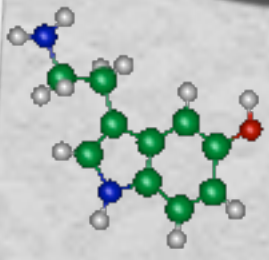
Behavior, Biology, and Chemistry:
Translational Research in Addiction

March 6-7, 2010

La Quinta Inn & Suites

Medical Center

San Antonio, TX



Acknowledgements

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* Committee Chairperson

Program Overview

Friday March 5, 2010

4:00 pm - 7:00 pm	Registration
7:30 pm - 10:00 pm	Opening Reception at Rio Rio on the San Antonio Riverwalk Buses depart from La Quinta at 7:00 PM

Saturday March 6, 2010

7:00 am - 5:00 pm	Registration
8:10 am - 8:15 am	Welcome and Opening Remarks
8:15 am - 10:35 am	Junior Investigator Oral Communications (Chair: Galen Wenger)
10:35 am - 11:00 am	Coffee Break
11:00 am - 12:10 am	Plenary Symposium (Part I): "D3 Receptors and Addiction" Speakers: Amy Newman, Bruce Jenkins (Chairs: Amy Newman and Wouter Koek)
12:15 pm - 1:45 pm	**Lunch**
1:45 pm - 2:55 pm	Plenary Symposium (Part II): "D3 Receptors and Addiction" Speakers: Robert Luedtke, Michael Nader (Chairs: Amy Newman and Wouter Koek)
3:00 pm - 4:00 pm	Special Lecture: Christian Heidbreder "Current perspectives on selective dopamine D3 receptor antagonists as pharmacotherapeutics for addictions and related disorders" (Chair: Amy Newman)
4:00 pm - 4:30 pm	Poster set-up
4:30 pm - 7:00 pm	Poster Session
7:00 pm - 9:00 pm	**Dinner** After Dinner Speaker: R. Adron Harris; "Small molecule seeks protein partners for meaningful relationship" (Chair: John Roache)
9:00 pm - 11:00 pm	Hospitality and Entertainment

Sunday March 7, 2010

8:00 am - 9:40 am	Open Oral Communications I (Chair: Thomas Prisinzano)
9:40 am - 9:55 am	Coffee Break
9:55 am - 11:15 am	Open Oral Communications II (Chair: Sandy Comer)
11:15 am - 11:30 am	Coffee Break
11:30 am - 12:30 pm	Special Lecture: F. Ivy Carroll "Development of selective kappa opioid receptor antagonists" (Chair: Andrew Coop)
12:30 pm - 1:30 pm	**Lunch** Presentation of travel awards and awards for oral and poster presentations Closing Remarks

See you at BBC 2011!

Program Details

Friday March 5, 2010 (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 6, 2010

Welcome and Opening Remarks (8:10 am - 8:15 am)

Junior Investigator Oral Communications (Chair: Galen Wenger)

8:15 am - 8:35 am	***Colin Cunningham***, University of Texas Health Science Center at San Antonio Behavioral effects of nicotine and varenicline: differences in nicotine acetylcholine receptor activation.
8:35 am- 8:55 am	***Brandi Blaylock***, Wake Forest University School of Medicine Effects of varenicline on the discriminative stimulus effects of nicotine in female cynomolgus monkeys.
8:55 am - 9:15 am	***Luis Natividad***, University of Texas at El Paso The rewarding effects of nicotine are enhanced in adolescent rats and adults that were pre-exposed to nicotine during adolescence.
9:15 am - 9:35 am	***Amy Eppolito***, University of Texas Health Science Center at San Antonio Effects of acute and chronic flunitrazepam on delay discounting in pigeons.
9:35 am - 9:55 am	***Adam Stewart***, Tulane University School of Medicine Repeated drug withdrawal paradigm in zebrafish, Danio rerio.
9:55 am - 10:15 am	***Michelle Baladi***, University of Texas Health Science Center, San Antonio Eating a high fat chow increases the sensitivity of rats to some behavioral effects of quinpirole.
10:15 am - 10:35 am	***Gregory Collins***, University of Michigan Response-maintaining effects of D2-like agonists in rats: Influence of operant history and conditioned reinforcement.

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

Saturday March 6, 2010 (continued)

Plenary Symposium (Chair: Amy Newman, Wouter Koek)

Dopamine D3 Receptors and Addiction

It is well documented that the mesolimbic DA system, and especially D1 and D2 receptors, is critically involved in drug reward and addiction. With the recent discovery of D3 receptors, a growing body of evidence strongly suggests that the D3 receptor is involved in addiction. There is now a critical need to understand the role of D3 receptor systems in the mechanisms of drug dependence and abuse and for development of new drugs as tools to study D3 receptor systems. This symposium will explore some of the recent data on D3 receptor localization and function in brain and discuss novel actions of drugs at D3 receptors.

Part I

11:00 pm - 11:35 am	***Amy H. Newman***; National Institute on Drug Abuse, Medicinal Chemistry Section Evolution of D3 receptor antagonists and partial agonists
11:35 am - 12:10 pm	**Bruce Jenkins**; Harvard Medical School/Massachusetts General Hospital phMRI of D3 receptors in rodents and primates

Lunch (12:15 pm - 1:45 pm)

Plenary Symposium (Chair: Amy Newman, Wouter Koek)

Dopamine D3 Receptors and Addiction
Part II

1:45 pm - 2:20 pm	***Robert R. Luedtke***; University of North Texas Health Science Center Functional selectivity of D3 dopamine receptor-selective ligands
2:20 pm - 2:55 pm	***Michael A. Nader***; Wake Forest University Interactions of cocaine history with the behavioral effects of D3 compounds in nonhuman primates

Special Lecture 3:00 pm - 4:00 pm (Chair: Amy Newman)

Christian Heidbreder: “Current perspectives on selective dopamine D3 receptor antagonists as pharmacotherapeutics for addictions and related disorders”

Reckitt Benckiser Pharmaceuticals

Saturday March 6, 2010 (continued)

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

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

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

5:30 pm - 6:30 pm Even numbered posters should be manned 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 for the best post-doctoral fellow poster presentation will be made.

Poster judging (for postdoctoral fellows and graduate students) will begin at 4:30 pm or 5:30 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 judge postdoctoral 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: John Roache)

R. Adron Harris: “Small molecule seeks protein partners for meaningful relationship”

University of Texas at Austin

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

Live music will be provided by the Tennessee Valley Authority (TVA). Come and enjoy the fun! In the ballroom at La Quinta.

Sunday March 7, 2010

Oral Communications I (Chair: Thomas Prisinzano)

8:00 am - 8:20 am	***Oscar Torres***, University of Texas at El Paso Nicotine withdrawal enhances anxiety-like behavior in female versus male rats.
8:20 am - 8:40 am	***Jonathan Pinkston***, University of Texas Health Science Center at San Antonio Adolescent-limited and life-persistent impulsive choice: studies with inbred mice.
8:40 am - 9:00 am	***Andrew Coop***, University of Maryland Phenylpropyloxyethylamines – a new class of opioids?
9:00 am - 9:20 am	***Martin Javors***, University of Texas Health Science Center, San Antonio Combination of breath carbon monoxide and saliva cotinine levels to estimate smoking.
9:20 am - 9:40 am	***Malliga Iyer***, National Institutes of Health Studies on racemic cis-benzofuro[2,3-c]pyridin-6-ols and cis-benzofuro[2,3-c]pyridin-8-ols: Probes for narcotic receptor mediated phenomena.

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

Oral Communications II (Chair: Sandy Comer)

9:55 am - 10:15 am	***Laura O'Dell***, The University of Texas at El Paso A psychobiological framework of the substrates that mediate enhanced tobacco abuse during adolescence.
10:15 am - 10:35 am	***Barry Setlow***, Texas A&M University Dopaminergic modulation of risky decision-making.
10:35 am - 10:55 am	***Allan Kalueff***, Tulane University School of Medicine Zebrafish behavioral and endocrine responses to drugs of abuse: LSD, MDMA, morphine, pentobarbital and benzodiazepines.
10:55 am - 11:15 am	***Claudia Miller***, University of Texas Health Science Center at San Antonio Do environmental exposures initiate and exacerbate addiction?

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

Special Lecture 11:30 am - 12:30 pm (Chair: Andrew Coop)

F. Ivy Carroll: "Development of selective kappa opioid receptor antagonists"

Research Triangle Institute

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

Presentation of awards for travel, oral, and poster presentations

Closing Remarks and Adjournment

See you at BBC 2011!

Abstracts

Oral Communications

1

Behavioral effects of nicotine and varenicline: differences in nicotine acetylcholine receptor activation.

Cunningham, Colin1; McMahon, Lance R1
1Department of Pharmacology, UTHSCSA, San Antonio, TX USA

Varenicline (Chantix®) is a recently approved pharmacotherapy that is reported to be more effective than nicotine in promoting smoking cessation. Pharmacologic mechanisms responsible for differences in clinical effectiveness are not well established. To examine differences in site of action and efficacy at nicotine acetylcholine receptors that could underlie differences in clinical effectiveness, nicotine and varenicline were compared in two behavioral assays in C57BL/6J mice. In mice responding under a fixed ratio 30 schedule of milk delivery, nicotine and varenicline dose-dependently reduced responding; nicotine was more potent than varenicline. The non-selective, non-competitive nicotine acetylcholine antagonist mecamylamine attenuated the effects of nicotine and varenicline to decrease responding, indicating that both drugs acted through mecamylamine (i.e. nicotine acetylcholine) sensitive receptors. In mice discriminating a relatively large dose (1.78 mg/kg s.c.) of nicotine from saline, nicotine and varenicline dose-dependently increased nicotine-appropriate responding; nicotine was again more potent than varenicline. There was striking consistency in the potency of nicotine to increase nicotine-appropriate responding among mice, whereas varenicline produced maximal increases in nicotine-appropriate responding at different doses among mice. When combined with nicotine, varenicline produced a leftward shift in the nicotine dose-response curve for not only discriminative stimulus effects, but also the effects of nicotine to decrease response rate. Collectively, these results suggest that varenicline acts through nicotine receptors to produce behavioral effects; however, differences in the pattern of discriminative stimulus effects among animals suggest that nicotine and varenicline diverge in their receptor mechanisms. Under the present experimental conditions, it appears that varenicline does not have lower nicotine agonist efficacy than nicotine. Alternatively, nicotine and varenicline could differ in their selectivity for nicotine receptor subtypes. Funded by USPHS grant DA25267.

2

Effects of varenicline on the discriminative stimulus effects of nicotine in female cynomolgus monkeys.

Brandi L. Blaylock1, Susan H. Nader1, Michael A. Nader1,2
Department of Physiology and Pharmacology1, Department of Radiology2
Wake Forest University School of Medicine, Winston-Salem, North Carolina

Nicotine (NIC) is a widely abused drug, primarily consumed through tobacco products. Varenicline (VAR), a nicotinic acetylcholine receptor partial agonist, is an FDA-approved pharmacotherapy for smoking cessation. Despite its clinical use, little preclinical research exists to understand how VAR reduces the abuse liability of NIC. Rodent studies have demonstrated that the nicotinic B2 subunit mediates dopamine release caused by NIC (Grady et al. 2001), which may also contribute to its' discriminative stimulus (Di Chiara and Imperato 1988, Corrigall et all 1994). VAR is a partial agonist at a4B2 nicotinic receptors. In rodent studies, VAR has been shown to decrease the reinforcing effects of NIC and fully (Rollema et al., 2007) or partially substitute (Ross et al., 2009) for nicotine. However, there are no published studies in nonhuman primates examining the effects of VAR on the discriminative stimulus effects of NIC. We investigated the effects of VAR (0.03-0.3 mg/kg, base) alone at several pretreatment times (10-120 min) and in combination with NIC (0.1-0.3 mg/kg, base) in a two-choice, food-reinforced, drug discrimination procedure in which ovariectomized female cynomolgus monkeys (n=4) were trained to discriminate 0.3 mg/kg NIC from saline (i.m., 10-min presession). Preliminary data suggest that VAR does not engender NIC-like discriminative stimulus effects, at any dose or pretreatment time, although it can have effects on response rates. Time course studies suggest that when administered 10 min and 60 min prior to NIC, VAR abolished responding and elicited emesis. When administered 120 min prior to the training dose of NIC, VAR (0.1-0.3 mg/kg) blocked the NIC-like discriminative stimulus effects without affecting response rates. These results suggest VAR synergistically interacts with NIC at early time points to potentiate the rate-decreasing effects of NIC, but antagonizes the discriminative stimulus effects of NIC at later time points. DA12460

3

The rewarding effects of nicotine are enhanced in adolescent rats and adults that were pre-exposed to nicotine during adolescence.

Natividad, Luis A., Torres, Oscar V., Escalante, Evelyn, O'Dell, Laura E.
Department of Psychology, University of Texas at El Paso

Much work has shown that adolescence is a unique period of development characterized by enhanced tobacco abuse that facilitates long-term susceptibility to tobacco dependence in adulthood. The goal of this study was to compare the rewarding effects of nicotine in adolescent and adult animals, and to determine whether exposure to nicotine during adolescence produces long-lasting changes in the rewarding effects of nicotine in adulthood. We used an extended-access model of nicotine self-administration (SA) whereby rats were given 23-hour access to increasing doses of nicotine separated by brief periods of nicotine abstinence. This model allowed us to compare nicotine intake in our treatment conditions across a range of nicotine doses and following repeated nicotine withdrawal. Adolescent and adult rats were implanted with a jugular catheter for nicotine SA. An additional group of adolescents were implanted with a subcutaneous pump that delivered nicotine for 14 days (4.2 mg/kg/day; base), and they received jugular catheters later in adulthood. All rats were given access to nicotine SA for 3 separate cycles that lasted 4 days, and during each cycle, rats received a higher dose of nicotine as follows: 0.03, 0.06, and 0.09 mg/kg (base)/0.1 ml infusion. Each 4-day cycle was separated by a forced period of abstinence where the rats were returned to their home cages for 3 days. Our results revealed that adolescents displayed higher nicotine intake relative to both adult groups. Also, pre-exposed adults displayed higher nicotine intake relative to adult rats that were not pre-exposed to nicotine during adolescence. Lastly, all groups of animals displayed enhanced responding for nicotine following each of the forced abstinence periods, and this effect was also higher in adolescent and pre-exposed adult animals. Taken together, our findings suggest that enhanced rewarding effects of nicotine during the adolescent period of development contribute to long-term vulnerability to tobacco dependence. This research was supported by the National Institute on Drug Abuse Grants (R01DA021274; LEO and F31DA021133; LAN) and the American Psychological Association- Diversity Program in Neuroscience (T32MH018882-20; LAN).

4

Effects of acute and chronic flunitrazepam on delay discounting in pigeons.

Eppolito, Amy K.1; France, Charles P.1,2; Gerak, Lisa R.1
Departments of Pharmacology1 and Psychiatry2, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Drug abusers tend to be more impulsive than nonusers on a variety of measures and there is growing evidence that impulsivity both contributes to and is a consequence of addiction. One aspect of impulsivity can be studied with delay discounting procedures in which subjects choose between an immediately available small reinforcer and a delayed larger reinforcer. Benzodiazepines are commonly abused especially in combination with illicit drugs; little is known about the effects of chronic benzodiazepine use on delay discounting. In this study, adult white Carneaux pigeons (n=6) responded for food in a delay discounting procedure in which delays were increased across the session comprising five 16-min cycles. On the first cycle no delays were imposed; beginning on the second cycle, a delay to the large reinforcer was introduced and thereafter doubled with each cycle. Pigeons responded predominantly for the large reinforcer (4.5 sec access to food) when there was no delay and switched responding to predominantly the small reinforcer (1 sec access) as the delay to the large reinforcer increased. Acute administration of the benzodiazepine flunitrazepam (0.32, 1.0 and 3.2 mg/kg) increased choice for the large reinforcer in spite of increasing delays; at the longest delay, 70% of responses were for the large reinforcer. Daily treatment with flunitrazepam resulted in tolerance to this effect such that, at the longest delay, the percent of responses for the large reinforcer decreased to 36%. When daily treatment ended, responding for the large reinforcer returned to control (15% of responses for the large reinforcer). Given that pigeons respond more for delayed reinforcers when treated with flunitrazepam, these data suggest that benzodiazepines might be effective in reducing impulsive behaviors. Because drug abusers discount delayed rewards more rapidly than nonusers, understanding how chronic drug use and withdrawal affect choice behavior could lead to improved treatments for addiction.

Oral Communications

5

Repeated drug withdrawal paradigm in zebrafish, Danio rerio

Stewart, Adam; Cachat, Jonathan; Goodspeed, Jason; Suciu, Chris; Roy, Sudipta; Wong, Keith; Gaikwad, Siddharth; Chung, Kyung Min; Elegante, Marco; Bartels, Brett; Elkhayat, Salem; Tien, David; Tan, Julia; Grimes, Chelsea; Denmark, Ashley; Gilder, Tom; Beeson, Esther; Wu, Nadine; DiLeo, John; Grossman, Leah; Frank, Kevin; Kalueff, Allan V.
Department of Pharmacology and Neuroscience Program, Tulane University School of Medicine, New Orleans, LA, USA

Zebrafish are emerging as a reliable and high-throughput animal model in neurobehavioral research. Recent studies suggest the potential of zebrafish as a model for drug reward and addiction. With their robust phenotypes, they are well suited for behavioral analysis in addiction and withdrawal research. One such method of behavioral analysis is the novel tank diving paradigm, which we used to explore zebrafish behavior in response to a series of repeated withdrawal experiments utilizing different pharmacological agents. After 1 week of chronic treatment, fish were placed into exposure tanks with fresh untreated water for 3 h at a time, twice per day for 1 week prior to testing. Our results show that repeated morphine and ethanol withdrawal induced a significant elevation in anxiety-related behaviors. The treated groups experienced a strong decrease in exploratory behavior (longer latency to, transitions to, and duration in the top of the tank), prolonged freezing, and increased erratic movements. These results are also of clinical relevance, especially in regards to alcoholism as it is a cyclical disease characterized by recurring periods of withdrawal and exposure. Our study further validates the merit of zebrafish in neurobehavioral research and reconfirms their utility for modeling drug withdrawal syndrome.

6

Eating a high fat chow increases the sensitivity of rats to some behavioral effects of quinpirole

Baladi, Michelle G1 and France, Charles P1,2
Departments of Pharmacology1 and Psychiatry2, University of Texas Health Science Center, San Antonio, TX, USA

Many drugs of abuse as well as drugs used clinically target dopamine systems and act either indirectly or directly at dopamine receptors. Dopamine D2 and D3 receptors are important drug targets and recent studies suggest that changes in food intake can markedly affect the behavioral effects of drugs acting at these receptors (e.g. quinpirole). The current study examined the impact of feeding condition on sensitivity to behavioral effects of quinpirole in rats with access to either a standard (5.7 % fat) or a high fat (34.3%) chow. When rats had access to a high fat chow, the quinpirole discrimination dose-response curve (mediated by D3 receptors) shifted leftward. In the same rats, both the ascending (mediated by D3 receptors) and descending (mediated by D2 receptors) limbs of the dose-response curve for quinpirole-induced yawning shifted leftward. These results indicate that access to a high fat chow increases sensitivity of both D2 and D3 receptors and furthermore suggests that eating high fat food might impact the clinical or abuse-related effects of drugs acting on dopamine systems.

7

Response-Maintaining Effects of D2-like Agonists in Rats: Influence of Operant History and Conditioned Reinforcement.

Collins, Gregory T and Woods, James H
Department of Pharmacology, University of Michigan, Ann Arbor, MI, 48019

D2-like agonists, such as quinpirole (QPRL), maintain responding in rats and monkeys, but are rarely abused in humans. The following studies were aimed at further elucidating the antecedents of QPRL-maintained responding in the rat, and examined the influence of operant history and conditioned reinforcement (cocaine-paired stimuli; CS) on the capacity of QPRL to maintain and/or induce responding. To determine whether rats would acquire a new response for QPRL rats were first trained to nosepoke for cocaine with substitutions of QPRL, cocaine, remifentanil, nicotine, or saline performed on a previously inactive lever and paired with a novel stimuli; nosepokes continued to result in CS presentation. Despite the rapid reallocation of responding from the nosepoke to the lever when cocaine or remifentanil were available, lever presses remained low, and nosepoking persisted when QPRL or nicotine were made contingent upon lever presses. To evaluate the influence of CS on the capacity of QPRL to maintain responding, substitutions were performed in the presence, or absence of injection-CS pairings. Although cocaine maintained similar rates of responding regardless of whether injections were accompanied by CS presentation, high rates of QPRL-maintained responding were only observed when CSs were paired with injections. Finally, to evaluate whether the response-contingent delivery of QPRL was necessary for QPRL to maintain responding, rats were pretreated with QPRL, cocaine, or saline and allowed to respond for the stimuli that were previously paired with either cocaine or food delivery. Similar to when QPRL was substituted for cocaine, pretreatment with QPRL resulted in dose-dependent increases in responding that were dependent upon CS presentation. Together these results suggest that the response-maintaining effects of QPRL are primarily mediated by a QPRL-induced enhancement of the conditioned reinforcing effects of previously cocaine-paired stimuli, and not a primary reinforcing effect of QPRL.

8

Nicotine withdrawal enhances anxiety-like behavior in female versus male rats.

Torres, Oscar V., Natividad, Luis A., Walker, Ellen M., Muñiz, Adrian K., and O'Dell, Laura E.
University of Texas at El Paso, Department of Psychology, El Paso, Texas 79968

Clinical reports have shown that during abstinence, female smokers display negative mood states, such as depression, anxiety, and craving that is higher relative to men. These negative mood states are believed to contribute to relapse behavior, and the possibility exists that intense stress produced by nicotine withdrawal may contribute to enhanced vulnerability to tobacco abuse in female versus male smokers. To address this issue, the present study compared anxiety-like behavior and plasma corticosterone levels (a blood maker of stress axis activation) in male and female adult rats experiencing nicotine withdrawal. To examine potential sex differences in nicotine metabolism, we also compared cotinine levels (a nicotine metabolite) in these animals. Rats were prepared with subcutaneous pumps that delivered either saline or nicotine (4.7 mg/kg/day). After 14 days of nicotine exposure, the pumps were removed to induce spontaneous withdrawal. Twenty-four hours later, anxiety-like behavior was assessed using elevated plus maze procedures. Immediately after behavioral testing, blood samples were collected and analyzed for corticosterone and cotinine levels using radio-immune assay procedures. Our results revealed that withdrawal produced a significant increase in anxiety-like behavior and plasma corticosterone levels in female rats experiencing withdrawal. However, these effects were reduced in male rats experiencing nicotine withdrawal. These behavioral findings do not appear to be related to sex differences in nicotine metabolism, since male and female rats displayed similar cotinine levels during nicotine withdrawal. Taken together, our data suggest that intense anxiety produced by withdrawal may contribute to enhanced susceptibility to tobacco abuse in female versus male smokers. Supported by the National Institute of Drug Abuse Grant R01DA021274.

Oral Communications

9

Adolescent-limited and life-persistent impulsive choice: studies with inbred mice.

Pinkston, JW, & Lamb, RJ
1Department of Psychiatry, University of Texas Health Science Center at San Antonio, San Antonio, TX, 2Department of Pharmacology, University of Texas Health Science Center at San Antonio

Impulsive choice reflects the preference for smaller, immediate rewards over larger, delayed rewards. In humans, impulsive choice is associated with increased risk of drug abuse, risky sexual practices, and other deviant behavior. In humans, impulsive choice generally peaks during adolescence and declines by middle adulthood. Developmental changes in impulsivity, however, have received little attention in the laboratory. In the present experiment, we explore a novel procedure for evaluating developmental changes in impulsive choice using a rapid discounting task. The discounting task consisted of two phases. In the first phase, mice could choose between a 0.02-ml drop of milk or a 0.1-ml drop of milk, each delivered after a 1-s delay. After 7 sessions, the second phase began. Across days, the delay to the large drop of milk increased from 1 to 100 s. Mice from two inbred strains, C57/BL6J and DBA/J2, were studied on the discounting task at 6 and 12 weeks of age, corresponding to adolescent and adulthood, using a between-groups design. At the end of the first phase, mice of both ages showed a preference for the large reward. As the delay to the large volume of milk increased, preference shifted to the smaller, immediate volume for all mice. How fast the function shifted, however, differed among strains and age groups. Adolescent mice of both strains showed similar shifts in preference, but adult mice showed differences in their responses to increasing delay. Specifically, adult DBA mice revealed a function similar to adolescent mice of both strains, but C57 mice were less sensitive to increases in delay (less impulsive) than adolescent mice or adult DBA mice. The results show that C57 mice show normative developmental changes in impulsive choice similar to what has been reported in humans, i.e., impulsive in adolescence but not in adulthood. Interestingly, DBA make more impulsive choices across development. The patterns shown by DBA mice may reflect "life-persistent" impulsivity reported in a subpopulation of humans. Implications for understanding impulse control disorders will be discussed.

10

Phenylpropyloxyethylamines – a new class of opioids?

Andrew Coop
University of Maryland

11

Combination of Breath Carbon Monoxide and Saliva Cotinine Levels to Estimate Smoking.

M.A. Javors (1,2), J.P. Hatch (1,3), and R.J. Lamb (1,2)
Departments of Psychiatry (1), Pharmacology (2), and Orthodontics (3), University of Texas Health Science Center, San Antonio, Texas 78229

The purpose of this analysis was to evaluate the combination of breath carbon monoxide (BCO) and saliva cotinine (sCOT) to verify reported smoking. Both measurements are convenient, quantitative, and do not require invasive procedures such as blood draws. BCO levels were measured with a handheld breathalyzer and saliva cotinine levels with a high sensitivity ELISA assay. Average daily cigarettes were reported at study entry (AveRCigs) and reported cigarettes smoked during the past day (RCigs24) were collected at all visits (0-5). Participants were 72 males and 62 females between the ages of 19 and 67 years. At study entry, they reported smoking at least 15 cigarettes a day, had a BCO level $\geq$ 15 ppm, and were seeking to stop smoking. The levels of sCOT and sCOT/AveRCigs levels were higher in females than males and increased with age for all smokers. Detectable sCOT levels persisted for up to 5 days of abstinence in some, but not all, subjects while BCO levels were below 3 ppm in 89% smokers with a single day of abstinence. sCOT levels correlated best with cigarettes smoked during the period of 24-96 h preceding the collection of saliva, while BCO levels correlated best with cigarettes smoked only during the past 24 h. We conclude that the combination of BCO and sCOT levels should be used to assess smoking levels and patterns of nicotine intake when possible when interested in both last day and last several days of smoking.

12

Studies on racemic cis-benzofuro[2,3-c]pyridin-6-ols and cis-benzofuro[2,3-c]pyridin-8-ols: Probes for narcotic receptor mediated phenomena

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Racemic cis-benzofuro[2,3-c]pyridin-6-ols with suitable substitution pattern on nitrogen have shown high affinity for opioid receptors. Benzofuro[2,3-c]pyridinols are partial structures of oxide-bridged phenylmorphans, where the hydroxy group meta to the phenylmorphan core is a necessary requisite for opioid activity. An approach to the synthesis of a series of novel cis-1,2,3,4,4a,9a- hexahydrobenzofuro[2,3-c]pyridin-8-ols was undertaken. A series of compounds bearing a different substitution pattern on the tertiary nitrogen atom were synthesized. These compounds were tested in competitive binding experiments with the mu, delta and kappa opioid receptors. (±) cis-4a-(4- fluorophenethyl)-2-methyl-1,2,3,4,4a,9a- hexahydrobenzofuro[2,3-c]pyridin-8-ol had the highest affinity for mu receptor with Ki 0.35 μM. In continuation of above goals, an improved route for the synthesis of cis-benzofuro[2,3-c]pyridin-6-ols and cis-benzofuro[2,3-c]pyridin-8-ols was developed. Binding studies on the newly synthesized (±) cis-2-methyl-4a-phenethyl-1,2,3,4,4a,9a- hexahydrobenzofuro[2,3-c]pyridin-6-ol showed that it had nanomolar affinity at mu and delta opioid receptors. A structure activity relationship on the hexahydrobenzofuro[2,3-c]pyridin-6-ol series of compounds is being explored.

Oral Communications

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A psychobiological framework of the substrates that mediate enhanced tobacco abuse during adolescence

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Clinical work suggests that adolescents are more likely to use tobacco products and this causes a greater risk of long-term tobacco abuse. Pre-clinical studies also have shown that there are fundamental differences in the mechanisms that drive nicotine abuse in adolescents and adults. There are several potential factors that influence developmental differences to nicotine use, including sex differences, environmental conditions, genetic background, social factors, and constituents of tobacco other than nicotine that may contribute to enhanced nicotine use during adolescence. This presentation will focus on pre-clinical rodent studies that have led to our hypothesis that enhanced tobacco abuse during adolescence is driven by two factors: 1) the positive effects of nicotine during adolescence are greater than in adults and 2) the negative effects associated with nicotine and withdrawal from this drug are substantially lower than those experienced by adults. The overall result is that adolescents seek nicotine because the enhanced positive effects they experience are inadequately balanced against minimal negative effects. Behavioral evidence from animal studies will compare the positive and negative effects of nicotine in adolescent and adult rats. A theoretical framework will also be presented to explain why adolescents are different from adults with regard to neurochemical mechanisms involving enhanced excitatory and underdeveloped inhibitory influences on dopamine transmission in the mesolimbic reward system. Lastly, the clinical implications of our hypothesis will suggest that the current diagnostic criteria for nicotine dependence that was developed for adults may be inappropriate for adolescents who experience less withdrawal, and as a result, may be less responsive to tobacco cessation treatments that focus on reducing withdrawal. This work was supported by NIH grant R01DA021274.

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Dopaminergic modulation of risky decision-making.

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People are faced with daily choices among competing alternatives, some of which are accompanied by adverse consequences. Most people are able to accurately assess the risks and rewards of such alternatives and decide adaptively; however, drug users often display maladaptive decision making, such that choices are biased toward risky options. This type of decision-making is commonly studied in laboratory tasks in humans; however, there have been few animal models that examine how risk of adverse consequences (punishment, as opposed to reward omission) influences decision-making. Our lab recently developed such a task, in which rats choose between small "safe" rewards and large rewards accompanied by varying risks of punishment. Here we report the results of studies using this task that were designed to determine how dopamine modulates risky decision-making.
Male Long-Evans rats were trained in the task in standard operant chambers, in which they were given choices between pressing one of two levers, one of which resulted in a small, "safe" reward (1 food pellet), and the other which resulted in a large (3 food pellets) "risky" reward. Choice of the large reward was accompanied by a risk of mild footshock, the probability of which increased over the course of a session in consecutive trial blocks (0, 25, 50, 75, 100%). Once stable performance was obtained, rats were given acute systemic injections of various dopaminergic agents, using a within-subjects design.
Amphetamine dose-dependently decreased preference for the large risky reward, an effect that was mimicked by a D2, but not D1, agonist, and attenuated by a D2, but not D1 antagonist. The antagonists had no effects on their own. In a separate group of rats, in situ hybridization was used to show that greater D2 mRNA expression in dorsal striatum was associated with greater risk aversion (decreased preference for the large risky reward). These results implicate striatal D2 receptors as playing a modulatory role in risky decision-making.

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Zebrafish behavioral and endocrine responses to drugs of abuse: LSD, MDMA, morphine, pentobarbital and benzodiazepines

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The zebrafish (Danio rerio) is rapidly becoming a popular model species in behavioral neuroscience research. Zebrafish behavior is robustly affected by environmental and pharmacological manipulations, which can be examined using exploration-based paradigms, paralleled by analysis of endocrine (cortisol) stress responses. As in humans and rodent models, exposure of zebrafish to psychotropic drugs evokes behavioral symptoms characterized by increased or decreased anxiety. Our examination of the effects of exposure to lysergic acid diethylamide (LSD; 70-250μg/L), 3,4-methylenedioxymethamphetamine (MDMA; 2.5-5mg/L), morphine (1-2mg/L), pentobarbital (5-20mg/L) chlordiazepoxide (10mg/L), and diazepam (30-150mg/L) in adult zebrafish demonstrated relationships between anxiogenic, anxiolytic, and sedative-like behavioral phenotypes, identified using the Novel Tank Diving Test and the Light-Dark Box Test, and increases or decreases in circulating cortisol levels, quantified via ELISA assay. Taken together, these findings suggest the existence of readily-identifiable endophenotypes of drug exposure in zebrafish, further validating the utility of this animal model in translational research of drug abuse.

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Do environmental exposures initiate and exacerbate addiction?

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Synthetic organic chemical production exploded following World War II. Exposures to these evolutionarily novel substances are now ubiquitous. A typical air sample taken indoors, where we spend 90% of our time, reveals hundreds of volatile organic compounds (VOCs), including pesticides, plasticizers, fragrances and cleaning agents. As these VOCs, mainly sourced from petrochemicals, increased, buildings were also "tightened" to increase energy efficiency, which decreased fresh air and created the "sick buildings" where millions now live and work. The resulting chronic low-level exposures have caused a subset of individuals to become ill with new multi-system symptoms and new sensitivities or intolerances. Individuals from more than a dozen industrialized nations report that everyday exposures that never bothered them before, including certain fragrances, engine exhaust, tobacco smoke and cleaning agents, as well as foods, medications, alcoholic beverages and caffeine, trigger multi-system symptoms lasting minutes to days. These include pronounced stimulatory and withdrawal symptoms to structurally diverse substances (reminiscent of cross-addiction). These observations violate the rules of toxicology and allergy—they are "compelling anomalies," Kuhn's term for observations that do not fit existing scientific paradigms and thus drive the search for new theories or explanations. We propose a new theory of disease, "Toxicant-induced loss of tolerance" or "TILT." TILT, the subject of a recent NIEHS/NIH meeting, is a fundamental breakdown in natural (versus acquired) tolerance resulting from environmental exposures. TILT appears to underlie certain cases of addiction and what has been termed "abdiction" (substance avoidance/aversion or chemical intolerance/sensitivity). Although avoidance and addiction are opposite behaviors, both successfully attenuate unpleasant withdrawal symptoms. The QEESI (Quick Environmental Exposure and Sensitivity Inventory), a published, validated instrument for clinicians and researchers, is currently used in several countries to assess chemical intolerance.

Abstracts

Poster Communications

1

Vulnerability to Addiction: Identifying Traits for Increased Risk

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Patterns of individual differences in novelty-seeking and impulsivity can be seen with respect to drugs of abuse such that individual sensitivity seems to determine the likelihood that drug use will escalate into dependence. We tested the hypothesis that the high novelty-seeking and high impulsive phenotypes are co-expressed in an individual Sprague-Dawley rat. Experimentally naïve male rats were initially placed in low-light open field monitors for 30 min and total horizontal activity was recorded to differentially identify novelty-seekers based on the degree of locomotor activation [high responder (HR) and low responder (LR), upper and lower 50% total horizontal activity, respectively]. HR/LR rats, maintained at ~90% free-feeding weight, were sequentially trained in the 1-choice serial reaction time task to nose-poke to receive food pellet rewards on a 5-sec inter-trial interval (ITI) schedule (16.6 ± 0.6 days to acquire task); responses during the ITI (premature responses) resulted in further delays of reward presentation and are indicative of impulsive action. Following stable performance (9.9 ± 0.7 days), rats were challenged in a session where the ITI was raised from 5 to 8 sec allowing us to detect impulsive action more easily as rats must now withhold responding for an extended period of time. The upper 25% and lower 25% were identified as high impulsive (HI) or low impulsive (LI), respectively; premature responses were significantly higher in HI vs. LI rats ($p<0.001$, t-test). HR rats were more likely to be HI (HR/HI) and LR rats were more likely to be LI (LR/LI), suggesting that novelty-seeking and impulsivity phenotypes are interrelated and may have similar neurochemical underpinnings. The HR/HI and LR/LI models established here provide an excellent opportunity to examine the factors that contribute to individual differences in the progression of addiction. Supported by: Jeane B. Kempner Scholarship, DA06511, DA024157, DA000403

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Gene transfer of cocaine hydrolase blocks cocaine seeking in an animal model of relapse.

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Cocaine dependence is a persistent and pervasive disorder characterized by high rates of relapse. In a previous study, direct administration of a quadruple mutant butyrylcholinesterase termed albumin-cocaine hydrolase acutely blocked cocaine seeking in an animal model of relapse and abolished cocaine-induced seizures and lethality. In the present experiment we extended these results to a gene transfer paradigm using an efficiently transduced though slightly less active quadruple mutant termed "AME". Our objective was to determine if a single iv delivery of helper-dependent vector encoding this enzyme would attenuate the reinstatement of cocaine-seeking behavior for a prolonged interval of time. Male rats were trained to self-administer 0.4 mg/kg cocaine under a fixed-ratio 1 (FR1) schedule of reinforcement and were allowed to self-administer iv cocaine for approximately 10 days. Immediately after the final cocaine self-administration session, rats were injected with saline or AME vector, and their cocaine solutions were replaced with saline. They were then allowed to extinguish lever pressing on the previously drug-paired lever for 14 days. Subsequently, they were tested for reinstatement responding during an 8-day reinstatement procedure based on ip priming injections of saline (S), cocaine (5, 10, and 15 mg/kg, C), and amphetamine (A) according to the following sequence: S, C, S, C, S, C, S, A. Cocaine priming injections were then given every 7 days for 4 weeks to assess the long-term effects of the viral vector on cocaine seeking. Results indicated that injection of the vector produced substantial and sustained cocaine hydrolase activity in plasma and diminished cocaine- (but not amphetamine-) induced reinstatement responding for 4 weeks following treatment (compared to saline-treated controls). These results demonstrate that viral transfer of cocaine hydrolase may be useful in treating relapse to cocaine addiction during extended periods of withdrawal.

3

Structure Activity Evaluation of the Role of the Carbonyl group of the Arylamide Moiety in D3 Receptor Selective Substituted Phenylpiperazines.

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It has been established that the dopaminergic system, within the nucleus accumbens, plays a key role in the CNS reward system. There is now experimental evidence to suggest that the D3 dopamine receptor subtype stimulation and/or regulation may play a pivotal role in the a) regulation of extracellular dopamine levels and b) reinforcing and drug seeking effects associated with the abuse of psychostimulants, such as cocaine. Although the D2 and the D3 dopamine receptor subtypes are structurally similar, we have identified D3 dopamine receptor selective agents with varying intrinsic activities. The present study was undertaken to a) more precisely define the pharmacophore that contributes to subtype binding selectivity for D3 dopamine receptors and b) potentially optimize bioavailability of our novel D3 dopamine receptor selective compounds. We have previously discovered novel substituted phenylpiperazines linked with an arylamide moiety that are highly selective for the D3 dopamine receptor. Several of these compounds (e.g. PG01037) have provided excellent tools for investigation in animal models of psychostimulant abuse. However, continued lead optimization is required for potential clinical utility. Hence, a series of substituted amine-analogues lacking the carbonyl group was prepared and evaluated for binding at D3 and D2 dopamine receptors. We discovered that compounds lacking the carbonyl group remained equiactive at D2 but displayed a >100-fold reduction in affinity for D3 dopamine receptors. This study supports a pivotal role for the carbonyl group and further reveals a point of separability between D3 and D2 dopamine receptors in this series of compounds.

4

Design and Synthesis of N-(3-Fluoro-4-(4-(2,3-dichloro or 2-methoxyphenyl) piparazine-1-yl)-butyl)-heterobiaryl and biaryl carboxamides as Selective Dopamine D3 Receptor Ligands

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The dopamine D3 receptor, a member of the dopamine D2-like receptor family, has become a target of intensive research over the past decade, due to its potential as a target for the development of medications for neuropsychiatric disorders and drug abuse. We have previously discovered novel and D3-selective ligands that are currently being used as in vivo tools for further investigation of the role that D3 receptors play in addiction. Based on structure-activity relationship studies in the 4-phenylpiparazine class of compounds, herein we report the design and synthesis of novel N-(3-Fluoro-4-(4-(2,3-dichloro or 2-methoxyphenyl)piparazine-1-yl)-butyl)-heterobiaryl and biaryl carboxamides demonstrating both high affinity and selectivity for D3 receptors. In this series, we have discovered some of the most D3-selective compounds reported to date, (e.g. BAK-02-66 and BAK-03-05 D3Ki = 6.1 nM and 4 nM, respectively and >1000-fold selective over the D2 receptor subtype.) The ease of synthesis and encouraging invitro profiles of these compounds make them promising new leads for in vivo investigation.

Poster Communications

5

Interactions between delta opioid receptor (DOR) and kappa opioid receptor (KOR) in peripheral sensory neurons.

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The delta opioid receptor (DOR) has become an attractive target for analgesic drug action in different pain paradigms. DOR agonists produce antinociceptive responses in acute and chronic models of pain in rats and may be effective in treating neuropathic pain. However, in general, the efficacy of DOR agonists (as compared to mu opioid receptor agonists) to promote analgesia is weak to moderate. An understanding of the mechanisms to increase peripheral opioid receptor function would be expected to lead to improved peripheral opioid therapy for the treatment of pain.

Here we show that co-administration of kappa opioid receptor (KOR) antagonists differentially alter the analgesic efficacy of DOR agonists both in vitro (in primary cultures of pain-sensing neurons) and in vivo (with peripherally-restricted opioid administration in a behavioral model of pain). In vitro, concentration curves for inhibition of PGE2-stimulated adenylyl cyclase for the DOR agonist, DPDPE, were shifted to the left 10-fold, whereas curves for the agonist DADLE were shifted to the right 20-fold and the response to agonist SNC80 was abolished in the presence of the KOR antagonist, nor-BNI. Further, in contrast to nor-BNI, DPDPE responses in the presence of the KOR antagonist 5'-GNTI were reduced dramatically. In vivo, we found a profound enhancement of the DPDPE response upon occupancy of KOR with nor-BNI, whereas occupancy of KOR with 5'-GNTI reduced the DPDPE response to produce thermal anti-allodynia following peripheral application of PGE2. In addition, the DOR-KOR heterodimer selective ligand, 6'-GNTI, produced a concentration dependent inhibition of PGE2-stimulated AC in vitro and reversal of PGE2-mediated thermal allodynia in vivo. We propose that KOR antagonists alter DOR agonist responses via allosteric interactions between protomers of DOR-KOR heterodimers. Conformational changes in KOR, due to ligand occupancy, lead to conformational changes in DOR, which lead to changes in agonist affinity and/or intrinsic efficacy. Supported by DA026619.

6

Effects of a monoamine oxidase inhibitor on the duration of action of N,N-dimethyltryptamine.

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N,N-dimethyltryptamine (DMT) is a compound that produces intense, short-lasting hallucinations that has been used for religious and recreation reasons for centuries. Due to the significant amount of monoamine oxidase (MAO) in the liver and gut, DMT is rapidly degraded in the body. This degradation renders DMT orally inactive, unless taken with a MAO inhibitor. Both DMT and potent MAO inhibitors are the components responsible for the psychotropic effects in the religious plant tea ayahuasca. In a drug discrimination paradigm, male Sprague-Dawley rats were trained to discriminate DMT (5 mg/kg, ip) from saline with a 5 minute pretreatment time. The time course was evaluated from 5 to 60 minutes. Tranylcypromine, a nonselective MAO inhibitor, was then administered with DMT to evaluate the difference in the duration of activity. Tranylcypromine was also tested alone for substitution for DMT. Tranylcypromine partially substituted in DMT trained animals, with the highest drug- appropriate responding (52%) at the 1 mg/kg dose. DMT produced a time-dependent decrease in drug-appropriate responding with 97% at 5 minutes and no effect (1%) by 60 minutes. When tranylcypromine (1 mg/kg) was administered with DMT, drug-appropriate responding remained about 100% for up to 30 minutes, followed by a time-dependent decrease for up to 120 minutes. At 120 minutes after administration of DMT, drug-appropriate responding was still 33%. Following intraperitoneal injection, the effects of DMT have a rapid onset and are gone by 60 minutes. When a MAO inhibitor, tranylcypromine, is given in combination with DMT, the duration of action is lengthened to 120 minutes. Tranylcypromine, when administered alone, did produce some DMT-like effects as evidenced by the partial substitution.

7

Chronic morphine impaired performance of rats in a modified attentional set-shifting test.

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Increased perseverative errors (PE, repeated responses to previously correct cues) are a key feature of clinical disorders with frontal lobe damage (e.g., schizophrenia and Alzheimer's disease, substance abuse). To mimic the Wisconsin Card Sorting Test that detects PE in humans, we modified Birrell & Brown's (2000) attentional set-shifting test to a three-choice set-shifting and perseveration test (SSPT). The SSPT utilizes successive sets of three cups that differ in both digging medium and odor. On each trial, only one cue (medium or odor) is paired with food. The rats learn to select the baited cup by either medium or odor, and to switch between cues in a series of discriminations: compound discrimination (CD), intradimensional (ID) shift, extradimensional (ED) shift. To reduce variables between and within subjects, we tested rats' preference for 21 odors, and identified three equally-preferred odors and an odor intensity that is recognizable but not aversive. We then used these three odors and the intensity to evaluate chronic effects of morphine on rats' (N=6-/group) SSPT performance. Chronic morphine (10 mg/kg, b.i.d, 14 days) seemed to have minimal effect on simple discrimination training and it did not affect performance in the first SSPT on Day7. However, it impaired performance in the second SSPT on Day14, specifically, increased perseverative errors in CD and trials to criterion in CD and ID1. Moreover, fewer rats finished each stage after 14-day morphine treatment. The results indicate that chronic morphine may impair ability to inhibit inappropriate behavior and ignore irrelevant cues or interference when acquiring a new strategy. Supported by TTU Research Enhancement Fund

8

Nicotine and varenicline share discriminative stimulus properties and act through mecamylamine-sensitive receptors in rhesus monkeys.

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Varenicline is reported to be more effective than nicotine in promoting smoking cessation. To examine pharmacologic factors (i.e., site of action and efficacy at nicotine acetylcholine receptors) that could underlie differences in the clinical effectiveness of these smoking cessation pharmacotherapies, these and other drugs were studied in rhesus monkeys (n=3) discriminating nicotine (1.78 mg/kg, s.c.) from saline. Nicotine and varenicline (ED50 = 0.50 mg/kg and 0.56 mg/kg respectively) produced ≥91% nicotine-lever responding. Cocaine produced a maximum of 50% nicotine-lever responding; however, other drugs that act through non-nicotine receptors (midazolam and ketamine) produced ≤4% nicotine-lever responding. The nicotine antagonist mecamylamine dose-dependently antagonized the effects of nicotine and varenicline. These data show that the nicotine discrimination assay is mediated by nicotine receptor agonism and, to some extent, monoamine agonism. The qualitatively similar effects of varenicline and nicotine and the similar antagonism of these agonists suggest that common, mecamylamine-sensitive receptors mediate the effects of both agonists. Under these conditions in vivo, there is no evidence that nicotine and varenicline differ in their nicotine agonist efficacy. Studies were funded by USPHS grant DA25267.

Poster Communications

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The rewarding effects of nicotine are enhanced in diabetic rats.

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Previous reports have suggested that diabetic patients are prone to using tobacco products. However, it is unclear whether diabetic persons experience enhanced rewarding effects of nicotine as compared to healthy controls. Thus, the goal of this study was to compare the rewarding effects of nicotine using self-administration procedures in diabetic rats displaying a blood glucose level of approximately 500 mg/dl as compared to control rats with normal glucose levels of 150 mg/dl. Male Wistar rats were first trained to perform operant responses for food and water in the chambers where they would receive 23-h access to nicotine. Following food and water training, the rats were given vehicle or various doses of streptozotocin (STZ), which is a drug that produces toxicity to pancreatic insulin- producing cells. Rats were then implanted with a jugular catheter and following a brief recovery period, were reintroduced to the self-administration chambers to assess baseline food and water levels. Rats were then given access to a lever that produced nicotine infusion on an FR1 schedule of reinforcement (0.03 mg/kg/0.1 ml infusion; base). Rats were allowed to self-administer this dose of nicotine for 10 days. Blood glucose levels and body weight were examined every day prior to the start of each session. The results revealed that STZ-treated rats displayed enhanced nicotine intake, as well as robust increases in food and water intake relative to controls. These findings suggest that diabetic animals display enhanced rewarding effects of nicotine. Taken further, our studies suggest that strong rewarding effects of nicotine may contribute to enhanced tobacco abuse in diabetic patients. Supported by the UTEP Biology Undergraduate Research Training Program (BURS).

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Assessing cognitive performance of "diabetic" and "normal" Tg2576 mice using multiple measures of learning and memory.

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Both the ability of an animal and the cognitive demands of a learning/memory task should be considered when assessing animal cognition. Tg2576 mice are a transgenic model used to study Alzheimer's disease (AD) and cognitive impairment, and in addition, diabetes has been demonstrated to be a risk factor for the development of AD. Therefore, we used multiple measures of learning and memory to assess our ability to detect cognitive deficits in Tg2576 mice and the exacerbated deficits in "diabetic" Tg2576 mice. Training/testing progressed across three measures of learning and memory beginning with the 8-arm radial arm maze (RAM), followed by the Morris watermaze (WM), and finally in the repeated acquisition of operant chains (Chaining) as described by Wenger et al., (2004). RAM revealed no sign of learning during ten weeks of training/testing and no performance differences between the "diabetic" and "normal" mice. In contrast, WM revealed differences between the groups after the first day of training/testing. "Diabetic" mice took longer (secs) and traveled farther (cm) to find the escape platform than "normal" mice. However, the two groups did not differ in swim speed (cm/sec) so impaired performance of "diabetic" mice cannot be attributed to motor dysfunction. Finally, mice were trained/tested in Chaining for 30 days. Briefly, Chaining requires mice to emit cued sequences of responses (chains) on three "nosepokes" (left, right, center) for reinforcement and a successfully emitted sequence triggers an increase in the chain length. Most diabetic (90%) and all normal (100%) mice emitted a single response chain. Only "diabetic" mice were impaired in emitting a two response chain (70%). Both groups were impaired at emitting a three response chain (60%) at the start of study. However, 90% of "normal" were successful after 30 days of training, while diabetic mice did not improve. A similar pattern was observed for a four response chain. Both groups were initially impaired (40%) but by the end of study 70% of "normal" mice could emit a four response chain while no improvement was observed in diabetic mice.

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Novel Drug Abuse Therapies through Derivatization of Salvinorin A

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The neoclerodane, Salvinorin A, is the main constituent isolated from the leaves of Salvia divinorum. Chewing of the leaves or smoking of the leaves of Salvia divinorum will produce hallucinogenic-like experiences. It is interesting that these effects seem to be different from that of the classic hallucinogen, LSD. Several neoclerodanes have been found to have opioid receptor activity in vivo and in vitro. Savinorin A had been found to be a selective and a potent kappa opioid receptor agonist. To explore further pharmacology of Savinorin A, a series of derivatives must be synthesized that display enhanced pharmacological properties such as increased duration of action and water solubility. This work will allow for the emergence and development of opioid receptor agonists and ligands as drug abuse therapies.

12

The busy rat: a new model of relapse

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Relapse is a defining characteristic of addiction. To better understand and reduce relapse, the reinstatement animal model has been widely used. In this procedure, drug self-administration is established and extinguished, usually in a different context. Subsequent exposure to stimuli that predict drug availability or coincide with drug delivery can elicit a resumption of responding for the drug, even in it's continued absence. This procedure has helped frame our understanding of relapse, but has some important limitations. Humans rarely reduce or eliminate drug use under extinction. Rather, in many cases, alternative events such as job or familial obligations require increased behavior allocation, supplanting the drug-seeking and consumption behaviors, even while drug is still accessable at the same cost. There is evidence that behavioral and biological mechanisms responsible for reducing behavior depend on the way in which it is suppressed, either by extinction or by providing an alternative. One example is induction – in animals, the amount of reinstated behavior is positively related to the duration of the extinction period. In contrast, in humans, the probability of relapse is inversely related to the duration of abstinence or reduced drug use. Neurobiological data also suggests that the influence of discrete brain regions such as the anterior cingulate cortex on behavior depends on whether the subject is exposed to extinction or a choice.

Poster Communications

13

Maze behavioral tests for zebrafish and other small teleosts.

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Behavioral tests involving zebrafish and other small teleost fish can be useful tools for pharmacology and toxicology screening. Drug treatments or chemical exposures affecting the mesolimbic system can alter fish emotional behavior. In this context, we utilize two different aquatic maze-based tests for fish: a novel environment based light-dark plus maze anxiety test, and a dichotomous choice T-maze associative learning task. We can measure the effects of acute or chronic compound exposures and dose-response relationships in these tests. In the light-dark plus maze, fish are introduced into the center of a plus shaped maze in which two side arms are lined with black and the other two white, and their movements are tracked for 5 min. With acute bath exposure to GABAA agonist chlordiazepoxide (5 mg/L), 0.5% ethanol, or 1 week dietary exposure to the cannabinoid agonist WIN 55,212 (1 ug/d), zebrafish spent more time in white arms and/or entered white arms more often than controls ($p < 0.05$, N=6-8). Nicotine (50 mg/L) exposure increased fish swimming activity as evidenced by more arm entries overall. Sub-chronic dietary exposures of zebrafish to the pesticides dieldrin or chlorpyrifos (10 ug/d) produced immobility and a trend toward less time spent in white arms ($p < 0.08$, N = 4-6). Baseline behavior in the light-dark plus maze is similar among zebrafish, goldfish and fathead minnows, and solvents can influence fish performance, so vehicle controls are essential. The associative learning task involves an extended training period (4 trials/day, 16 days) during which a food +/- stimulant reward is associated with a color choice through operant conditioning. In this task, with 5 min pre-exposure to caffeine (50 mg/L) food-restricted zebrafish acquired the association of a food reward with a color (+ green vs. - purple) sooner than controls in the first few training sessions. Future associative learning studies are planned to examine extinction of the association and reversal learning, as well as use of psychostimulant rewards. These robust tests based on stereotypical behavior in fish hold great potential for neuroscience and psychology research.

14

Acute administration of mirtazapine attenuates methamphetamine cue reactivity and cue-induced reinstatement in rats: a comparison of experimental techniques

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Extinction/reinstatement paradigms are a popular means to study the neurobiology of relapse during drug-withdrawal in self-administering laboratory rats. An alternative paradigm involves cue reactivity (CR) testing wherein rats lever press in the presence of drug-associated cues without undergoing extinction training. It is not known how these two paradigms compare, nor if the effectiveness of potential relapse reduction pharmacologicals would differ. To address both issues, we evaluated the functional efficacy of mirtazapine (mirt), an FDA-approved atypical antidepressant, in both CR and cue-induced reinstatement paradigms. To do so, rats were trained to self-administer methamphetamine (meth; 0.1mg/kg/0.1ml infusion) in a two lever operant chamber (FR1 days 1-7; FR5 days 8-14). Depressing the active lever resulted in meth infusion paired with a cue light and an in-house light indicating a time-out period; inactive lever responses had no consequence. Self-administering rats were divided into groups tested for CR and cue-induced reinstatement. During CR tests, rats lever pressed for 1hr in the presence of contingently presented cues and the absence of meth; rats were repeatedly tested with 2 consecutive days of self-administration between CR tests with mirt or its vehicle. For cue-induced reinstatement, a separate group of meth self-administering rats were extinguished to <20% of day 14 active lever responding (minimum of 10 days of extinction). A 15min pretreatment of mirt or vehicle was tested. For CR, vehicle, 0.5, 1.0, and 5.0mg/kg were randomly administered using a within subject design. For cue-induced reinstatement, a between subjects design with one dose/per rats was used. Rats readily reinstated lever pressing behavior when administered a vehicle pretreatment in either paradigm ($p<0.001$); 5.0mg/kg mirt attenuated both CR and cue-induced reinstatement by ~50% (vehicle vs. 5.0mg/kg; $p<0.01$). These studies highlight the overlapping nature of CR and cue-induced reinstatement protocols and reveal the potential of mirt as a relapse reduction pharmacotherapy.

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Synthesis of Nantenine enantiomers and their evaluation in a rate-suppression assay vs MDMA

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(+)-Nantenine, an aporphine alkaloid ex Nandina domestica, has been shown to antagonize behavioral and physiological effects of MDMA in mice. In our quest to decipher the role of various receptors in nantenine's anti-MDMA effects, (±)-nantenine was synthesized and its binding profile evaluated against a panel of CNS targets. To begin to understand the importance of the chiral center of nantenine with regards to its capacity to antagonize the effects of MDMA in vivo, (R)- and (S)-nantenine were prepared and evaluated in a food-reinforced operant task in rats. Pretreatment with either nantenine enantiomer (0.3 mg/kg ip) completely blocked the behavioral suppression induced upon administration of 3.0 mg/kg MDMA. (±)-Nantenine displayed high affinity and selectivity for the α1A adrenergic receptor suggesting that this α1 subtype may be significantly involved in the anti-MDMA effects of the enantiomers. Details of our synthetic and pharmacological experiments will be presented.

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Serotonin Uptake in Human Platelets and Lymphoblastoid Cells.

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The purpose of this study is to compare serotonin uptake into human platelets and lymphoblastoid (LB) cells as a function of the expression of the serotonin transporter. Experiments on platelets must be performed immediately after blood has been collected, while LB cells can be grown and cultured and experiments performed at the convenience of the investigator. This convenience will allow for greater flexibility and more efficient utilization of time if uptake in platelets and LB cells correlates at a high level. METHOD: Platelets were isolated from whole blood of human subjects and platelet uptake performed the same day. LB cells were isolated and immortalized, then frozen, grown in culture medium at a later time, and then uptake was measured when a sufficient number of LB cells had accumulated. Tritiated serotonin was used to detect uptake into platelets and LB cells. RESULTS: Our preliminary study indicates that (1) the measurement of serotonin uptake into human LB cells is feasible and that levels of maximal 5HT uptake in lymphoblastoid cells versus platelets among subjects appear to correlate. Km and Vmax of 5HT uptake was measured in LB cells using essentially the same procedure that we use for platelets. Km values for 5HT were similar in platelets (Km = 658 nM) and LB cells (Km = 1387 nM) for subject 2046. The platelet Km value of subject 2030 was also similar (890 nM). Platelet Vmax value for 2046 was significantly higher than the lymphoblastoid Vmax. The reason for this discrepancy is probably twofold: (1) fewer LB cells were used in each assay tube (106 for L cells versus 107 for platelets and (2) it may be that the level of expression of the 5HT transporter is lower in LB cells than platelets. Importantly, the Vmax of 5HT uptake into platelets and lymphoblastoid cells in a second human subject (2030) were both significantly lower than for subject 2046, suggesting that Vmax of uptake may correlate in the two cell types among human subjects. CONCLUSION: This result has led to the hypothesis that Vmax, but not Km, of 5HT uptake into platelets and LB cells correlates among all subjects. In summary, these results demonstrate that 5HT transporters are expressed in cultured LB cells and that the Km and Vmax of 5HT uptake is quantifiable, providing feasibility for the proposed experiments.

Poster Communications

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Blockade of adrenergic receptors during chronic unpredictable stress prevents the detrimental effects on cognitive flexibility in rats.

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Chronic stress is a risk factor for depression and anxiety disorders, and cognitive impairments related to prefrontal cortical dysfunction (e.g., preservative behavior and cognitive set-shifting deficits) are a component of such illnesses. We have shown acute elevation of noradrenergic (NA) activity in rat medial prefrontal cortex (mPFC) enhances extra-dimensional (ED) cognitive set-shifting on an attentional set-shifting test (AST), and that this effect was blocked by mPFC microinjections of the α1-adrenergic receptor antagonist, benoxathian (Lapiz et al., 2006). Subsequent studies found rats exposed to chronic unpredictable stress (CUS) exhibit replicable cognitive impairments on the ED task (Bondi et al., 2008). Many of the effects of chronic stress have been attributed to glutamate-mediated excitotoxic cell damage, thus NA facilitation of glutamate transmission in mPFC may be beneficial acutely, yet detrimental when evoked repeatedly by stress. To address this hypothesis, we assessed the effects of chronic stress on cognitive function after blocking the acute NA facilitatory actions elicited by each stress session during a 14d CUS paradigm. Adult male Sprague-Dawley rats were exposed to a different acute stressor each day of CUS in the stress condition, while controls were handled daily with no stress. Half of the rats in each group received a systemic injection of vehicle (50% DMSO, 3.0 ml/kg, i.p.), while the others were administered a combination of the α1-adrenergic receptor antagonist, prazosin (2.5 mg/kg), and the β1/2-adrenergic receptor antagonist, propranolol (10 mg/kg), 20 min before each stress or handling session. Rats were then tested on day 17, three days after the last stress and drug treatment. As shown previously, CUS caused a deficit of ED set-shifting in vehicle-treated rats. However, antagonist treatment prevented the detrimental effects of CUS, as these rats performed comparably to unstressed controls on the ED task. Thus, preventing the activation of an acute NA facilitatory influence by each stressor prevented the detrimental effects of chronic stress on cognition, supporting the hypothesis that the cognitive deficit may be a consequence of cumulative excitatory neurotoxicity in mPFC.

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The Role of Dopamine in Operant Conditioning

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Dopamine is a neurotransmitter that regulates many physiological processes including reward, motivation, movement, learning and memory, and reinforcing effects of addictive drugs. Increased levels of dopamine are associated with the intake of addictive drugs such as cocaine and alcohol. The association made between the act of drug intake and subsequent rewarding experience is a type of operant learning and this is believed to play a significant role in the development of drug addiction. However, the underlying cellular mechanisms of this learned behavior are not well understood. Using Drosophila melanogaster as a model system, we have investigated the mechanism underlying operant learning and memory. In a conditioned courtship assay, a male fly's courtship behavior is influenced by persistent rejection of a mated female. The male fly learns to associate unsuccessful courtship and copulation with the female fly and displays a generalized aversion (via courtship suppression) toward a virgin female. The goal of this study is to elucidate dopamine's roles in this operant conditioning. For this task, we are currently testing flies lacking D1 receptor dDA1 (dumb), D2 receptor dDR2 (dd2r), or dopamine transporter DAT (fmn). Once we identify important components mediating operant learning and memory, their functional sites (brain structures) and underlying cellular mechanisms will be clarified. Knowledge obtained in this study should shed light onto the complex and multifaceted pathway of drug addiction.

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Oxycodone fails to act as a reinforcer among buprenorphine/naloxone-maintained chronic pain patients

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While significant misuse of opioid drugs among chronic pain patients occurs, the risk factors and motivation for abuse may differ from that of other opioid abusers. This study sought to examine the reinforcing effects of oxycodone among chronic pain patients who meet DSM-IV criteria for prescription opioid abuse. Eighteen opioid-dependent patients with chronic pain (11M, 7W; 8 Latinos, 7 African-American, 3 Caucasian) were admitted to an inpatient unit of the NYSPI where they resided for this 7-week study. Participants received several doses of oral oxycodone (0, 10, 20, 40, 60 mg/70 kg) while maintained on three doses of sublingual buprenorphine/naloxone (2/0.5, 8/2, 16/4 mg). All of the medications were administered under randomized, double-blind conditions using a cross-over design. Several dependent measures, including pupil diameter and subjective responses (e.g., "I feel High," "I feel a Good Effect"), indicated that psychoactive doses of oxycodone were used. However, unlike recreational opioid users without pain, and more similar to non-drug abusing individuals, none of the active doses of oxycodone were self-administered above placebo levels. Furthermore, participants did not report that they liked the drug or that they would be willing to take it again. These data suggest that the pattern of subjective responses after opioid agonist administration may differ in opioid abusing chronic pain patients compared to opioid abusers without pain. In addition, the data suggest that sublingual buprenorphine/naloxone may be an ideal medication for reducing prescription opioid abuse among chronic pain patients.

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REPEATED INTERMITTENT TREATMENT WITH WAY 163909 INDUCES BEHAVIORAL TOLERANCE TO ITS ANORECTIC AND HYPOMOTIVE EFFECTS

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The serotonin (5-HT) 5-HT2C receptor (5-HT2CR) is distributed widely in the corticolimbic circuit and 5-HT2CR dysfunction is implicated in several psychiatric disorders. Selective 5-HT2CR agonists are proposed as potential therapeutic medications; however, repeated treatment with 5-HT2CR agonists is generally thought to rapidly induce tolerance which may prove counterproductive under pharmacotherapeutic conditions. In the present study, we are exploring the behavioral and molecular response to repeated treatment with a selective 5-HT2CR ligand. Male, Sprague-Dawley rats were treated daily for seven days with the selective 5-HT2CR agonist WAY 163909 (10 mg/kg), inverse agonist SB 206553 (4 mg/kg), antagonist SB 242084 (1 mg/kg), or vehicle (45% β-cyclodextrin; n=10/trt group). Weights were recorded daily; on day 8, all animals were challenged with WAY163909 (10 mg/kg) or vehicle, and locomotor activity was measured. Repeated administration of WAY 163909 induced tolerance to its anorectic effects. Acute administration with WAY 163909 significantly reduced total ambulations compared to vehicle controls ($p<0.05$, two way ANOVA). Repeated WAY 163909 administration blunted the decrease in ambulations induced by acute WAY 163909 challenge ($p<0.05$, two way ANOVA). Repeated administration of the 5-HT2CR inverse agonist SB 206553 or antagonist SB 242084 did not alter acute WAY 163909-evoked hypomotility, indicating that alterations in 5-HT2CR function that may occur upon repeated exposure to these ligands does not interfere with agonist activation of the receptor. Thus, the data presented here indicate a loss of 5-HT2CR function upon repeated agonist exposure, potentially as a result of underlying receptor desensitization mechanisms. Supported by: DA06511, DA020087, DA024157

Poster Communications

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The involvement of ghrelin in ethanol-induced expression of c-Fos in the pIIIu of mice

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Ghrelin is a stomach-derived hormone involved in hunger and cravings. There is a high density of ghrelin receptors (GHSR1a) in the perioculomotor urocortin-containing population of neurons (pIIIu, previously known nonpreganglionic Edinger-Westphal nucleus). Previous studies from this lab have shown that the pIIIu is important in alcohol intake. Recent studies have shown that alcoholic patients have lower plasma ghrelin levels (Badaoui et. al., 2008) and that GHSR knock-out mice or mice injected with a GHSR antagonist have decreased alcohol intake (Jerlhag et al., 2009). Based on this evidence, it is hypothesized that ghrelin can regulate alcohol intake by acting on GHSR1a receptors in pIIIu. A recent experiment in this lab showed that the GHSR antagonist, D-Lys-GHRP-6, decreased alcohol consumption. In the study described here, brains from these behaviorally-tested mice were investigated for levels of c-Fos (an immediate early gene responsive to neuronal activation). Male C57BL/6J mice (12-13 weeks old) were injected with saline for three days and allowed 2h access to 20% ethanol. On day 4, saline or D-Lys-GHRP-6 was injected (400 nmoles, ip), and the mice were allowed access to ethanol for 4h prior to sacrifice. Brain slices (30 microns) were processed for c-Fos immunohistochemistry. Significantly lower levels of ethanol-induced c-Fos were seen in the pIIIu of these mice. These data are in agreement with the idea that ghrelin antagonists can act in pIIIu to inhibit ethanol consumption. Further understanding of the role of ghrelin in ethanol consumption could lead to the development of therapies for alcoholism in the future.

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Evaluation of nicotinic ligands in discrimination assays: concepts from receptor theory

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This study evaluated the behavioral effects of nicotine and nicotinic ligands in relation to pharmacological concepts and receptor theory. The effects of nicotine, other nicotinic agonists, and antagonists were evaluated in drug discrimination assays in male Sprague-Dawley rats. We utilized experimental parameters to evaluate efficacy, potency, and receptor selectivity, which have been used in the characterization of other receptor systems, especially the opioid receptor system. Multiple nicotinic agonists generalized to the nicotine discriminative stimulus, and the potency rank order did not change with training drug or dose. Most agonists produced high levels of generalization, suggesting that nicotine discrimination assays are a low-efficacy requiring behavior. In addition, the rank order of agonist potency was not altered under conditions of different training doses of nicotine or following training with different nicotinic agonists, implying that discriminative effects of nicotinic ligands are mediated through one receptor subtype. Antagonism studies demonstrated that the nonselective nicotinic antagonist mecamylamine shifted both the discriminative and rate decreasing effects of nicotine, but the α4β2 antagonist DHβE attenuated only the discriminative stimulus effects of nicotine, suggesting that a non-α4β2 receptor mediates the rate decreasing effects of nicotine. The pA2 analysis of DHβE antagonism demonstrated that the high doses of nicotine are working through more than one receptor subtype and that the antagonism of cytisine's discriminative effects is likely not competitive at the same receptor site. These interpretations of our nicotine data often parallel behavioral effects found in the opioid receptor system. Overall, these data and the presented points highlight the usefulness of pharmacological concepts and receptor theory in evaluating multiple receptor systems and can be used to improve our understanding of the behavioral effects of nicotine.

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Methamphetamine-Induced Place Reinforcement Learning within the Ventral Tegmental Area, the ventral Hippocampus, and the Nucleus Accumbens

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Addiction induced health problems are on the rise in the United States. Almost all addictive drug use including methamphetamine (METH) and cocaine involve the functioning of the midbrain dopamine neurotransmitter system. By contrast the hippocampus is thought to be an area that mediates learning and memory. It receives behaviorally relevant dopamine projections from ventral tegmental area (VTA). Since addiction is a learning process, the hippocampus is hypothesized to be part of the learning process we call addiction. Our laboratory previously reported that METH applied into the dorsal hippocampus using reverse dialysis produced positive place preference conditioning. In a replication of our previous findings the current study specifically targeted the ventral hippocampus (vHippo) due to its proximity to the VTA and nucleus accumbens (NAc). We used a rat model of intracranial conditioned place preference (IC-CPP) to address two main questions: i) to establish whether intra-VTA-METH induces a place preference, and ii) whether the VTA-vHippo-NAc order of exposure to METH influences positive place preference. Our results showed that: i) METH directly infused into the VTA induced positive place preference in both previously cocaine self administering and cocaine naïve subjects. This effect diminished with repeated exposure to the drug implying novelty plays a role during the acquisition process, and that the learning extinguishes. ii) IC-CPP in the order of VTA (1st)-vHippo (2nd)-NAc (3rd) produced positive place preference in all three brain regions. With repeated exposure to METH within both VTA and vHippo, place reinforcing capacity of METH was attenuated while it is maintained within the NAc; suggesting that acquisition and adaptation of METH induced place preference takes place through the VTA-vHippo connection while the expression of memory aspect of METH-CPP takes place within the nucleus accumbens of the ventral striatum. Our results suggest that pharmacotherapies for drugs of abuse to specifically target the VTA-vHippo connection. [A portion of this abstract presented at the 2009 society for Neuroscience conference in Chicago, IL]

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Is Induction of Hippocampal Opioid-Receptor Dependent Associative Long-Term Potentiation Recorded In Vivo Dependent on Protein Synthesis?

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Mossy fiber and lateral perforant path LTP require protein synthesis for induction, and induction in both pathways is dependent of the stimulation of opioid receptors, presumably by endogenous opioids contained in the presynaptic terminals. Concerning the locations of their terminations on the CA3 dendrite, the mossy fiber synapse is located most proximally to the cell body and the lateral perforant path is located most distally, while the C/A-CA3 and the medial perforant pathways are located between these areas, respectively. It can therefore be assumed that if mRNA information runs along the dendrite during associative LTP induction, then LTP at other afferents to hippocampal area CA3 could also depend on protein synthesis for its induction when potentiated in an associative paradigm. In this experiment stimulation of the mossy fiber pathway served as the strong input to area CA3 dendritic layer, and for associative LTP induction, it was paired with a low intensity stimulation from either the medial perforant pathway, the lateral perforant pathway, or the commissural associative-CA3 pathway after infusion of the protein synthesis inhibitor Anisomycin. The control group received Ringer's solution. The effects of Anisomycin on LTP induction in the low intensity pathway appeared to be based on the dependence of the pathway to either NMDA receptor or opioid receptor activation. The lateral perforant pathway, which expresses the opioid receptor dependent for LTP, did not show any induction of associative LTP when paired with stimulation from the mossy fiber pathway. By contrast the medial perforant and commissural/associative pathways, which express NMDA receptor-dependent LTP induction, exhibited associative LTP, but with a reduced amplitude compared to the Ringer's control. The data imply that protein synthesis may be a necessary component for opioid dependent associative LTP induction to area CA3 of the hippocampus, and protein synthesis may play a smaller role in the determination of the amplitude of induction of NMDA dependent associative LTP. This in vivo model of opioid-dependent

Poster Communications

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Delta9-tetrahydrocannabinol attenuates i. v. heroin self-administration in rhesus monkeys

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The cannabinoid receptor agonist delta9-tetrahydrocannabinol (THC) enhances the antinociceptive effects of mu opioid receptor agonists, raising the possibility of using a combination of THC and opioids for treating pain. This drug combination would be most useful if other (abuse related) effects of opioids were not increased by THC. This study examined the effects of non-contingent and contingent THC on i.v. heroin self-administration in rhesus monkeys. Monkeys could self administer different unit doses of heroin (0.0001-0.1 mg/kg/infusion) or saline under a fixed ratio 30 schedule, generating an inverted U-shaped dose effect curve. In one experiment (n=4), non-contingent THC (0.1-1.0 mg/kg) administration (30 min prior to sessions) dose-dependently decreased the number of heroin infusions in all monkeys, shifting the heroin dose effect curve downward. In a second experiment (n=4), monkeys self-administered THC alone (0.0032- 0.032 mg/kg/infusion), heroin alone, or a mixture of THC and heroin. THC alone did not maintain responding above that obtained with saline; however, increasing the THC dose in the mixture dose-dependently decreased the number of (heroin and THC) mixture infusions. Collectively, these data indicate that in rhesus monkeys, THC does not enhance the reinforcing effects of heroin. Combined with the findings that THC increases the antinociceptive effects and decreases the discriminative stimulus effects of mu opioid receptor agonists, these data suggest that a combination of THC and an opioid could be effective for treating pain with no greater, and perhaps less, abuse liability as compared with an opioid only. [Supported by NIDA grants DA05018 and 17918 (CPF)].

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A Multistudy Analysis of the Effects of Early Cocaine Abstinence on Sleep

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Objective: To describe the sleep patterns of early cocaine abstinence in chronic users by polysomnographic and subjective measures.
Methods: 28 cocaine-dependent participants (ages 24-55) underwent polysomnographic sleep (PSG) recording on the 1st, 2nd and 3rd weeks of abstinence on a research dedicated inpatient facility. Objective measures of total sleep time, total REM time, slow wave sleep, sleep efficiency and a subjective measure (sleep quality) along with demographic data were collected from three different long term research studies over a five year period. Data was reanalyzed to allow greater statistical power for comparisons.
Results: Progressive weeks of abstinence had main effects on all assessed PSG sleep measures showing decreased total sleep time and REM sleep and a slight increase in slow wave sleep. Total sleep time and slow wave sleep were negatively associated with years of cocaine use but total sleep time was positively associated with the amount of current ethanol use. Sex differences were found with females having more total REM time and an increase in a near significance level in slow wave sleep. Improving quality of sleep was reported with increasing abstinence.
Conclusions: Chronic cocaine users show a general deterioration in objective sleep measures over a three-week period despite an increase in subjective overall sleep quality providing further evidence for "occult insomnia" during early cocaine abstinence.

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The Roles of Nicotinic and Muscarinic Cholinergic Receptors in Cost-Benefit Decision Making

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Risky and impulsive decision making are common in drug-addicted individuals. Although the roles of several neurotransmitter systems in such behavior have been thoroughly investigated, little is known about the involvement of the cholinergic system. The overall goal of these experiments was to determine how cholinergic signaling is involved in cost-benefit decision making. Male Long-Evans rats (n = 15) were trained in a "probability discounting" task, in which they chose between small guaranteed and large probabilistically delivered food rewards. A separate group of rats (n = 16) were trained in a "delay discounting" task, in which they chose between small immediate and large delayed food rewards. Once stable performance was achieved, the effects of acute administration of nicotinic and muscarinic receptor agonists and antagonists were tested in each task. Because there were considerable individual differences in performance in some cases, rats were divided into high and low "risk-taking" or "impulsive" groups on the basis of their performance in the probability or delay discounting tasks, respectively. In the probability discounting task, acute administration of the AChE inhibitor donepezil decreased choice of the large risky reward in "risk-taking" rats and increased choice of the large reward in "risk-averse" rats. Acute administration of nicotine increased choice of the large risky reward in both groups, whereas administration of the nicotinic receptor antagonist mecamylamine increased choice of the large risky reward in only "risk-averse" rats. In the delay discounting task, donepezil had no effects, but nicotine and mecamylamine both decreased choice of the small immediate reward in "impulsive" rats. Choice preference was not significantly altered following acute administration of either a muscarinic agonist or antagonist in either task. These experiments suggest that the cholinergic system (particularly acting through nicotinic receptors) is involved in cost-benefit decision making. Given that drugs targeting the cholinergic system are already in use for treatment of a variety of clinical conditions, the results suggest that these drugs may prove useful for treatment of decision-making deficits present in addiction.

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Amphetamines trigger ubiquitination of the dopamine transporter.

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The dopamine transporter (DAT) belongs to the SLC6 family of neurotransmitter transporters that includes the GABA, serotonin and norepinephrine transporters. DAT, similar to other members of the family, is a polytopic protein embedded in the plasma membrane by 12 transmembrane domains and intracellular N- and C-terminal tails. During the last decade, it has been described that several neurotransmitter transporters including DAT, SERT, NET and GlyT1 are tightly regulated by Protein Kinase C (PKC). Activation of PKC leads to several changes on transporter such as: a reduction of glycine uptake, phosphorylation of ser/thr residues, ubiquitination of lysine residues and transporter internalization. We have shown for the dopamine and norepinephrine transporters that PKC-dependent ubiquitination is the signal for transporter internalization and that ubiquitin moieties attached to transporter are necessary to target DAT to the lysosomes for degradation. Although a large number of studies are dedicated to understand the effects of amphetamines (amphetamine and methamphetamine) on dopaminergic neurotoxicity, the effects of these drugs on DAT endocytosis are poorly understood. To get insights into the DAT modifications triggered by amphetamines, we incubated cells expressing DAT in epithelial cells with either AMPH or METH and looked at DAT ubiquitination. After purification and western blot analysis, it was found that AMPH or METH treatment induced DAT ubiquitination in a concentration and time dependent fashion. Whether amphetamines enhance DAT ubiquitination in the brain is currently underway.

Poster Communications

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Acute administration of drugs of abuse modulates risky decision making.

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People are faced with daily decisions among competing alternatives, some of which may be accompanied by adverse consequences. Most people are able to accurately assess the risks and rewards of such alternatives and decide accordingly; however, drug users often display maladaptive decision making, such that choices are biased toward risky options. This type of decision-making is commonly studied in laboratory tasks in humans (e.g. the Iowa Gambling Task). There have been few attempts using animal models to determine how risks of adverse consequences (punishment, as opposed to reward omission) influence decision making. Our lab has recently developed such a task, in which rats choose between small "safe" rewards and large rewards that are accompanied by varying risks of punishment. Previous work in our lab showed that amphetamine decreased risk taking in this task, whereas cocaine rendered rats insensitive to changes in the risk of punishment. The purpose of this study was to extend our investigation of the effects of drugs of abuse on risky decision making, using acute administration of nicotine, morphine, and ethanol.

Male Long-Evans rats were trained in the risky decision making task in standard operant chambers, in which they were given choices between pressing one of two levers, one of which resulted in a small, "safe" reward and the other which resulted in a large, "risky" reward; the choice of the large reward was accompanied by the possibility of a mild footshock, the probability of which increased over the course of the session in consecutive blocks of trials (0, 25, 50, 75, 100%). Nicotine caused a dose-dependent decrease in risk taking (fewer choices of the large risky reward). Morphine increased risk taking, whereas ethanol had no effect on choice behavior. Finally, as found previously, amphetamine dose-dependently decreased risk taking. These results suggest that acute intake of drugs of abuse can modulate risk taking in a drug-specific manner, either increasing or decreasing choices of highly rewarding, but risky, options.

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Cholinergic transmission in the nucleus accumbens is lower in adolescent versus adult rats experiencing 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. However, the neurochemical mechanisms that mediate these developmental differences are unknown. The goal of this study was to compare acetylcholine (ACh) levels in the NAcc of adolescent and adult rats experiencing withdrawal. Male Wistar adolescent (PND 28-30) and adult (PND 60-70) 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, dialysate samples were collected following systemic administration of the nicotinic-receptor antagonist mecamylamine (1.5 mg/kg or 3.0 mg/kg) to precipitate withdrawal. ACh levels were also compared in these groups following systemic administration of the ACh-esterase inhibitor, methanesulfonyl fluoride (MSF; 2.0 mg/kg). This was done to examine whether our results were due to age-dependent differences in the metabolism of ACh. Dialysate levels of ACh were quantified using HPLC-EC methods. Results indicate that adult rats experiencing withdrawal displayed a dose-dependent increase in ACh levels (128% and 149%) relative to baseline, consistent with other laboratories. However, adolescent rats displayed less of an increase in ACh levels (112% and 130%) as compared to adults. Both age groups displayed a similar change in ACh levels following MSF administration. Therefore, these results suggest that cholinergic systems play a role in mediating developmental differences to nicotine withdrawal. Our results further suggest that adolescent rats maybe less sensitive to the modulatory effects of nicotinic receptors in mediating withdrawal as compared to adults. Supported by NIDA Grant R01DA021274 and a diversity supplement fellowship to this award.

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The differential effects of varenicline between ethanol dependent and non-dependent rats

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The partial nicotinic α4 β2 receptor agonist varenicline (Chantix®) has been used in tobacco cessation therapies with clinical efficacy. The goal of the present study was to test the effects of varenicline on acute oral ethanol intake in rats. Eighteen male Wistar rats were first trained to self-administer a10% ethanol solution using a saccharin fading procedure. Once the animals' ethanol intake was stable, they were separated into 2 groups. One group of rats was exposed to intermittent ethanol vapor (14 hours on/10 off each day) and maintained at an average blood alcohol level of 172-365 mg/dl for approximately 2 months (dependent group). The other group of animals was maintained in control chambers that did not deliver ethanol (non-dependent group). The effects of varenicline were then compared across these groups by giving the rats a dose of varenicline (0, 0.3, 1.0, or 2.0 mg/ml, sc) six hours after the removal from vapor. Thirty min after the injection, the animals were tested for ethanol self-administration. The varenicline doses were given across 4 separate tests days in a Latin-square design. The results revealed that varenicline reduced acute ethanol intake in non-dependent rats but not in dependent rats. These findings suggest that varenicline may have differential effects in dependent versus non-dependent rats and may not be efficacious in ethanol dependent populations. (Supported by the NIH/NIGMS/Bridges to the Baccalaureate Program (2R25GM049011; AM).

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Pramipexole is rewarding and induces risk-taking behavior in rats.

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Dopamine D3 receptor-preferring agonists (e.g., pramipexole (PPX), Mirapex®) are clinically used to treat motor dysfunction in Parkinson's disease (PD). A subpopulation of these patients develop dopamine dysregulation syndrome and/or impulse control disorders. We developed a preclinical model of this phenomenon, and evaluated the effects of PPX on reward and risk-taking behaviors in parkinsonian-like (PD-like) rats and controls. Rats were rendered PD-like by bilateral injections of 6OHDA into the dorsolateral striatum; controls received vehicle. To measure reward, we used conditioned place preference (CPP) wherein rats tend to spend more time in an environmental context previously associated with an unconditioned stimulus (e.g., an abuse drug). We determined that CPP was induced following four pairings with PPX (±mixture; 2mg/kg ip) in PD-like rats ($p=0.009$) but not in sham controls ($p=0.125$). CPP was also induced with the positive control methamphetamine (+racemer; 1mg/kg ip) in both groups (PD-like $p= 0.01$; sham $p=0.046$). To indicate risk-taking, we used a probability discounting paradigm with intracranial self-stimulation (ICSS) of the lateral hypothalamus as the positive reinforcer. In this paradigm, rats choose between a small reinforcer that is always delivered and a large reinforcer that is delivered with varying probabilities. Risky behavior is defined as a preference for the large, uncertain reinforcer. Rats were trained in a six phase lever-pressing protocol to determine large and small reinforcers based on ICSS current strength, and to establish a stable baseline of risk-taking behavior (i.e., as the probability decreased for delivery of the large reinforcer, preference for the large reinforcer decreased). Subsequent PPX treatment (2mg/kg ip, 2Xday for 13 days) increased risk-taking in both PD-like rats and controls. At the lowest probabilities, increases were ~40% above baseline. In most rats, risk-taking was normalized by 2-3 weeks after terminating PPX treatment. These novel studies revealed that PPX is sufficiently rewarding to induce place conditioning and it can enhance risky behaviors.

Poster Communications

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Activation of estrogen receptor alpha enhances bradykinin signaling in female rats.

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Women are at increased risk for numerous pain disorders, including those mediated by the trigeminal (TG) and dorsal root ganglia. While many factors are likely involved in this sex difference, many studies have shown a direct enhancement of pain by estrogen. We have previously shown that activation of estrogen receptors (ER) rapidly enhances signaling of the inflammatory mediator bradykinin (BK) both in vitro and in vivo. Here we further characterize the effect of ER activation on BK signaling. Our data indicate that activation of ERα, but not ERβ or GPR30, rapidly enhance BK signaling, and this enhancement is likely mediated via non-genomic mechanisms. In primary sensory neuronal cultures derived from ovariectomized female rats, treatment with 17β-estradiol (E2) or the subtype-selective ligands PPT (ERα), DPN (ERβ), or G-1 (GPR30) had no effect on basal inositol phosphate (IP) accumulation. However, pretreatment with E2 or PPT, but not DPN or G-1, dose-dependently enhanced the maximal response to BK from 34% ± 3% above basal to 71% ± 13% above basal (mean ± SEM, n=3; vehicle vs PPT, respectively, $p<0.01$). Similarly, BK-mediated thermal allodynia was also rapidly enhanced by E2 and PPT, but not by DPN or G-1. Injection of E2 (50 ng) or PPT (2 ng), but not DPN (400 ng) or G-1 (1 μg), into the rat ipsilateral, not contralateral, hindpaw 15 min before injection of BK (1μg) significantly decreased the paw withdrawal latency to radiant heat ($p<0.001$ vs vehicle). Pretreatment with the protein synthesis inhibitor anisomycin (5 μg) had no effect on E2 or PPT enhancement of BK induced thermal allodynia, but both were blocked by the ER α/β antagonist fulvestrant (1 μg). Together these data demonstrate that ERα activation on peripheral sensory neurons rapidly enhances BK signaling, and this enhancement is peripherally restricted and likely non-genomic. Further investigation into the mechanisms underlying ERα-mediated enhancement of BK may lead to novel targets for pain therapeutics.

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The Role of Dopamine in Impulse Control

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Impulsivity is commonly observed in people with drug addiction and neurological disorders such as attention deficit hyperactivity disorder (ADHD) and autism. It occurs as a result of the brain's diminished ability to inhibit or to control behavioral or cognitive activity. One of the major neurotransmitters that play a role in addiction is dopamine. It affects neurological processes that control movement, attention, reward, learning and memory. The major goal of this study is to elucidate the role of dopamine in impulse control in the genetic model system Drosophila melanogaster. The fumin (fmn) mutant flies, which lack dopamine transporter, show increased activity levels and display hyperkinetic behavior under the influence of ethanol. Furthermore, when subjected to the No-Go test, wherein wild-type flies exposed to strong air flow inhibit motor activity, fmn mutant flies show impulsive hyperkinetic behavior. These observations together indicate the critical role of dopamine in impulse control. The studies are in progress to identify when (developmental or physiological or both processes) and where (particular brain structures) dopamine transporter deficiency (thus, hyper-dopamine tone) is critical for the impulsivity phenotype using pharmacological and genetic approaches. The outcome of this study has significant implications in understanding neurological disorders such as ADHD and substance dependence.

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Cocaine sensitization produces region-specific changes in expression of hyperpolarization activated cation current (Ih) channel subunits in the Mesocorticolimbic System

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Cocaine sensitization refers to an increase in motor-stimulant response that occurs with repeated, intermittent exposure to psychostimulants. The process of sensitization is associated with neuronal adaptations in the mesocorticolimbic area, which arises in the ventral tegmental area (VTA) and innervates mainly the nucleus accumbens (nAcc) and the prefrontal cortex (PFC). The Hyperpolarization-activated Cyclic-Nucleotide current (Ih) is an ubiquitous voltage dependent current generated by nonselective cation channels (HCN). Ih plays an important role in maintaining neuronal excitability thus, contributing to various physiological functions in the brain. Its biophysical properties depend on the expression profiles of the underlying channel's subunits (HCN1-4). Previous studies suggest an important role of Ih in the reinforcing actions of drugs of abuse such as ethanol. Here we investigated whether cocaine sensitization induces region-specific changes in expression of the Ih channel subunit HCN2. Adult Sprague Dawley rats (150-200g) were administered cocaine (15mg/kg, i.p.) for 7 days. Sensitized animals with their respective saline controls were anesthetized, decapitated and brains removed 24hrs after last injection. Brains were placed in ice-cold 1X PBS. Coronal sections of 400 μm at the level of the VTA, PFC, nAcc and hippocampus (Hip) were dissected following Paxinos and Watson Atlas. Brain slices were then mounted onto a glass surface and immediately frozen with dry ice. Micropunches from these areas were taken using a stainless-steel micropunchneedle (1mm diameter). Dissected tissue was homogenized in Lysis buffer II and resolved in 8% SDS-PAGE. Proteins were then transferred to a nitrocellulose membrane using Trans-Blot transfer medium (BioRad). HCN2 detection was performed using anti-mouse monoclonal HCN1 and HCN2 antibodies (Antibodies Incorporated/ NeuroMabs Labs). Our results demonstrate that HCN2 subunit is express in the nAcc and has a tendency to increase after cocaine sensitization ($p<0.05$). Changes in HCN expression in the VTA, PFC and hippocampus are currently under analysis. It is suggested that these changes in HCN subunits may contribute to the modulation of excitability induced during drug addictive processes.

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Dopamine transporter and receptor ligands modify the discriminative stimulus effects of rimonabant in Δ9-THC treated rhesus monkeys.

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In clinical studies, the antidepressant and catecholamine transport inhibitor bupropion worsens mood during marijuana withdrawal. To examine the catecholaminergic mechanism(s) by which bupropion enhances marijuana withdrawal, the effects of bupropion and pharmacologically related drugs were tested in a pre-clinical assay of cannabinoid withdrawal, i.e., rhesus monkeys that discriminated the cannabinoid antagonist rimonabant (1 mg/kg i.v.) while receiving daily treatment with Δ9-THC (1 mg/kg/12 h s.c.). Bupropion (1-5.6 mg/kg s.c.) and the non-selective monoamine transporter ligands cocaine (0.1-1 mg/kg s.c.) and amphetamine (0.1 and 0.32 mg/kg s.c.) produced a maximum of 31%, 54%, and 33% responding on the rimonabant lever, respectively. Cocaine and amphetamine produced leftward shifts in the rimonabant dose-response curve. The selective dopamine uptake blockers RTI 113 and RTI 177 also shifted the rimonabant dose-response curve leftward, whereas transporter ligands with relatively low affinity for dopamine transporters (desipramine and imipramine) did not modify the rimonabant discriminative stimulus. The involvement of dopamine receptor subtypes was then investigated; the dopamine D1- and D2-like receptor agonist apomorphine (0.32-1 mg/kg s.c.) produced a maximum of 38% rimonabant-lever responding and shifted the rimonabant dose-response curve leftward. The D2-like antagonist haloperidol (0.01-0.1 mg/kg s.c.), on the other hand, produced a maximum of 4% responding on the rimonabant lever and did not modify the rimonabant discriminative stimulus. These data provide evidence for interactions among cannabinoid and dopamine receptor systems in vivo and suggest that dopamine receptor agonism enhances cannabinoid withdrawal. Some clinically used drugs (bupropion) appear to enhance marijuana withdrawal by increasing dopamine neurotransmission. Supported by USPHS grant DA 19222 and DA 26781.

Poster Communications

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Natural Products as a Source of Novel Opioid Receptor Ligands

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Alkaloids extracted from the opium poppy, such as morphine, have been used for centuries to treat pain and induce sleep. Today, these compounds are among the most potent analgesics used in the clinic. The opium alkaloids and many of their synthetic derivatives are selective agonists at μ opioid (MOP) receptors. Administration of MOP agonists, while producing analgesia, is associated with several clinically significant side effects such as respiratory depression and constipation. Chronic use of MOP agonists leads to tolerance and dependence, which can further lead to opioid abuse. A unique way to circumvent these problems is to find compounds that differ from the opium alkaloids in their interactions with MOP receptors. One approach towards this goal is to turn to natural products as a source of novel structural scaffolds with activity at opioid receptors.
Dioclea grandiflora Mart. ex. Benth. (Leguminosae) is a vine that is native to northeastern Brazil. It is used in traditional medicine to treat kidney stones. The flavonoid natural products dioclein and dioflorin are two minor constituents that have been isolated and characterized from this plant. Both of these flavonoids produce antinociception in rodent models, where the activity of dioflorin is comparable to that of morphine. Interestingly, the effects of dioclein are attenuated by the administration of naloxone, an opioid receptor antagonist. Although these results indicate that the mechanism of action of these flavonoids proceeds through opioid receptors, this information has not been investigated in vitro, and no structure activity relationship (SAR) studies of these molecules have been published. Here, we report our efforts towards the design and synthesis of dioclein and several dioflorin analogs. These compounds are potential analgesics with improved side effect profiles and reduced or eliminated abuse liability.

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Utilizing Cycloaddition and Olefination Chemistry in the Synthesis of Conformationally Constrained Salvinorin A Analogues.

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There are three main types of opioid receptors: μ (MOP), δ (DOP), and κ (KOP), which belong to the superfamily of seven transmembrane-spanning (7TM) G-protein coupled receptors. Various findings have implied that KOP receptors are involved with the alteration of the effects that psychostimulants have on the central nervous system (CNS). The neoclerodane diterpene salvinorin A is isolated from the Mexican sage Salvia divinorum (Lamiaceae) and is a selective κ opioid receptor agonist. In addition, salvinorin A is a potent hallucinogen and, despite its dysphoric effects, it has gained an increase in popularity. Here we report our efforts toward the synthesis of conformationally constricted analogues of salvinorin A using olefination and cycloaddition chemistry in order to explore their potential as drug abuse therapies. Currently, there is a need for a more in depth understanding of the interactions of salvinorin A at the KOP receptor. While several molecular models have been proposed, there is no X-Ray crystal structure available, thus validation through structure-activity relationships continues to be necessary.

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Protein kinase Mzeta inhibitor (ZIP) decreases AMPA mediated currents of VTA neurons and alters cocaine sensitization in male rats.

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Chronic cocaine use produces long lasting changes in reward circuits that may underlie the transition from casual to compulsive patterns of drug use. Single or chronic in vivo cocaine injections cause a robust AMPAR-mediated potentiation of excitatory synapses in dopamine (DA) cells of the ventral tegmental area (VTA) that lasts several days. This long term potentiation (LTP) is postulated to increase dopamine release in the NAcc and other addiction-related brain regions and may underlie the behavioural responses observed in addiction. PKMZ is known to be involved, at least in the hippocampus, in synaptic plasticity maintaining LTP. Superfusion of ZIP, an inhibitory peptide specific for PKMZ, reverts LTP. Using ZIP, we studied the behavioural effects of reversing LTP in the VTA in an animal model of addiction named, cocaine sensitization. Male Sprague-Dawley rats (250g) received 15mg/kg of cocaine ip. for five days. Intra-VTA ZIP microinfusions were given after the last day of cocaine administration. A cocaine challenge (15mg/kg) 24 hours later showed that ZIP infusions erased the already established sensitization but had no effect on the expression measured after a one week withdrawal period. ZIP infusions on days 6 and 7 had no effect on the initiation or the expression of cocaine sensitization. This evidence suggests that in the VTA persistent phosphorylation of PKMζ mediates a necessary process for sensitization if no withdrawal period is permitted. To elucidate the mechanism for the previously observed behavioural response, we employed voltage clamp recordings of dopaminergic cells from single or chronically cocaine (15mg/kg i.p.) or saline injected rats. Pharmacologically isolated EPSC's were electrically evoked by placing a stimulating electrode ≈100 μm rostral to the patched cell. Once a stable EPSC was achieved 5uM ZIP was superfused into the slide. EPSC's were recorded for 15 minutes. ZIP superfusion decreases AMPA currents EPSC's (≈25% from baseline, $p<0.05$), in cocaine treated animals when compared to control rats. These results support the vision that addiction involves an aberrant learning process and implies that if this acquired plasticity is reverted, changes in the behavioural response may take place. (Supported by GM-08224 to CAJR)

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Female rats display enhanced rewarding and reduced aversive effects of ethanol relative to males and female rats lacking ovaries.

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Alcoholism is a major public health concern, particularly for women who are more vulnerable to alcohol abuse relative to men. Despite the magnitude of this problem, the mechanisms mediating sex differences are not well understood. To address this issue, the present study compared the rewarding and aversive effects of ethanol in female, ovarectomized (OVX) female and male rats using place-conditioning (PC) procedures. All rats were tested for their initial preference for 2 distinct compartments of our conditioning apparatus. Conditioning was conducted over 10 consecutive days where separate groups of rats (n=6--26) received an injection of ethanol (0.5, 1.0, 2.0 or 2.5 mg/kg; IP) and were immediately placed into their initially non-preferred side for 30 min. On alternate days, they received saline and were confined to the other side for 30 min. Following conditioning, the amount of time spent on each side was recorded for 15 min. To address sex differences in ethanol metabolism, blood alcohol levels (BALs) were compared in another group of female, OVX female and male rats (n=19-20 per group) 30 min after ethanol administration (0.5, 1.0, 2.0 or 2.5 mg/kg; IP). The results revealed that the rewarding effects of ethanol were enhanced in female rats, as evidenced by an upward shift in the dose-response curve in intact females relative to males. Aversive effects of ethanol were observed at high doses, and this effect was significant at a lower dose in male versus female rats. Taken together, the results suggest that female rats display enhanced rewarding and reduced aversive effects of ethanol relative to males. These sex differences appear to be mediated via ovarian hormones, since female rats lacking ovaries displayed similar behavioral effects as males. Also, our behavioral results do not appear to be related to group differences in metabolism because enhanced rewarding effects of ethanol were observed in female rats that displayed similar BALs as males. Taken together, these findings suggest that the enhanced rewarding and reduced aversive effects of ethanol may contribute to increased vulnerability to alcoholism in females. This research was supported by the UTEP Biology Undergraduate Scholars Program (EE) and the Minority Access to Research Careers Program (BSB).

Poster Communications

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Modeling single (acute) withdrawal in zebrafish, Danio rerio

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Although addiction pathogenesis has been extensively studied in both the clinical and murine
literature, zebrafish neurobehavioral and endocrine responses to different drugs of abuse have only lately been implicated. Recently, we demonstrated that zebrafish behavior is robustly affected following different environmental and pharmacological manipulations. Such sensitivity to the various treatments implicates zebrafish as a valid model of drug abuse, addiction, and withdrawal-induced neurocognitive changes. We showed that acute discontinuation of chronic anxiolytic agents (ethanol, diazepam, morphine) produced anxiety-like behavioral and endocrine responses. Our results reinforce the anxiogenic effect of diazepam withdrawal, while also demonstrating an increased anxiogenic response following acute caffeine withdrawal and naloxone-induced acute morphine withdrawal. Taken together, our studies reconfirm the anxiogenic effects of withdrawal from various psychotropic agents, further supporting zebrafish as a novel tool in addiction research.

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Discriminative stimuli of neuroactive steroids and benzodiazepines are similar but not identical in rats

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Neuroactive steroids and benzodiazepines are positive GABAA modulators with similar anxiolytic, sedative and anticonvulsant effects; however, their actions at different modulatory sites on GABAA receptors might confer differences in behavioral effects. This study compared the neuroactive steroid pregnanolone and the benzodiazepine midazolam to determine whether their effects can be differentiated using drug discrimination, a procedure with high pharmacological selectivity. Two groups of rats discriminated either 3.2 mg/kg pregnanolone or 0.56 mg/kg midazolam while responding under a fixed ratio 10 schedule of food presentation. Pregnanolone, midazolam and flunitrazepam produced greater than 80% drug-lever responding in both groups. Pregnanolone was more potent in rats discriminating pregnanolone, but the potencies of midazolam and flunitrazepam were not different between groups. Pentobarbital produced greater than 80% drug-lever responding in pregnanolone-discriminating rats and not in midazolam-discriminating rats. Ketamine and morphine produced little drug-lever responding in either group. Flumazenil antagonized midazolam and flunitrazepam, but not pregnanolone, in both groups. Despite many similarities between the pregnanolone and midazolam discriminative stimuli, two important differences were observed, suggesting that effects of positive GABAA modulators can be differentiated depending on their site of action. This study suggests that neuroactive steroids and benzodiazepines might vary in therapeutic profile. Supported by USPHS DA017240.

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Differential effects of eating a high fat chow on the sensitivity of adolescent and adult rats to cocaine

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Dopamine systems are an important target of many drugs. Many factors, including age, drug history, and diet, can modulate the activity of dopamine systems and, thereby, modify the behavioral effects of drugs acting on those systems. The present study examined the impact of eating a high fat chow on the sensitivity of adolescent and adult rats to the indirect-acting dopamine receptor agonist cocaine. Three groups of adolescent (PND 25) and three groups of adult (PND 75) rats had one of the following feeding conditions: free access to standard chow; free access to high fat chow; or restricted access to high fat chow so as to match body weight to rats with free access to standard chow. After 7 days on their respective diets, sensitivity to cocaine was assessed. The locomotor-stimulating effects of cocaine were enhanced in adolescent rats eating a high fat chow compared with rats eating a standard chow; however, there was no difference in the cocaine-stimulated locomotion among groups of adult rats. These results suggest that sensitivity to cocaine is increased by eating a high fat chow in adolescent rats, and this change in sensitivity is not due to differences in body weight.

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Notes

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