predict the construct of psychopathy.

## 2

**Knockdown of 5-HT2C receptor in the nucleus accumbens enhances trait impulsivity and confers enhanced sensitivity to obesogenic food**

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Serotonin (5-HT) is important in the control over the affective and motivational aspects of palatable food reward. The 5-HT2C receptor (5-HT2CR) regulates neurobehavioral processes which may underlie important chronic health maladies including obesity, eating disorders and drug addiction. Impulsivity is a multifaceted personality trait that is broadly defined as action without sufficient foresight and functions both as a predisposing factor and consequence of addiction. The purpose of this study was to assess the role of nucleus accumbens shell (NAcSh) 5-HT2CR in sensitivity to natural reward as well as expression of the impulsivity endophenotype. Recombinant AAV vectors were constructed with a separate expression cassette for eGFP and shRNA directed at the 3' untranslated region of the rat 5- HT2CR to decrease expression of all endogenous 5-HT2CR isoforms. The 5-HT2CR shRNA-AAV-eGFP viral vector was bilaterally infused into the NAcSh of male rats, while control animals received bilateral intra-NAcSh infusions of AAV-eGFP. Knockdown of NAcSh 5-HT2CR increased preference for 1% sucrose solution vs control. Exposure to a sub-preference concentration of sucrose (0.05%) following 4 days withdrawal resulted in sucrose preference in rats with a loss of NAcSh 5-HT2CR. Knockdown of the NAc 5-HT2CR also enhanced bingeing on an obesogenic food vs. controls. Premature responses were elevated in 5-HT2CR knockdown rats, indicating enhanced impulsivity. Sensitivity to palatable/obesogenic food is enhanced following knockdown of the 5-HT2CR in the NAcSh. 5-HT2CR in the NAcSh may play a role in regulation of responsiveness to rewarding stimuli and exerts inhibitory control over trait impulsivity.
Supported by: Jeane B. Kempner Scholarship, DA06511, DA024157, DA020087, DA000403

## 3

**Synthesis, Characterization and Evaluation of Diphenyl and Dimethyl Carbamate Derivatives of 6b-Naltrexol in Vitro and in Vivo as Possible Treatments for Opioid Addiction**

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We are currently studying the structural requirements that promote neutral antagonism (NA) of the mu opioid receptor (MOR) in our quest for discovering novel agents to combat addiction. We previously synthesized a series of sulfonate ester and carbamate derivatives of 6β-naltrexol and evaluated their opioid receptor subtype and functional selectivities. From these in vitro data we identified two lead compounds: one compound was characterized as a pure antagonist and another as a pure inverse agonist. In this presentation, we will discuss the scale-up, purification and characterization of our two lead compounds. In vitro studies along with preliminary in vivo data describing the antinociception profile of the NA lead compound will also be presented. In conclusion, the current research contributes to our understanding of the structure-activity profile for antagonist functional binding at the MOR.

## 4

**Bidirectional effects of methamphetamine on dopamine neuron excitability.**

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The commonly abused drug methamphetamine increases extracellular levels of dopamine in the brain by decreasing dopamine uptake and promoting release through reverse transport. Microdialysis and electrochemical measurements suggest that this occurs not only in terminal areas (such as the nucleus accumbens) but also in somatodendritic regions. Unfortunately, no study to date has investigated the physiological effects of acute methamphetamine exposure on dopamine neuron excitability. We used whole cell patch clamp electrophysiology in slices of mouse midbrain to investigate the cellular and synaptic consequences of acute methamphetamine administration. Low concentrations of methamphetamine enhanced the amplitude of dopamine-mediated inhibitory postsynaptic currents (IPSCs) by approximately 60%. Despite increased autoreceptor-mediated inhibition, dopamine neuron pacemaker firing rate was surprisingly increased by low concentrations of methamphetamine due to an offsetting excitatory conductance mediated through the dopamine transporter. Higher concentrations of methamphetamine had the opposite effect, dramatically decreasing the amplitude of dopamine IPSCs while slowing firing rate. In slices from mice that had been treated in vivo with methamphetamine (2 mg/kg i.p., every second day for nine days), a high concentration of methamphetamine increased firing rate, in contrast to the decrease in firing observed in slices from naïve and saline-treated mice. These results suggest a mechanism by which intermediate concentrations of methamphetamine could increase phasic dopamine neurotransmission. Sustained excitation by dopamine transporter-mediated currents offsets autoreceptor-mediated inhibition, allowing neuronal firing to continue despite increased extracellular dopamine levels. Further, methamphetamine-mediated excitation could be enhanced with drug experience, potentially increasing the reinforcing properties of the drug with repeated exposure. Supported by NIDA grant K01 DA21699.

# Poster Communications

## 5

**Exogenous melatonin modulates methamphetamine-induced sensitization and reward in C3H/HeN mice**.

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Studies in mice demonstrated the variation of stimulatory and rewarding properties of psychostimulants according to time of day. This time dependent difference suggests the involvement of a regulatory mechanism subject to circadian rhythms. One candidate for this role is melatonin, a pineal hormone that follows a circadian rhythm with highest levels observed at night. Our hypothesis is that exogenous melatonin given during the day modulates methamphetamine (MTA) induced locomotor sensitization and reward. To test this hypothesis we subjected C3H/HeN mice to 6 daily doses of 1.2 mg/kg MTA (ip) or vehicle (VEH) followed by 2h locomotor tests during ZT 5-7 (ZT 0 = lights on). When challenged with MTA (1.2 mg/kg, ip), MTA pretreated mice exhibited robust sensitization as compared with mice that received VEH pretreatments. Repeated co-treatments with melatonin (3 mg/kg, sc), administered 30 min prior to each daily MTA pretreatment, significantly increased sensitization compared to vehicle co-treatments administered prior to MTA. We assessed the rewarding properties of MTA using a conditioned place preference test. Following drug-free habituation and pre-test sessions, the animals were conditioned for 6 days with alternating treatments of MTA (1.2 mg/kg, ip) and VEH. Mice were tested for place preference 1 day after the last conditioning session. Animals spent significantly more time in the MTA-paired chamber during the post-test compared to the VEH-paired chamber. This model system appears suitable for testing the effects of exogenous melatonin on the rewarding properties of MTA in the C3H/HeN mouse. Supported by DA 021870

## 6

**Effects of metyrapone and oxazepam combinations on methamphetamine cue-reactivity in rats**

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We have previously reported that the combination of low doses of metyrapone (MET), a corticosterone synthesis inhibitor, and oxazepam (OX), a benzodiazepine, reduces intravenous cocaine self-administration (SA) and the cue-induced reinstatement of extinguished cocaine seeking in rats. This experiment explored the effects of various individual and combination doses of MET and OX on methamphetamine (METH) cue-reactivity (CR). Adult male rats were implanted with jugular catheters and trained to SA METH (0.06 mg/kg/inf) during daily 2-h sessions. METH delivery was paired with the presentation of a tone and a houselight (conditioned reinforcer). Once stable baselines of SA were observed, rats were placed into forced abstinence in their home cages for 14 days. During CR testing on the 15th day, the rats were placed in the operant chambers and responding only resulted in the presentation of the conditioned reinforcer, which reliably maintained METH seeking (i.e., lever pressing) following vehicle pretreatment. Pretreatment with OX (2.5, 5, and 10 mg/kg) dose-dependently attenuated CR responding. Pretreatment with MET (25, 50, 75 and 100 mg/kg) attenuated responding at the highest dose tested. Pretreatment with combinations of 25 mg/kg MET/5mg/kg OX or 50 mg/kg MET/10mg/kg OX resulted in dose-dependent attenuations of METH seeking. These data suggest that the combination of MET and OX may be useful in treating the relapse to METH use.

## 7

**Opioid Receptor Probes Derived from Cycloaddition of the Hallucinogen Natural Product Salvinorin A.**

Cunningham, Christopher W.; Lozama, Anthony; Caspers, Michael J.; Douglas, Justin T.; Dersch, Christina M.; Rothman, Richard B.; Prisinzano, Thomas E.
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Salvinorin A is a neoclerodane diterpene that acts as the principal psychoactive constituent of the hallucinogenic sage, Salvia divinorum Epling & Játiva (Lamiaceae). The hallucinatory effects are mediated through activation of the kappa opioid (KOP) receptor, indicating that salvinorin A is the first reported non-nitrogenous opioid receptor ligand. The structural features responsible for KOP receptor binding and efficacy are unknown, however, prompting our investigation into the structure-activity relationships (SAR) of salvinorin A. To date, the majority of salvinorin A analogues have investigated the C-2 acetate function, leaving the role of the C-12 furan moiety less understood. Toward developing SAR of this position, we have developed microwave-assisted Diels-Alder cyclization methodology that has resulted in C-12 ring constrained analogues of salvinorin A. In addition to introducing electron-withdrawing and bulky substituents into this region of chemical space, aromatization of the bent oxanorbornadiene system resulted in non-heterocyclic aromatic derivatives bound directly to C-12. Extensive NMR analysis investigated the degree of stereo- and regiocontrol associated with the [3+2] cycloaddition reaction for various reactants. Although dimethyl- and diethylcarboxylate analogues retain some affinity and selectivity for KOP receptors and are full agonists, their aromatized counterparts have greatly reduced affinity for KOP receptors. The results of these studies will be used to further develop SAR for KOP receptor binding and efficacy, and the chemical methodology developed here can be a useful approach toward rapidly probing the SAR of other furan-containing natural products.

## 8

**Implementing "Cocaine Use Reduction with Buprenorphine (CURB)" in the Veterans Health Care System**

Dawes, Michael[1,2] ; Potter, Jennifer[2]; Roache, John[2]; Trivedi, Madhukar[3];
1 South Texas Veterans Health Care System, Audie L. Murphy VA Hospital, 7400 Merton Minter, San Antonio, TX 78229; 2 University of Texas Health Science Center at San Antonio, Department of Psychiatry, 7703 Floyd Curl Drive, San Antonio 78229; 3 UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, Texas 75390-9119 (This study is conducted under the auspices of the Texas Region Node, PI Madhukar Trivedi, MD; Lead Investigator Walter Ling, M.D., UCLA, Pacific Region Node; Andrew Saxon, MD, and Larissa Mooney, MD, Co-Lead Investigators, Pacific Region Node)

**Background**: While Cocaine Dependence poses a serious health risk in the US, Veterans account for a sizable portion of this population. In the absence of any approved medications for Cocaine Dependence, buprenorphine (a partial mu-opioid agonist and kappa-opioid antagonist) is beginning to be studied for its effectiveness. **Study Objectives:** The primary aim of this multi-site study is to investigate the safety and effectiveness of buprenorphine in the presence of naltrexone for the treatment of cocaine dependence. Secondarily the safety and effectiveness will be evaluated between VA and non-VA participants. **Study Population:** Participants will be 300 cocaine-dependent adults; about 20% will be from the Veterans Health Care System. Participants will meet all eligibility criteria, including current or past year opioid abuse or current or lifetime opioid dependence (by DSM-IV). Participants will be either male, or females who are non-pregnant and non-lactating, of all racial/ethnic groups, aged 18-65. **Treatment**: Participants meeting eligibility will be inducted onto naltrexone (by mouth), then will be randomly assigned (1:1:1) to one of three treatment arms: (1) 4mg buprenorphine plus long-acting naltrexone (BUP4 + NTX); (2) 16mg buprenorphine plus long-acting naltrexone (BUP16 + NTX), or (3) placebo plus long-acting naltrexone (PLB + NTX), with these medications to be provided for 8 weeks. Also, participants will be scheduled for once-weekly cognitive-behavioral therapy (CBT). This poster will discuss the rationale and study design of this multi-center trial, and the implications of implementing the CURB study in a VHA context, with veterans' unique health issues.

# Poster Communications

## 9

**Psychomotor stimulant like behavioral effects of nicotinic agonists**

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We recently demonstrated that nicotinic agonists can engender full [nicotine (NIC)] and partial [(+) epibatidine (EPI), (–) EPI, anabaseine (ANA), isoarecolone (ISO), varenicline (VAR)] dose–dependent substitution for the discriminative–stimulus (DS)effects of methamphetamine (MA) in 0.3 mg/kg MA–trained rats. The present studies were undertaken to evaluate the effects of various nicotinic agonists in squirrel monkeys trained to discriminate 0.1 mg/kg (n = 4) of i.m. MA from vehicle. Subjects initially were trained under a 10–response fixed–ratio schedule to press one lever after i.m. injection of the training dose of MA and another lever after i.m. injection of vehicle. When responding was stable, cumulative i.m. dosing procedures were used to study the effects of MA and various nicotinic agonists including NIC, (+) EPI, (–) EPI, ISO, ANA, anabasine, VAR, (–) cytisine (CYT), lobeline (LOB), and DMAB–anabaseine (GTS–21). Overall the nicotinic agonists produced a wide range of MA–like discriminative stimulus effects including full [NIC, (+) EPI], partial [(–) EPI, ISO, ANA, anabasine, VAR], and no substitution [CYT, LOB, GTS–21] for MA's DS effects. In additional interaction experiments, pretreatment with VAR (0.0032–0.1 mg/kg) and CYT (0.032–0.32 mg/kg) antagonized the MA–like substitution produced by NIC (>10–fold rightward shift) suggesting that partial nicotinic agonists may be useful in reducing the abuse–related effects of nicotine. The above results further suggests that nicotinic mechanisms contribute to the abuse–related effects of MA, and that targeting nicotinic activity may be a useful avenue for developing treatments for the management of monoaminergic stimulant abuse and addiction (supported by DA026548).

## 10

**Effects of chronic morphine treatment and discontinuation on delay discounting in pigeons**

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Drug abusers tend to be more impulsive than nonusers on a variety of measures. For example, opioid abusers discount delayed reinforcers more rapidly than nonusers. This study examined the effects of repeated morphine treatment and its discontinuation on delay discounting. During sessions comprising 5 cycles, pigeons (n=6) responded on one key to receive 1.5 s access to grain or on a second key to receive 4 s access. During the first cycle, both reinforcers were delivered immediately; beginning on the second cycle, delivery of the large reinforcer was delayed with the length of the delay doubling across cycles. Before treatment, pigeons responded exclusively for the large reinforcer when there was no delay. As delay increased, pigeons switched responding to the small reinforcer. Daily treatment began with 10 mg/kg of morphine administered 6 hrs before sessions and increased every 14 days up to 100 mg/kg of morphine administered twice daily. Repeated morphine treatment altered response distribution in all pigeons, although that effect varied among individuals; changes in discounting persisted throughout treatment (i.e., no tolerance). Prior to repeated treatment, 10 mg/kg of morphine decreased the number of trials completed to <50% of the maximum, whereas during treatment, a 10-fold larger dose only modestly decreased the number of trials completed. Discontinuation of treatment further altered response distribution and disrupted responding; the latter effect waned within 5 days while changes in discounting persisted for several weeks. Although tolerance developed during repeated morphine treatment, as evidenced by reduced effectiveness of morphine to disrupt responding, tolerance did not develop to the effects of morphine on discounting. Disruptions in responding following discontinuation of treatment dissipated within one week, whereas changes in discounting persisted for much longer; such lingering behavioral effects could contribute to relapse long after treatment is terminated.

CPF is supported by a Senior Scientist Award DA17918.

## 11

**Selective stimulation of the 5-HT2CR signalosome is associated with elevated basal and reduced 5-HT2CR-evoked levels of novelty-induced motility**

Fink, Latham HL[1,2], Stutz, Sonja J[1,2], Bubar, Marcy J.[1,2], Cunningham, Kathryn A[1,2]
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Dysregulation in limbic-corticostriatal serotonin (5-HT) 2C receptor (5-HT2CR) function is implicated in a variety of neuropsychiatric conditions. However, little is known about 5-HT2CR regulation in vivo or how disruption of 5-HT2CR regulation may contribute to neuropsychiatric illness. To gain a better understanding of 5-HT2CR regulation, we tested the hypothesis that stimulation of the 5-HT2CR signalosome following repeated, intermittent pretreatment with the selective 5-HT2CR agonist WAY 163909 will result in a pattern of behavioral tolerance and associated molecular adaptations. Male Sprague-Dawley rats were pretreated once daily for 7 days with saline (1 ml/kg, i.p.) or WAY 163909 (10 mg/kg, i.p.). Weights were recorded daily. On day 8, rats received either a challenge injection of saline or WAY 163909 (10 mg/kg, i.p.), and motility induced upon exposure to a novel environment was measured (90 min). Brain tissue was immediately collected and frozen for future biochemical analysis of 5-HT2CR mRNA and protein expression. Interestingly, upon injection with saline, rats exposed to repeated WAY 163909 pretreatment exhibited elevated ambulations compared to saline-treated controls ($p<0.05$). As expected, acute challenge with WAY 163909 suppressed motility relative to vehicle controls, while repeated pretreatment with WAY 163909 blunted the WAY 163909-evoked hypomotility ($p<0.05$). Thus, repeated WAY 163909 pretreatment increases motility in response to a novel environment, suggesting desensitization of both tonic and agonist-induced 5-HT2CR signaling. Quantitative RT-PCR analyses revealed that repeated WAY 163909 administration did not alter 5-HT2CR mRNA levels in the nucleus accumbens, dorsal striatum, or hippocampus, indicating that alterations in 5-HT2CR transcription in these brain regions do not underlie the increase in novelty-induced motility associated with repeated WAY 163909. Studies are underway to examine WAY 163909-induced alterations in expression of the 5-HT2CR protein and protein complexes through Western blot and co-immunoprecipitation assays. Supported by: DA06511, DA020087, DA024157 and DA07287

## 12

**Anxiogenic effects of amphetamine in zebrafish.**

Gaikwad Siddharth, Hart Peter, Riehl Russell, Green Jeremy, Karl Vollmer, Jessica Cosgrove, Allain Alexander, Roth Andrew Razavi Roshan, Kyzar Evan, DiLeo John, Rhymes Kate, Hook Molly, Pham Mimi, Monnig Louie, Kalueff Allan V.
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Adult zebrafish have become a widely used animal model in neurobehavioral research, as their rich behavioral repertoire allows for the characterization of complex behavioral phenotypes. In our current study, we have used new techniques to visualize and quantify amphetamine evoked anxiety-related behavior in zebrafish. The novel tank test is a paradigm that utilizes the innate diving response and aversion to novel environments to assess stress responses in fish. Initially, the fish will bottom-dwell; however, as the fish acclimates to the environment, it gradually explores the upper portions of the tank. When exposed to amphetamine (0.5, 2, 5, 10, 20, or 40 mg/L, for 20 min by immersion), zebrafish exhibit markedly reduced transitions to and time spent in the upper half of the tank. This anxiety-like response is dose-dependently modulated, as higher doses increase avoidance behavior and decrease exploration. Interestingly, challenge from 10 mg/L of amphetamine evoked a marked increase in c-fos expression in the brain, suggesting the wide spread activatory/pro-arousal effects of amphetamine on neural pathways. Interestingly, these effects of amphetamine resemble those of another similar agent, cocaine, in zebrafish (Stewart et al., 2011). Overall, our results suggest that adult zebrafish can be useful in identifying and characterizing behavioral phenotypes precipitated by pharmacological manipulation, including anxiogenic effects of selected drugs of abuse, such as amphetamine. Furthermore, this model may also be useful in screening the effects of novel compounds on zebrafish anxiety-related behavior.

# Poster Communications

## 13

**The 5-HT2C Receptor Inverse Agonist SB 206553 Decreases Inward Rectification and Increases the Number of Action Potentials by Neurons in the Basal Forebrain**

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Administration of the 5-HT2C inverse agonist SB 206553 (SB206) attenuates methamphetamine-seeking in rats (Graves & Napier, CPDD Abstract 2009). The nucleus accumbens (NAc) expresses constitutively active 5-HT2C receptors and these receptors regulate dopamine efflux in this region (Navailles et al., Neuropsychopharm, 33:237 2008). We sought to determine the impact of SB206 on 'basal' neuronal function within the NAc. Whole-cell patch clamp recordings from NAc neurons were conducted in ex vivo brain slices obtained from young (4~6 weeks of age) treatment-naïve rats. Under current-clamp conditions, cells were injected with current pulses at 0.05nA increments (-0.5 to 0.5nA, 500 ms), and the effects of bath-applied SB206 (10μM) were determined. Results show (i) attenuated inward rectification (n=9; Drug x Current interaction, $p=0.009$), (ii) depolarized resting membrane potential (by ~5mV; n=8; $p<0.05$), (iii) reduced afterhyperpolarization amplitude (by ~2mV; n=12; $p<0.01$), and (iv) and a trend to increase the number of action potentials evoked by depolarizing currents. Ongoing studies suggest a similar excitatory profile of SB206 (i.e., enhanced firing) in the ventral pallidum (n=2), an output region for the NAc that is involved in cocaine-seeking (McFarland & Kalivas, J Neurosci, 21:8655 2001). Explanations for these findings include: (i) SB206 is providing orthosteric competition for endogenous 5-HT and inhibiting agonist-induced 5-HT2C activity or (ii) SB206 is inhibiting constitutively active 5-HT2C receptors. Constitutively active 5-HT2C receptors are represented in a subpopulation of receptors. As every neuron recorded thus far responded to SB206 with a similar profile, the results likely reflect orthosteric inhibition of endogenous 5-HT, as opposed to negative intrinsic efficacy. Future studies will determine if 5-HT2C receptor function is altered in rats trained to self-administer methamphetamine.

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## 14

**Role of Cytochrome P450 Enzymes in Oxidized Linoleic Acid Metabolite Mediated Inflammatory Pain**

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We recently demonstrated that oxidized linoleic acid metabolites (OLAMs) are formed upon exposure of cell membranes to noxious heat (>43C), and by utilizing antibodies to OLAMs we saw a significant reversal in thermal allodynia induced by CFA in vivo. Thus production of OLAMs contributes to the thermal and inflammatory responsiveness of TRPV1. However, the mechanism by which linoleic acid is converted to OLAMs is unknown. It has been speculated that cytochrome p450 (CYPs) and lipoxygenase (LOX) enzymes play an important role in the metabolism of linoleic acid. We have observed detectable levels of several subtypes of these enzymes in the nociceptive neurons from rat trigeminal ganglia. To evaluate possible role of CYPs and/or LOX in the activation of sensory neurons by linoleic acid, rat trigeminal ganglia (TG) were cultured for 24hrs on coverslips and real time calcium imaging was employed to investigate CYP and LOX inhibitors on calcium influx due to LA. Pretreatment with the CYP inhibitors rather than LOX inhibition blocked LA-evoked increases in [Ca]i. Furthermore HPLC data utilizing extracts from inflamed rat vibrissal pads shows an increase in LA metabolites under inflammation, and that the production of these metabolites can be reduced pretreatment with a broad CYP inhibitor, Ketoconazole. In concert with in vitro data, behavioral data suggest that CYP inhibition in vivo, using two broad cytochrome P450 inhibitors Ketoconazole and Voriconazole, is capable of an approximately fifty percent reversal of thermal allodynia under a peripheral inflammatory model. Taken together, these findings indicate that TG neurons are capable of synthesizing endogenous TRPV1 agonists following application of linoleic acid via a CYP pathway, and that in an inflammatory model inhibition of CYPs partially reverses inflammatory induced thermal allodynia. Elucidating the role of these enzymes in pain mechanisms may provide potential strategies for development of novel non-opioid analgesics with fewer debilitating side effects and whose use will have no potential for addiction.

## 15

**Dynamic regulation of serotonin (5-HT) 2A/2C receptors and estrogen receptor (ER) systems on serotonin transporter (SERT) interaction**

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Dysregulation of serotonin neurotransmission, including alterations in the 5-HT transporter (SERT) and receptors, contributes to vulnerability to drug addiction. Fluctuations of ovarian estrogenic hormones during a female's life are associated with mood disorders - perhaps with differential success rates among women undergoing treatment for drug addiction. We have previously identified that exposure to estradiol (E2) reduces 5-HT uptake via SERT (Koldzic-Zivanovic et al, 2004). Direct interactions between ERß and SERT, their receptor populations, and/or shared intracellular signaling pathways may be involved. Here, we tested the hypothesis that E2 regulates SERT through ERß-SERT interactions. We investigated the interactions between the 5-HT2R family (5-HT2AR, 5-HT2CR) and ERß mechanisms, and the potential functional consequences of E2-mediated regulation of ERs, 5-HT2Rs or SERT expression and function in the RN46A neuronal cell line. Since E2 and 5-HT rapidly elevated intracellular calcium, such elevation could result from activation of 5-HT2AR and/or 5-HT2CR. As the interaction of ERß and 5-HT2 receptors in this cell line has not been investigated, we plan to study this interaction and possible consequences on these receptors or SERT regulation. Membranes from RN46A cells were isolated by differential centrifugation. Membrane-enriched protein fractions were separated by SDS-PAGE, transferred to PVDF membranes and analyzed by immunoblot. ERß, SERT, 5-HT2AR and 5-HT2CR were detected using rabbit polyclonal, mouse monoclonal, rabbit polyclonal, and mouse monoclonal antibodies, respectively. We detected immunoreactive bands corresponding to the ERß (~56 KDa), SERT (~70 KDa) and 5-HT2CR (~46 KDa) in RN46A cell membranes, indicating that ERß, 5-HT2CR, and SERT are expressed in membrane fractions of RN46A cells. We are now primed to investigate the pertinent protein:protein interactions to elucidate the functional consequences. Support by: DA020087, DA024157, ES15292

## 16

**Anxiolytic effects of ketamine in zebrafish.**

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Zebrafish (Danio rerio) have become a widely used animal model in neurobehavioral research. Here, we have used several paradigms to describe behavioral effects of ketamine in adult zebrafish. The novel tank paradigm utilizes the innate diving response elicited by zebrafish in novel environments, in which bottom-dwelling behavior indicates anxiety or avoidance responses. As the subject habituates to the environment, it will gradually explore the upper portion of the environment, signifying anxiolysis. Ketamine (20 and 40 mg/L, 20-min exposure by immersion) exhibited increased duration of time spent in the upper half of the novel tank, decreased latency to initial entry of the top, as well as decreased freezing. Additionally, several other endpoints automatically measured through video-tracking (Noldus EthoVision XT7) showed reduced velocity and meandering. In a similar paradigm, the shoaling test, zebrafish inter-subject distance is used as an endpoint of anxiety, as shoaling fish tend to remain within close proximity. Ketamine evoked longer distances between fish in a dose-dependent manner, with distance increasing with higher doses (20 and 40 mg/l). In the light-dark box test, natural aversion to the illuminated portion of the environment can be used to measure anxiety-like behavior. Similar to our other findings, ketamine reduced anxiety, increasing the number of transitions to and time spent in the light section. Finally, ketamine produced overt circling behavior in the open field test, as fish treated with 20 and 40 mg/L showed robust rotations (strikingly resembling ketamine-evoked rotation responses in rodents). Ketamine also markedly reduced whole-body cortisol levels in zebrafish, confirming its anxiolytic-like profile. Taken together, these findings demonstrate high sensitivity of zebrafish-based models to anxiolytic effects of selected drugs of abuse, such as ketamine.

# Poster Communications

## 17

**Utility of Transgenic Mice to Aid Therapeutic Development in Nicotine Addiction: The Case of the Orexin System**

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Orexin (or hypocretin) transmission plays a key role in motivated behavior for drugs of abuse. Indeed, previous studies from our laboratory have shown that the orexin-1 antagonist SB-334867 attenuates nicotine-induced lowering of brain-stimulation reward thresholds and decreases intravenous nicotine self-administration (SA) in rats. However, SB-334867 is derived from a class of compounds that has affinity for other non-orexin receptor classes as well, raising the possibility that its effects may partly derive from "off-target" actions at non-OX1 receptors in the brain. Additionally, the role of orexin-2 (OX2) receptors in nicotine reinforcement is unknown. To unambiguously verify a role for OX1 and OX2 receptors in nicotine dependence, we first assessed nicotine SA behavior in male wildtype (WT) and OX1 receptor knockout (KO) mice (Exp 1). In a separate cohort of mice (Exp. 2), extinction sessions commenced following nicotine SA where lever press responding had no programmed consequences. After extinction, animals received a single reinstatement session where a nicotine-paired cue light was presented and subsequent operant responding resulted in cue light presentation. SB-334867 was administered IP 15 min prior to the SA session in all experiments. To investigate the effects of OX2 signaling in nicotine SA behavior, Exp. 3 entailed the exact same procedure as Exp. 1 except the selective OX2 receptor antagonist, SR-8809, was injected in place of SB-334867. Our results demonstrate that OX1 receptors are critical for maintaining sensitivity to the reinforcing effects of nicotine and expression of relapse-like behavior. Additionally, our data provides strong evidence that SB-334867 acts selectively at OX1 receptors in vivo. Moreover, the decrease in nicotine responding was most dramatic when both orexin receptor subtypes were inactivated. Collectively, our findings highlight the potential utility of OX1 and OX2 receptor antagonists for the treatment of tobacco dependence, and report a novel behavioral procedure that can support the identification of selective orexin receptor antagonists in the effort to treat nicotine addiction in smokers.

## 18

**Sex differences in anxiety-like behavior and locomotor activity following prenatal and postnatal methamphetamine exposure in rats.**

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Our previous studies demonstrated that methamphetamine (MA) administered during gestation and lactation periods impairs maternal behavior, alters the functional development of rat pups and affects cognitive functions and nociception in adulthood. The aim of the present study was to investigate the impact of prenatal and postnatal MA exposure on behavior and anxiety in adult male and female rats. Mothers were daily exposed to injection of MA (5 mg/kg) or saline (S): prior to impregnation and throughout gestation and lactation periods. On postnatal day 1, pups were cross-fostered so that each mother raised 6 saline-exposed pups and 6 MA-exposed pups. Based on the prenatal and postnatal exposure 4 experimental groups (S/S, S/MA, MA/S, MA/MA) were tested in the Open field (OF) and in the Elevated plus maze (EPM). Locomotion, exploration, comforting behavior were evaluated in the OF, while anxiety was assessed in the EPM. Our results showed that prenatal MA exposure did not affect behavior and anxiety in adulthood, while postnatal MA exposure (i.e. MA administration to lactating mothers) induced long-term changes. Adult male and female rats in diestrus postnatally exposed to MA via breast milk (S/MA and MA/MA) had decreased locomotion and exploratory behavior in the OF and showed increased anxiety in the EPM when compared to rats postnatally exposed to saline (S/S and MA/S). In adult females in proestrus, postnatal MA exposure did not affect behavior and anxiety in the OF and EPM. In conclusion, the present study demonstrates that postnatal exposure to MA via maternal breast milk decrease locomotion and exploration, but increase anxiety to novel environment in adult females rats in diestrus and adult males. On the other hand, these behavioral changes were not present in rats prenatally exposed to MA and postnatally exposed to saline. Supported by: CN LC 554, MSM 0021620816 and GACR P303/10/0580.

## 19

**Genetic Deletion of Both MT1 And MT2 Melatonin Receptors Attenuated Methamphetamine-Induced Locomotor Sensitization in C3H/HeN Mice**

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The magnitude of drug-induced locomotor sensitization varies according to time of day. Although circadian regulatory systems have been implicated in governing these variations, questions remain regarding the underlying cellular and molecular mechanisms. We sought to determine how the pineal hormone melatonin, which is synthesized following a circadian rhythm which peaks at night, interacts with the MT1 and MT2 melatonin receptors to influence the sensitizing properties of methamphetamine (METH). Locomotor sensitization was induced in C3H/HeN mice by 6 daily METH pretreatments (1.2 mg/kg, i.p.) during the daytime (ZT 5-7, ZT 0=lights on), followed by 4 days of drug abstinence. The mice were tested for sensitization by digital video analysis of their locomotor activity following a METH challenge injection (1.2 mg/kg, i.p). The expression of sensitization was determined by comparing the distance traveled by mice pretreated with METH to that of vehicle-pretreated controls following the METH challenge. Wild-type mice repeatedly pretreated with METH expressed significantly more locomotor activity when challenged with METH than did control animals subjected to repeated vehicle injections, indicating the strong expression of sensitization. Likewise, knockout mice lacking either the MT1 or MT2 receptors also exhibited robust sensitization. In contrast, repeated METH pretreatments did not induce sensitization in MT1/MT2 double knockout mice. Our results indicate that the MT1 and MT2 melatonin receptors play key roles in METH-induced sensitization and may contribute to diurnal variations in drug-induced responses. Supported by 021870.

## 20

**Identification of novel peptide modulators of glycine and gabaa receptors using phage display**

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Ethanol is a widely-used drug of abuse with a complex, poorly understood neurochemical mechanism of action. It has actions on a wide variety of biochemical targets, among them a number of ion channels and enzymes. However, the relevance of each target to the expression of ethanol-induced behaviors remains unknown. Separating the individual contributions of alcohol effects on various targets will allow for a better understanding of ethanol effects and for the rational development of novel therapies for alcohol abuse. One way to accomplish this is to mimic, or alternatively antagonize, the effects of alcohol on individual targets to determine the behavioral consequences of alcohol actions at each target. One method for identifying compounds that act selectively at specific putative alcohol targets is phage display. Phage display is a cell-based selection system that enables the identification of small peptides binding with high affinities to a single target. This approach has been used to identify novel peptides that bind to proteases, cell-specific peptides, and specific neuronal cell types. In addition to identifying binding motifs, phage display has yielded peptides that not only show affinity for specific targets, but also have modulatory effects on function. Combining phage display selection with a functional characterization technique, whole-cell voltage clamp electrophysiology, allowed us to identify highly specific peptides capable of functionally modulating the selected targets. Our project focuses on identifying novel peptide modulators of two major ethanol targets: glycine and GABAA receptors. Both receptors are involved in inhibitory neurotransmission and are thought to mediate some of the behavioral effects of ethanol. Phage display was used to identify peptides that bind to specific subtypes of glycine and GABAA receptors. Use of a negative selection protocol ensured high specificity of binding. Peptides selected from these screens were then studied for their functional effects on the respective receptor subtypes and also tested on closely-related channels to determine their specificities. Our findings suggest that phage display can be successfully implemented to identify subtype-specific modulators for ligand-gated ion channels.

# Poster Communications

## 21

**Neural substrates of methamphetamine induced place reinforcement learning: The role of the hippocampus-VTA Loop**

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The reinforcing effects of addictive drugs including methamphetamine (METH) involve the midbrain ventral tegmental area (VTA). The VTA is a primary source of dopamine (DA) to the nucleus accumbens (NAc) and the ventral hippocampus (VHC). These three brain regions are functionally connected through the hippocampal-VTA loop, which includes two main neural pathways: the bottom-up pathway and the top-down pathway. In this paper we take the view that addiction is a learning process. Therefore, we tested the involvement of the hippocampus in reinforcement learning by studying conditioned place preference (CPP) learning by sequentially conditioning each of the three nuclei in either the bottom-up order of conditioning; VTA, then VHC, finally NAc, or the top-down order; VHC, then VTA, finally NAc. Following habituation, the rats underwent experimental modules consisting of two conditioning trials each followed by immediate testing (test 1 and test 2) and two additional tests 24 h (test 3) and/or one week following conditioning (test 4). The module was repeated three times for each nucleus. The results showed that METH, but not Ringer's, produced positive CPP following conditioning each brain area in the bottom-up order. In the top-down order METH, but not Ringer's, produced either an aversive CPP or no learning effect following conditioning each nucleus of interest. In addition, METH place aversion was antagonized by co-administration of the NMDA antagonist MK801 suggesting that the aversion learning was an NMDA receptor activation dependent process. We conclude that the hippocampus is a critical structure in the reward circuit and hence suggest that the development of target specific therapeutics for the control of addiction emphasize on the hippocampus-VTA top-down connection.

## 22

**Peroxisome proliferator-activated receptor gamma activation relieves expression of behavioral sensitization to methamphetamine and of neuropathic pain in mice**

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Peroxisome proliferator-activated receptor (PPAR) is a ligand-activated transcription factor that regulates lipid metabolism and neuroinflammation. And its ligands (pioglitazone and ciglitazone) are clinically used for the treatment of some diseases, including type 2 diabetes. Behavioral sensitization and neuropathic pain has recently been implicated in an inflammation of central nervous system. We examined the involvement of PPAR gamma, one of the isotypes of PPAR, in development of behavioral sensitization to the stimulant effect of methamphetamine (METH) and of partial sciatic nerve ligation (PSL)-induced neuropathic pain in mice.
<Behavioral sensitization to METH> Repeated administration of METH (once a day for 5 days) enhanced the locomotor-activating effect of METH, which was reproduced by METH challenge on withdrawal day 7 (test day 12). The protein level and activity of PPAR gamma were significantly increased in the nuclear fraction of whole brain after 5 days of METH administration (test day 5) and on withdrawal day 7 (test day 12). Concurrent administration of PPAR gamma agonist (icv) with METH prevented the expression of behavioral sensitization to METH challenge on withdrawal day 7, but not the sensitization that occurred during repeated administration of METH. In addition, the magnitude of expression in behavioral sensitization was augmented by PPAR gamma antagonist (icv, once daily for 6 days) during the withdrawal period. <PSL-induced neuropathic pain> Administration of PPAR gamma agonist for the first week after PSL attenuated thermal hyperalgesia and tactile allodynia induced by PSL in a dose-dependent manner, which was blocked by PPAR gamma antagonist. PSL-induced up-regulation of tumor necrosis factor alpha and interleukin 6, which are essential for neuropathic pain, was suppressed by PPAR gamma agonist for the first week.
These results suggest that PPAR gamma has a significant role in the expression of behavioral sensitization to METH and of PSL-induced neuropathic pain.

## 23

**The role of non-GABAA receptors in the discriminative stimulus effects of the neuroactive steroid pregnanolone in rats**

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Neuroactive steroids, such as pregnanolone, are positive modulators of γ-aminobutyric acid (GABAA) receptors. In contrast to other positive modulators (e.g., benzodiazepines), which act mainly at GABAA receptors, neuroactive steroids are thought to act at other receptors, including n-methyl-D-aspartate (NMDA) and serotonin3 (5-HT3) receptors. The current study exploited the pharmacological selectivity of drug discrimination procedures to determine whether non-GABAA receptors contribute to the effects of the neuroactive steroid pregnanolone. Separate groups of rats discriminated either 3.2 mg/kg of pregnanolone (n=13) or 0.32 mg/kg of the benzodiazepine midazolam (n=11) while responding under a fixed ratio 10 schedule for food. The long acting NMDA receptor antagonist dizocilpine, the 5-HT3 receptor agonist CPBG, and the mu opioid receptor agonist morphine were studied alone; each drug that did not occasion drug-lever responding was also studied in combination with midazolam. When administered alone, midazolam and pregnanolone produced ≥80% drug-lever responding in both groups. Dizocilpine occasioned responding predominantly on the drug lever, whereas CPBG and morphine produced vehicle-lever responding up to doses that markedly decreased response rates, regardless of the training drug. When CPBG or morphine was administered in combination with midazolam, there was no change in the midazolam dose-effect curve in either group. Given that CPBG is without effect under these conditions, it is unlikely that 5-HT3 receptors contribute substantially to the discriminative stimulus effects of pregnanolone. Similarities in effects of dizocilpine obtained in the two groups suggest that NMDA receptors do not differentially contribute to the discriminative stimulus effects of pregnanolone, although additional studies with other NMDA antagonists are warranted to exclude a role of NMDA receptors in the discriminative stimulus effects of pregnanolone. Supported by USPHS grant DA017240.

## 24

**Do mu-opioid receptors exhibit differential signaling efficiency in the brain versus the spinal cord?**

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Mu-opioid receptor (MOR) activation of the family of Gαi/o proteins is necessary for the production of opioid analgesia. Historically, studies of the role of these Gα subtypes in opioid analgesia have evaluated antinociception in the tail-flick test after manipulation of Gα expression levels at higher brain centers, e.g. in the PAG. Unfortunately, these studies have reported complex and conflicting results. Previous in vitro data suggests that coupling of MOR in brain is essentially limited to Gαo. The aim of the present work was to evaluate the role of Gαo in MOR-mediated antinociception in vivo using a Gαo null mouse strain. Wild type and heterozygous (+/-) Gαo null mice were evaluated for morphine (i.p.) antinociception in both the warm-water tail withdrawal (measuring spinal antinociception) and hot plate (measuring supraspinal antinociception) tests. Loss of Gαo resulted in a significant (~4-fold) rightward shift in the morphine dose-effect curve in the hot plate, but not the tail withdrawal test. In contrast, antinociception produced by the partial agonist nalbuphine in the tail-withdrawal test was significantly attenuated (~10-fold) in Gαo +/- mice. In whole brain homogenates from the Gαo +/- mice loss of half the Gαo protein resulted in a significant decrease in high-affinity MOR expression, with no change in total MOR. In contrast, in the spinal cord, even with such a significant loss of Gαo, high-affinity MOR binding was retained. Together, these findings suggest that MOR-Gαo coupling in the spinal cord may be more efficient than in the brain. Supported by DA007267 (JTL) and DA04087 (JRT).

# Poster Communications

## 25

**Regulation of DOR and DOR-KOR heteromer signaling in peripheral sensory neurons**

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In normal (uninflamed) tissue, opioid receptors, such as delta (DOR), kappa (KOR), and mu (MOR) are functionally quiescent in that they do not inhibit adenylyl cyclase activity or neuropeptide release and do not elicit an analgesic response. However, brief exposure to the inflammatory mediator, bradykinin (BK), induces functional competence. Although opioid receptors can regulate multiple signaling pathways in cells, it is unknown whether other signaling pathways, such as Extracellular Signal-Regulated Kinase (ERK) need BK pretreatment for the opioid receptor system to become functionally active. This study determined whether DOR and DOR-KOR heteromers require BK pretreatment to activate ERK in sensory neurons from rat trigeminal ganglion. Cells were pretreated with BK (10 μM, 15 min) followed by incubation with maximal concentrations of either the DOR selective agonist (DPDPE, 100 nM) or the DOR-KOR heteromer selective agonist (6'GNTI, 1 μM). As previously reported, DOR-mediated inhibition of adenylyl cyclase activity required BK pre-treatment and was pertussis toxin (PTx)-sensitive. DPDPE-stimulated ERK activity was also PTx sensitive, however, it was not dependent upon BK pretreatment. Interestingly, DPDPE-mediated ERK activation became insensitive to PTx following BK pretreatment. In contrast to the effects on cAMP accumulation, ERK activation by the heteromer selective ligand, 6'-GNTI, was not dependent on BK pretreatment. Further, in contrast to activation of DOR alone, activation of ERK by the DOR-KOR heteromer was insensitive to PTx. These data suggest that 1) not all opioid receptor signaling pathways (i.e. ERK activation) require BK pretreatment to induce functional competence and 2) mechanisms underlying DOR–mediated ERK activation appear to differ from that of the DOR-KOR heteromer. Understanding mechanisms by which opioid receptors on peripheral sensory neurons are regulated could provide new approaches for the pharmacological treatment of pain. *Supported by DA026619 and DA024865*

## 26

**PPARγ as a Therapeutic Target in Drug Abuse**

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Psychostimulant abuse, addiction, and relapse during abstinence remains a confounding public health issue in the United States and safe, effective pharmacotherapies are still needed for treatment. Here we explore a novel therapeutic target, peroxisome proliferator-activated receptor (PPAR), using a preclinical model of addiction in vivo. This ligand activated transcription factor belongs to the nuclear receptor family and its gamma isoform (PPARγ) plays a vital role as a primary lipid sensor and regulator of lipid metabolism. Thus, there are several FDA approved ligands that are clinically used for the treatment of diseases such as type 2 diabetes. However PPARγ is also widely distributed in the CNS and is highly expressed in neurons. Our lab has already demonstrated that PPARγ rescues hippocampal cognitive impairment in an animal model of Alzheimer's. This rescue partly involves the recruitment of hippocampal ERK MAPK activity to the nucleus (Rodriguez et al., 2010). Given the important role for learning and memory in the process for which drug abuse transitions into addiction, and our recent evidence that neuronal PPARγ is involved in restoring cognitive deficits through ERK MAPK, we hypothesize that neuronal PPARγ represents a potential therapeutic target for maintaining drug abstinence during stimulant withdrawal.

## 27

**Developmental differences in nicotine withdrawal are mediated via enhanced excitatory and reduced inhibitory mechanisms that regulate dopamine transmission in the mesolimbic pathway.**

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The mechanisms that mediate developmental differences in nicotine withdrawal appear to involve dopamine transmission in the mesolimbic pathway. This is based on our finding that nicotine withdrawal produces a lower decrease in extracellular levels of dopamine in the terminal region of the nucleus accumbens (NAcc) in adolescent versus adult rats. It has been suggested that during adolescence, inhibitory gamma-aminobutyric acid (GABA) systems that modulate dopamine are not fully developed, whereas excitatory glutamate systems are overdeveloped. This study examined whether changes in NAcc dopamine are mediated via inhibitory GABA and/or excitatory glutamate in the dopamine cell body region of the ventral tegmental area (VTA). Male adolescent and adult rats were prepared with subcutaneous pumps that delivered nicotine for 13 days. We then monitored changes in extracellular levels of the amino acids in the VTA following administration of the nicotinic-receptor antagonist mecamylamine to precipitate withdrawal. The results revealed that adults exhibited a 38% maximal increase in VTA GABA and a 44% decrease in glutamate. However, adolescents did not display changes in VTA GABA and only a 10% maximal decrease in glutamate. These results provide a potential mechanism involving VTA amino acid neurotransmission that mediates developmental differences in nicotine withdrawal. Specifically, they suggest that reduced inhibition and enhanced excitation of the dopamine cell bodies in the VTA contribute to adolescent insensitivity to nicotine withdrawal. This research was supported by NIDA Grants (R01DA021274; LEO and F31DA021133; LAN) and the APA-Diversity Program in Neuroscience (T32MH018882-20; LAN).

## 28

**Neonatal Pain and Stress Alters Sensitivity to the Discriminative Stimulus Effects of Morphine in Rats**

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Neonatal pain and stress (NPS) is a severe problem that affects up to 15% of newborns born in the United States. Changes in brain opioid systems have been demonstrated in response to NPS, but few studies have examined the impact of these changes on opioid pharmacology into adulthood. In these pilot studies, we were interested in examining the effects of NPS on the discriminative stimulus effects of opioids. We studied two groups of adult male rats: the first group had undergone a colorectal distension procedure on PND's 4, 6, and 8 to induce NPS, while the second group was a control group that was not exposed to this procedure. Rats were initially shaped to lever-press under an FR1 reinforced by food presentation, then gradually shaped to a terminal FR20. No learning or performance decrements were evident as a function of NPS. Discrimination training established morphine (1.5 mg/kg, SC) and saline as cues to designate which of the two levers would be reinforced in a given session. Once discriminative responding was stable and reliable, dose effect functions for morphine were acquired in both groups of rats, and repeated in the presence of the non-selective opioid antagonist naltrexone (0.1 mg/kg, SC). Interestingly, the NPS rats discriminated lower doses of morphine than the controls, and the antagonist effects of naltrexone against the interoceptive effects of morphine were less pronounced in NPS rats. These findings demonstrated that the changes in brain opioid systems elicited by NPS have persistent pharmacological consequences into adulthood, and may suggest that NPS increases sensitivity to abuse-related effects of opioids across development.

# Poster Communications

## 29

**Cocaine Activates ER Stress in the Striatum of Rats.**
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Previous research has shown that endoplasmic reticulum(ER) stress proteins, ATF3 and ATF4, are induced in the striatum by amphetamine. It was also shown that viral vector induction of these proteins in the striatum increases sensitivity to amphetamine in a locomotor activity paradigm. However, what is not known is whether drugs of abuse induce other ER stress genes or just ATF3 and ATF4 in the striatum. Rats were subjected to acute or repeated cocaine administration, sacrificed, and brain tissue was collected and processed for protein or mRNA. ER stress gene expression was measured using Western blots and quantitative real time PCR and the acute and repeated groups were compared to saline-injected control groups. Acute cocaine administration activated all three ER stress pathways (ATF6, PERK, and IRE1a) in the rat striatum via inductions of Bip, CHOP, GADD34, ATF6, and XBP1 mRNA. However, repeated cocaine administration activated only the IRE1a pathway via induction of Bip and XBP1 mRNA. These results suggest that cocaine induces ER stress pathways in the striatum of rats at sub-toxic doses and that the ER stress pathways may be targets for novel addiction therapies.

## 30

**Kappa opioid receptor (KOR) signaling on peripheral sensory neurons in vitro and in vivo.**
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Considerable interest has developed in understanding the regulation of peripheral opioid receptors to avoid central nervous system-mediated side effects of opioid pharmacotherapy. However, little is known about peripherally restricted KOR-mediated analgesic responses. Here we studied KOR agonist-signaling responses in primary cultures of sensory neurons and in a rat model of thermal allodynia. The KOR agonist, U50488, did not inhibit PGE2-stimulated adenylyl cyclase (AC) activity in vitro nor PGE2-induced thermal allodynia in vivo unless cells/tissue were pretreated with the inflammatory mediator bradykinin (BK). In contrast, U50488 stimulated ERK activity without BK pretreatment, which suggests that not all KOR-mediated responses require pre-exposure to an inflammatory mediator. Interestingly, the duration of U50488-induced anti-allodynia was inversely related to dose, suggesting that the KOR system desensitizes or that KOR activation may initiate both pro- and anti-nociceptive pathways. These results indicate that KOR signaling to AC and ERK is differentially regulated in peripheral sensory neurons. A better understanding of how KOR are regulated on peripheral sensory neurons may provide for improved approaches for the treatment of pain that are devoid of CNS adverse effects.
Supported by DA026619.

## 31

**Cannabinoids as Peripheral Analgesics**
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Peripherally active but centrally inactive analgesics have great clinical implication as they can prevent centrally-mediated side effects. Cannabinoids are a class of novel analgesics known to activate TRPV1 and TRPA1 channels that are involved in detecting peripheral pain including orofacial pain. Both TRP channels are expressed on a major class of nociceptors. Our recent data demonstrate that cannabinoid, arachidonyl-2-choloroethylamide (ACEA) activates TRPV1 while WIN-55,212 (WIN) and AM1241 activate TRPA1 (Akopian et al., 2008). TRPV1 is also activated by capsaicin (CAP) while TRPA1 is activated by mustard oil (MO). We have previously shown that pretreatment of rat skin biopsies with WIN significantly inhibits CAP-evoked CGRP release (Patwardhan et al., 2006). Objectives were to evaluate peripheral effects of cannabinoids via TRPV1 and TRPA1 in vivo.
**Methods**: 1) The effect of ACEA pretreatment on MO-evoked CGRP release from rat hindpaw skin; 2) The effect of a peripheral dose of ACEA pre-injection on MO-induced nocifensive behavior (intraplantar injections/ grooming and flinching assay) and 3) The effect of a peripheral dose of WIN and AM1241 on CAP-induced nocifensive behavior. Data were analyzed using ANOVA.
**Results**: ACEA(100 μM) significantly inhibited MO (0.1%)-evoked CGRP release from skin terminals and this effect was reversed by a TRPV1-selective antagonist, capsazepine(CPZ; 100 μM). ACEA (100 μg) significantly inhibited MO (0.1%)-induced nocifensive behavior in WT mice and this effect was fully abolished in TRPV1-/- mice. Moreover, ACEA when injected into the contralateral paw did not inhibit MO-induced nocifensive behavior in WT mice indicating a local site of action. Also, WIN (2.5 μg) and AM1241(40 μg) significantly inhibited CAP (0.5 μg)-induced nocifensive behavior in WT mice. This effect was completely reversed in TRPA1-/- mice. WIN and AM1241, when injected into the contralateral paw did not inhibit CAP-induced nocifensive behavior suggesting a local site of action. **Conclusions**: Overall, these studies provide insight into the potential mechanisms by which cannabinoids mediate peripheral anti-nociceptive effects via TRP channels.

## 32

**The kinetics of priming-induced functional competence of delta opioid receptors**
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Unreliable opioid receptor analgesia in the periphery represents a major challenge in pain management. However, analgesic effects of opioids are enhanced in inflamed tissues, providing insight into how opioid efficacy may be improved via ancillary pathways. Consistent with these findings, our lab has shown that prior administration of the inflammatory mediator, bradykinin (BK), promotes delta opioid receptor (DOR)-mediated effects in a primary culture of trigeminal ganglion (TG) and a behavioral model of pain. To further characterize the kinetics of this interaction, we measured the ability of a delta agonist, DPDPE, to reduce prostaglandin E(2) (PGE2)-mediated responses across a range of BK pre-treatments (0-90 min). In behavioral experiments, rats received hindpaw injections of VEH or BK prior to co-administration of DPDPE and PGE2. With BK priming, DPDPE attenuated PGE2-induced thermal allodynia, but only following 15-30 min BK pretreatments. Subsequent studies in TG cultures mimicked the in vivo effects, with DPDPE producing a significant reduction in PGE2-stimulated cAMP accumulation when primed for 15-30 min, but not when BK was applied for 60 min. This time-dependent loss of competence may reflect BK receptor desensitization and future studies will address the mechanisms underlying these effects with the intention of identifying potential adjuvants that may promote persistent peripheral opioid receptor analgesia.

# Poster Communications

## 33

**Phenylpropyloxyethylamines: Opioids lacking a tyrosine mimetic**

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14-Phenylpropyloxymorphinans are agonists that exhibit extreme potency at mu receptors, suggesting that the 14-phenylpropyloxy group has a major effect on receptor binding and is responsible for the dramatic increase in potency. Our hypothesis is that both a basic amine and a phenylpropyloxy group alone are required for opioid activity, and the aromatic A-ring, that was historically considered essential, is not required. By removing the A-ring, this allows the skeleton to adopt an alternate binding mode with the receptor interacting with different residues, thereby potentially causing alternate receptor trafficking events and post-receptor mechanisms, all of which are involved in the development of tolerance. During initial studies, conformationally sampled pharmacophore approach was utilized to confirm that the aromatic moiety in the novel series does not mimic the A-ring. In order to further substantiate our hypothesis, a series of phenylpropyloxyethylamines and cinnamyloxyethylamines were synthesized, and analyzed for opioid receptor binding affinity. Opioid binding studies showed that the optimal N-substituent is the N-phenethyl analog, 2-(cinnamyloxy)-N-methyl-N-phenethylethanamine which has an affinity of 1680 nM for mu-opioid receptors. Subsequently, rings B, C, and D from the morphine skeleton were systematically re-introduced as ring-constrained analogs. Binding studies showed that the B-ring analog containing N,N-dimethyl substituent produced the highest affinity of 2340 nM, while the C and D-ring analogs were fully inactive. However, upon introduction of an indole group into the C-ring analog, N,N-dimethyl-1-(3-(3-phenylpropoxy)- 2,3,4,9-tetrahydro-1H-carbazol-3-yl)methanamine, the affinity was increased to 1110 nM. Furthermore, by combining the B-ring with the optimal N-substituent, phenethyl, we were able to achieve 726 nM (same as codeine) affinity at mu indicating that cis-N-methyl-N-phenethyl-2-(3-phenylpropoxy)cyclohexanamine is a viable lead compound for optimization studies.

## 34

**Peptide disruption of the serotonin (5-HT) 5-HT2C receptor interaction with protein phosphatase and tensin (PTEN) is functionally important to the 5-HT2CR signalosome**

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Central 5-HT2CR plays an important role in psychological disorders marked by impulsive-compulsive traits (e.g., addiction, eating disorders), and strategies to augment 5-HT2CR signaling may prove therapeutically useful in such disorders. We sought here to detail the structural and functional significance of the protein:protein interaction between 5-HT2CR and PTEN, and to test the hypothesis that a small peptide fragment of the 5-HT2CR (3L4F; Pro283-Arg297) will compete with the PTEN for binding to 5-HT2CR and enhance 5-HT2CR signaling. 5-HT2CR function was established in agonist- stimulated mobilization of intracellular calcium (Cai++) in CHO cells stably expressing the 5-HT2CR. We also developed a novel split luciferase complementation assay (LCA) to validate that the 5-HT2CR and PTEN proteins are in direct contact in live cells. The 3L4F peptide enhanced 5-HT-stimulated intracellular Cai++ efflux in a concentration-related manner. The LCA detected a direct interaction between 5-HT2CR:PTEN, and preliminary studies suggest that the 3L4F peptide impairs production of the luminescence emitted upon formation of the 5-HT2CR:PTEN complex. These data suggest that the 5-HT2CR:PTEN complex is essential to the efficiency of 5-HT2CR signaling. Molecules that inhibit the 5-HT2CR:PTEN association will be novel pharmacological tools to enhance 5-HT2CR function and may prove therapeutically promising in eating and addictive disorders in which disrupted 5-HT2CR signaling is implicated.
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## 35

**Identification of novel peptide modulators of glycine and $GABA_A$ receptors using phage display**

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Ethanol is a widely-used drug of abuse with a complex, poorly understood neurochemical mechanism of action. It has actions on a wide variety of biochemical targets, among them a number of ion channels and enzymes. However, the relevance of each target to the expression of ethanol-induced behaviors remains unknown. Separating the individual contributions of alcohol effects on various targets will allow for a better understanding of ethanol effects and for the rational development of novel therapies for alcohol abuse. One way to accomplish this is to mimic, or alternatively antagonize, the effects of alcohol on individual targets to determine the behavioral consequences of alcohol actions at each target. One method for identifying compounds that act selectively at specific putative alcohol targets is phage display. Phage display is a cell-based selection system that enables the identification of small peptides binding with high affinities to a single target. This approach has been used to identify novel peptides that bind to proteases, cell-specific peptides, and specific neuronal cell types. In addition to identifying binding motifs, phage display has yielded peptides that not only show affinity for specific targets, but also have modulatory effects on function. Combining phage display selection with a functional characterization technique, whole-cell voltage clamp electrophysiology, allowed us to identify highly specific peptides capable of functionally modulating the selected targets. Our project focuses on identifying novel peptide modulators of two major ethanol targets: glycine and GABAA receptors. Both receptors are involved in inhibitory neurotransmission and are thought to mediate some of the behavioral effects of ethanol. Phage display was used to identify peptides that bind to specific subtypes of glycine and GABAA receptors. Use of a negative selection protocol ensured high specificity of binding. Peptides selected from these screens were then studied for their functional effects on the respective receptor subtypes and also tested on closely-related channels to determine their specificities. Our findings suggest that phage display can be successfully implemented to identify subtype-specific modulators for ligand-gated ion channels.

## 36

**Acupuncture attenuates stress-induced relapse to cocaine-seeking behavior**

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We have previously demonstrated that acupuncture suppresses the enhancement of dopamine release in the nucleus accumbens induced by drugs of abuse, including cocaine. It has been suggested that the nucleus accumbens is an important part of the anatomical circuitry underlying stress-induced reinstatement of drug-seeking. The aim of this study was to evaluate the effects of acupuncture on footshock-induced reinstatement of cocaine-seeking behavior and gene expression in the nucleus accumbens during footshock-induced reinstatement. Male Sprague-Dawley rats were trained to self-administer cocaine (1.0 mg/kg) intravenously on fixed ratio 5 for 14 d. After a 5 d period in the home cage, each rat experienced an extinction session followed by footshock stress (15 min exposure to intermittent electric shock, 0.5 mA). Acupuncture was applied at bilateral Shenmen (HT7) or Yangxi (LI5) points for 1 min after the 15-min period in which footshock was delivered. Expression of c-fos and activation of phosphorylated cAMP response element-binding protein (pCREB) in the nucleus accumbens were measured using different groups of rats using the same footshock challenge design. Results showed that acute stress reinstated cocaine-seeking behavior and enhanced c-fos expression and pCREB activation in the nucleus accumbens in rats trained to self-administer cocaine. Most importantly, these results showed that acupuncture at HT7, but not at control point (LI5), significantly reduced footshock-induced reinstatement of cocaine-seeking behavior and footshock-induced c-fos expression and pCREB activation in the nucleus accumbens in rats that previously self-administer cocaine, suggesting that acupuncture can attenuate stress-induced relapse by regulating neuronal activation within the nucleus accumbens.

# Poster Communications

## 37

**A Novel Fluorescent Probe for the SERT Based on Citalopram**

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(+/-)-Citalopram (1-(3- (dimethylamino)propyl)-1- (4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, CIT) is a selective serotonin reuptake inhibitor (SSRI) that is used clinically to treat depression and anxiety. Previously, we explored a series of CIT-based analogues wherein halo, aryl and arylalkyl functional groups were introduced into the 4- or 5-positions of the CIT isobenzofuran ring (Zhang et al. 2010). These analogues were evaluated for binding at serotonin (SERT), dopamine (DAT) and norepinephrine (NET) transporters, in rat brain. Most of the analogues not only retained high binding affinities (Ki=1-40 nM) at SERT, but also were highly SERT selective over DAT and NET (>100-fold). These SAR results suggested that the 4- or 5-position of the CIT isobenzofuran ring was potentially suitable for functionalizing with a linked fluorophore. Hence, ZP229, a 5-aniline analogue, was chosen to serve as a template for our first SERT fluorescent ligand. The resulting Rhodamine-labeled compound, ZP455, has proven to be a useful tool for visualizing SERT in HEK 293 cells.

## 38

**Wheel running interacts with escalation of cocaine intake in adolescent and adult female rats**

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AIM: Concurrent access to an exercise wheel has been shown to reduce cocaine self-administration under maintenance conditions and to suppress cocaine-primed reinstatement in adult rats. In the present study, the effect of wheel running on the escalation of cocaine intake during long access (LgA) conditions was assessed. METHOD: Adolescent (N = 12) and adult (N = 13) female rats were allowed to acquire wheel running and establish a running baseline over 3 days. Rats then were catheterized and allowed to self-administer cocaine (0.4 mg/kg, iv) during 6-hr daily sessions for 16 days with concurrent access to either an unlocked or a locked wheel during each session. Subsequently, for 10 additional sessions, wheel access conditions during cocaine self-administration sessions were reversed (i.e., locked wheels became unlocked and vice versa). RESULTS: Preliminary results indicate that unlocked wheel access may be more effective at attenuating cocaine intake in adolescent compared to adult female rats. Concurrent access to an unlocked exercise wheel decreased responding for cocaine and attenuated escalation of cocaine intake irrespective of when access occurred. However, when the wheel was subsequently locked for groups with initial access to an unlocked wheel, cocaine intake increased. These effects were seen in both adolescents and adults but may be more pronounced in adolescents. CONCLUSIONS: Wheel running was sufficient to reduce cocaine intake during LgA conditions, but concurrent access to running was necessary. Rat models suggest that exercise has the potential to be a useful intervention to reduce cocaine-seeking behavior and may be more effective in adolescents than adults. Research supported by R01 DA003240, K05 DA015267 (MEC), P20 DA024196 (Kelvin O. Lim, director; MEC, co-director).

**Preparing Effective Oral Presentation Slides**

**adapted from http://www.sfn.org/am2011/index.aspx?pagename=resources_presentation#posters**

Clear Purpose - An effective image should have a main point and not be just a collection of available data. Central theme of the image should be readily identified.

Readily Understood - The main point should catch the attention of the audience immediately. Audience is not paying attention to the speaker when trying to figure out the image - minimize this.

Simple Format - With a simple, uncluttered format, the image is easy to design and directs audience attention to the main point.

Free of Nonessential Information - If information doesn't directly support the main point of the image, reserve this content for questions.

Digestible - Excess information can confuse the audience. With an average of seven images in a 10-minute paper, roughly one minute is available per image. Restrict information to what is extemporaneously explainable to the uninitiated in the allowed length of time - reading prepared text quickly is a poor substitute for editing.

Unified - An image is most effective when information is organized around a single central theme and tells a unified story.

Graphic Format – Use graphs to emphasize qualitative relationships "Drug X dose-dependently and markedly increased behavior". Avoid presenting data in Tables.

Designed for the Current Oral Paper – Avoid extraneous information; show evidence and conclusions directly related to the subject of the paper; it is not necessary to communicate how much work was done.

Experimental - In a 15-min presentation, there is not enough time to teach methods. Only mention what is necessary to develop the theme.

Visual Contrast - Contrasts in brightness and tone between illustrations and backgrounds improves legibility. The best color combinations include white letters on black or black on yellow. Never use black letters on a dark background. Many people are red/green color blind - avoid using red and green next to each other.

Integrated with Verbal Text - Images should support the verbal text and not merely display numbers. Conversely, verbal text should lay a proper foundation for each image. As each image is shown, give the audience a brief opportunity to become oriented before proceeding.

Clear Train of Thought - Ideas developed in the paper and supported by the images should flow smoothly in a logical sequence, without wandering to irrelevant asides or bogging down in detail. Everything presented verbally or visually should have a clear role supporting the paper's central thesis.

If using PowerPoint, consider the following:

Use standard fonts, such as Times, Helvetica, or Arial and Symbol. Space is lost and the amount of information per slide is reduced by repeating graphics (including logos), busy backgrounds, and decorative typefaces.

Enhance the legibility of text and diagrams by maintaining color and intensity contrast. Use white or light yellow text and lines on black backgrounds, and/or use black on white or clear backgrounds. Avoid using colors that do not provide enough contrast red or dark green on blue, and avoid yellow on white.

Test your completed presentation on a separate PC-compatible computer to ensure that fonts are standard and components, such as movies, have been included rather than merely linked.

**Preparing Effective Posters**

An effective poster is self-contained and self-explanatory. Viewers can proceed on their own while leaving the author free to discuss points raised in inquiry.

The poster session offers a more intimate forum for discussion than a slide-based presentation, but discussion becomes difficult if the author must explain the poster to a succession of viewers. Time spent at a poster presentation is not determined by the author, but by the viewer – be prepared for 3 min or less.

An effective poster balances figures and text and is not a page-by-page printout of a journal paper or a slide show. Minimize text! Put yourself in the viewers shoes – how much text are you willing to read?

Layout - Organize illustrations and text using a grid plan. Arrange materials in columns rather than rows. Place the most significant findings at eye level immediately below the title bar; place supporting data and/or text in the lower panels. Use line borders to separate areas. Avoid reflective, plastic-coated paper. Use muted background colors - shades of gray are also effective.

Title - Title, author(s), and affiliation should be at least one-inch high.

Illustrations - design figures for viewing from a distance and use clear, visible graphics and large type. Colors are effective if used sparingly; use dark colors on white or pale backgrounds and light colors on dark backgrounds. Figures should illustrate no more than one or two major points. However, simple figures are unnecessary. Make clear main points. Illustration sequences can be specified with numbers or letters. Omit "Fig." or "Figure" - this is unnecessary and occupies excess space.

Text - Each figure or table should have a heading of one or two lines in very large type stating the "take-home" message. Provide additional essential information in the figure itself set in 16 point or larger type. Minimize narrative. Integrate text that would normally appear in the body (Results and Discussion) of a manuscript in figure legends. Concisely describe not only the content of the figure, but also the derived conclusions. Place brief details of methodology at the end of each legend. Numbered or bulleted lists are effective ways to convey a series of points, even for Introduction and Discussion. Do not set entire paragraphs in uppercase (all capitals) or boldface type.

Place an introduction at the upper left and a conclusion at the lower right, both in large type. The abstract should not be included.

# BBC Judge's Evaluation Form

**Presentation number/Presenter:** _____

Please assign points for each section and an overall score - **(5) Strong** to **(1) Weak**

| | | STRENGTHS | POINTS | WEAKNESSES |
|---|---|---|---|---|
| ABSTRACT | **Text:** Logical? Clear? Well-organized? Complete? Sufficiently succinct? | | | |
| PRESENATION CONTENT | **Introduction:** Objectives/background clear? Appropriate rationale? | | | |
| | **Methods and Results:** Appropriate design? Appropriate detail? Clearly explained? | | | |
| | **Conclusions:** More than summary? Supported by the data? | | | |
| STYLE/ORGANIZATION/VISUALS | **Style:** Clear voice? Volume? Appropriate inflection? Eye contact? Pace? Energy? | | | |
| | **Organization/Timing:** Logical sequence? Appropriate time for different sections? | | | |
| | **Visuals/Graphs:** Visible? Clear? Not cluttered? Used appropriately? See guidelines | | | |

**OVERALL SCORE (CIRCLE ONE):** **POINT TOTAL**

| | | | | | |
|---|---|---|---|---|---|
| **5 Outstanding** | **4 Excellent** | **3 Very good** | **2 Good** | **1 Fair** | |

# Notes

# Notes