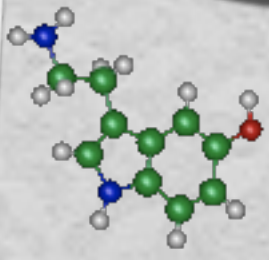
Behavior, Biology, and Chemistry:
Translational Research in Addiction

March 20-22, 2009

La Quinta Inn & Suites

Medical Center

San Antonio, TX



Acknowledgements

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

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Program Overview

Friday March 20, 2009

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 21, 2009

7:00 am - 5:00 pm	Registration
8:10 am - 8:15 am	Welcome and Opening Remarks
8:15 am - 10:35 am	Plenary Symposium: 5-HT_{2C} Receptors as Novel Targets for the Treatment of Addiction Speakers: Kelly Berg, Kathryn Cunningham, Amarnath Natarajan, Scott Gilbertson (Chair: Wouter Koek)
10:35 am - 11:00 am	Coffee Break
11:00 am - 12:00 am	Special Lecture: Richard Glennon “Behavioral Studies and SAR” (Chair: Andy Coop)
12:00 pm - 1:15 pm	**Lunch**
1:30 pm - 3:00 pm	Student Oral Communications I (Chair: Joe Martinez)
3:00 pm - 3:30 pm	Coffee Break (and set up posters)
3:30 pm - 4:45 pm	Student Oral Communications II (Chair: Alice Young)
5:00 pm - 7:00 pm	Poster Session
7:00 pm - 9:00 pm	**Dinner** Travel Award Presentation After Dinner Speaker Terry Kenakin; “The Chemist-Biologist Interface in Drug Discovery: Forming a Basis of Understanding” (Chair: WIlliam Clarke)
9:00 pm - 11:00 pm	Hospitality and Entertainment

Sunday March 22, 2009

8:00 am - 9:15 am	Open Oral Communications I (Chair: Lisa Gerak)
9:15 am - 9:35 am	Coffee Break
9:35 am - 11:05 am	Open Oral Communications II (Chair: Galen Wenger)
11:05 am - 11:30 am	Coffee Break
11:30 am - 12:30 pm	Special Lecture: Thomas Kosten “Pharmacotherapy of Stimulants: From Antabuse to Vaccines” (Chair: John Roache)
12:30 pm - 1:30 pm	**Lunch** Presentation of awards for oral and poster presentations Closing Remarks

See you at BBC 2010!

Program Details

Friday March 20, 2009 (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	Second (and 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 21, 2009

Plenary Symposium (Chair: Wouter Koek)

5-HT_{2C} Receptors as Novel Targets for the Treatment of Addiction

Over the past several years considerable evidence has accumulated which establishes a prominent role for the 5-HT_{2C} receptor in various aspects of addiction. Notably, the 5-HT_{2C} receptor regulates the activity of mesolimbic dopaminergic neurotransmission which is activated by addictive drugs. 5-HT_{2C} receptors display a highly dynamic repertoire of characteristics which may be exploited to develop novel medications for treatment of various drug addiction-related behaviors.

8:15 am - 8:50 am	***Kelly A. Berg***; University of Texas Health Science Center, San Antonio, TX 5-HT_{2C} receptor pharmacology: Roles for functional selectivity, constitutive receptor activity and mRNA-editing
8:50 am - 9:25 am	***Kathryn A. Cunningham***; University of Texas Medical Branch, Galveston, TX Serotonin 5-HT_{2C} receptor and its protein partners: Prospects for addiction pharmacotherapy
9:25 am - 10:00 am	***Amarnath Natarajan***; University of Texas Medical Branch Galveston, TX Mining protein:protein interactions for new therapeutic targets in addiction
10:00 am - 10:35 am	***Scott R. Gilbertson***; University of Texas Medical Branch, Galveston, TX Synthetic chemistry at a Health Science Institution; CNS receptors to proteases as targets

Saturday March 21, 2009

Special Lecture 11:00 am - 12:00 pm (Chair: Andrew Coop)

Richard Glennon: "Behavioral Studies and SAR"

Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA

Lunch 12:00 pm - 1:15 pm

Student Oral Communications I (Chair: Joe Martinez)

1:30 pm - 1:45 pm	***Ajna Hamidovic***, University of Chicago Variation in dopamine transporter gene associated with stimulant effects of d-amphetamine - a pharmaco- behavioral genetic study with healthy volunteers
1:45 pm- 2:00 pm	***Luke Sherrill***, University of Illinois Individual differences in sensitivity to cocaine and the potential role of disparities in 5-HT_1 and 5-HT_2 receptors
2:00 pm - 2:15 pm	***Adriane dela Cruz***, University of Texas Medical Branch Molecular events in the expression of cocaine CPP
2:15 pm - 2:30 pm	***Balaji Krishnan***, University of Texas Medical Branch Dopamine induced synaptic plasticity in the rat amygdala in saline- and cocaine-treated animals undergoing conditioned place preference
2:30 pm - 2:45 pm	***Kevin Murnane***, Emory University Effects of amphetamine derivatives on tissue content of monoaminergic and amino acid neurotransmitters in mice
2:45 pm - 3:00 pm	***Sarah White***, University of Arkansas for Medical Sciences Maternal administration of an anti-(+)-methamphetamine/(+)-amphetamine (METH/AMP) monoclonal antibody (mAb) reduces brain METH and AMP concentrations in pregnant rats

Student Oral Communications II (Chair: Alice Young)

3:30 pm - 3:45 pm	***Oscar Torres***, University of Texas at El Paso Nicotine withdrawal enhances anxiety-like behavior and expression of stress-related genes in female versus male rats
3:45 pm - 4:00 pm	***Luis Natividad***, University of Texas at El Paso Nicotine withdrawal produces fewer decreases in extracellular dopamine levels in the nucleus accumbens of adolescent versus adult rats
4:00 pm - 4:15 pm	***Paul Romanowich***, University of Texas Health Science Center at San Antonio The effect of ascending and descending schedules of incentives on smoking behavior
4:15 pm - 4:30 pm	***Antoniette Maldonado***, University of South Florida Binge ethanol during adolescence increases sweetened voluntary ethanol, but not saccharin, intake in young adulthood in male and female rats
4:30 pm - 4:45 pm	***Amanda Lipsitt and Hector Palacios***, University of Texas Health Science Center at San Antonio Mitochondria as a primary target for liver and neuronal lesions during chronic ethanol feeding in cynomolgus macaque monkeys

Saturday March 21, 2009 (continued)

Poster Session (5:00 pm - 7:00 pm)

Dinner (7:00 pm - 11:00 pm)

Recognition of travel awardees and presentation of awards.

After Dinner Lecture (Chair: WIlliam Clarke)

Terry Kenakin: "The Chemist-Biologist Interface in Drug Discovery: Forming a Basis of Understanding"

GlaxoSmithKline Research and Development Laboratories at Research Triangle Park, NC

Hospitality and Entertainment

Live music will be provided by the Tennessee Valley Authority (TVA). Come and enjoy the fun!

Sunday March 22, 2009

Open Oral Communications I (Chair: Lisa Gerak)

8:00 am- 8:15 am	***David Linsenbardt***, Binghamton University Agonism of the endocannabinoid system modulates binge-like alcohol intake in male C57BL/6J mice: involvement of the posterior ventral tegmental area
8:15 am - 8:30 am	***James Orfila***, University of Texas at El Paso The behavioral and neurochemical effects produced by kappa-opioid receptor stimulation are diminished in nicotine-dependent adolescent versus adult rats
8:30 am - 8:45 am	***Stephanie Groman***, University of California at Los Angeles Differences in D2 receptor availability and behavioral flexibility as a function of trait impulsivity in monkeys
8:45 am - 9:00 am	***Alex James***, University of California at Los Angeles Alpha-2A adrenoceptors play a role in behavioral flexibility in mice, but are not required for improvements caused by atomoxetine
9:00 am - 9:15 am	***Daniela Pereira***, University of Texas Health Science Center at San Antonio Salt addiction: the role of age

Sunday March 22, 2009 (continued)

Open Oral Communications II (Chair: Galen Wenger)

9:35 am - 9:50 am	***Stephen Brimijoin***, Mayo Clinic Chronic reduction of cocaine action in neostriatum by transduced hydrolase - a gene therapy approach
9:50 am- 10:05 am	***Edward Castañeda***, University of Texas at El Paso Cross-sensitization of rotational behavior and exocytotic dopamine release evoked by electrical stimulation in rats displaying amphetamine-induced sensitization
10:05 am - 10:20 am	***Martin Javors***, University of Texas Health Science Center at San Antonio Phosphatidylethanol levels in red blood cells as a marker of ethanol consumption
10:20 am - 10:35 am	***Manual Miranda***, University of Texas at El Paso Regulation of activity of the dopamine and glycine transporters by phosphorylation and ubiquitination
10:35 am - 10:50 am	***Neil Paterson***, PsychoGenics The triple uptake inhibitors DOV216,303 and JZAD-IV-22 differ in their discriminative stimulus and locomotor stimulant properties despite similar potency at three monoamine transporters
10:50 am - 11:05 am	***Andrew Coop,*** University of Maryland School of Pharmacy Therapeutics to prevent methamphetamine neurotoxicity

Special Lecture 11:30 am - 12:30 pm (Chair: John Roache)

Thomas Kosten: "Pharmacotherapy of Stimulants: From Antabuse to Vaccines"

Baylor College of Medicine and Michael E DeBakey VA Medical Center

Lunch 12:30 pm - 1:30 pm

Presentation of awards for oral and poster presentations

Closing Remarks and Adjournment

See you at BBC 2010!

Abstracts

Oral Communications

1

Variation in dopamine transporter gene associated with stimulant effects of d-amphetamine - a pharmacobehavioral genetic study with healthy volunteers

Ajna Hamidovic[1], Andrea Dlugos[1], Abraham A. Palmer[1,2], Harriet de Wit*[1]
1 Dept of Psychiatry, University of Chicago, Chicago, IL 60637, USA, 2 Department of Human Genetics, University of Chicago, Chicago, IL 60637, USA

Individuals vary in their subjective responses to stimulant drugs, due in part to variability in many genes, potentially including the dopamine transporter gene (*DAT1*). In the present study, we evaluated associations between mood, cognitive and cardiovascular responses to *d*-amphetamine and four polymorphisms in *DAT1*: rs3756450, rs460000, rs37022 and rs6869645. Healthy Caucasian male and female volunteers (N=152) participated in a double-blind, crossover design study where they received placebo, *d*-amphetamine 10mg and *d*-amphetamine 20mg. Following capsule administration, we measured self-report ratings of drug effects, performance on the Digit Symbol Substitution Task, blood pressure and heart rate. We first evaluated whether the genotypic groups differed on any measures in the absence of any drug administration, and then whether the genotypic groups differed in their responses to acute doses of *d*-amphetamine (10 or 20 mg) relative to placebo. In general, the genotypic groups did not differ in the placebo condition. However, subjects homozygous for the C allele of rs460000 exhibited a pattern of enhanced responsiveness to the stimulant and euphoric effects of acute amphetamine. Specifically, individuals with the C/C genotype (N=83) reported approximately twofold higher ratings of stimulation and euphoria relative to the A/A+A/C (N=69) group interestingly after both 10mg and 20 mg doses of the drug. The groups did not differ on either cognitive or cardiovascular responses to the drug. None of the remaining SNPs were associated with responses to *d*-amphetamine. In fact, recent findings implicate a SNP that is in perfect linkage disequilibrium with rs460000 in the etiology of Attention Deficit Hyperactivity Disorder. The current findings have important implications for understanding genetic determinants in variability in stimulant response.

2

Individual differences in sensitivity to cocaine and the potential role of disparities in 5-HT_1 and 5-HT_2 receptors.

Sherrill, Luke K[1] and Gulley, Joshua M[1,2]
[1]Department of Psychology and [2]Neuroscience Program, University of Illinois Urbana-Champaign, Champaign, IL USA;

Animals, like humans, exhibit marked individual differences in response to psychostimulants. For example, based on their locomotor response to cocaine, outbred rats can readily be classified as low or high cocaine responders (LCRs or HCRs, respectively). LCRs subsequently exhibit enhanced cocaine-induced sensitization, conditioned place preference, and self-administration under progressive ratio schedules of cocaine reinforcement. Here, we describe studies investigating if differences in 5-HT_1 and 5-HT_2 receptors in LCRs compared to HCRs contribute to the disparate behaviors we observe in these phenotypes. Adult, male Sprague-Dawley rats were first characterized as LCRs or HCRs based on their response to 10 mg/kg (i.p.) cocaine in an open-field arena. In experiment 1, rats were trained to discriminate cocaine from saline in a two-lever drug discrimination task. In experiment 2, a separate group of rats was trained in a delay-discounting task that assesses impulsive choice. The effect of pharmacological challenges with amphetamine (AMPH), cocaine and 5-HT_1- or 5-HT_2-selective ligands was also determined. Our results from experiment 1 indicate that low initial sensitivity to cocaine is associated with enhanced sensitivity to the interoceptive cue produced by low doses of cocaine. In addition, we found that LCRs, compared to HCRs, were even more sensitive to the cocaine cue when cocaine was given with the 5-HT_2 agonist DOI. In experiment 2, we found that LCRs chose the large reward more frequently across longer time delays and thus made less impulsive choices compared to HCRs. In addition, impulsive choice was selectively increased in LCRs following injections of AMPH or the 5-HT_{1A}-selective ligand 8-OH-DPAT. Taken together, our findings indicate that LCRs, compared to HCRs, display enhanced sensitivity to interoceptive cocaine cues and reduced impulsive behavior. In both cases, 5-HT receptor-selective compounds had a greater effect on these behaviors in LCRs. Thus, we hypothesize that phenotype differences in the function of 5-HT_1 and/or 5-HT_2 receptors underlie the differences in cocaine-induced locomotion after a single cocaine injection and also contribute to the enhanced vulnerability of LCRs to addiction-related behavior following repeated cocaine exposure.

3

Molecular events in the expression of cocaine CPP.

dela Cruz, Adriane M; Moron, Jose A; Cunningham Kathryn A.
Center for Addiction Research, UTMB, Galveston, TX

Early learning events in the progression of cocaine use to dependence can be modeled using the single-trial conditioned place preference (CPP) model. Identifying the molecular neuroadaptations that underlie this persistent memory is critical to understanding the ability of cocaine-associated environments to stimulate craving and relapse in humans. Altered phosphorylation and expression of p42/44 MAP kinase (ERK) and the AMPA glutamate receptor subunit 1 (GluR1) in several brain areas has been suggested as critical to memory development and expression. To test the hypothesis that these neuroadaptations are critical early cocaine learning events, we investigated the expression and phosphorylation of ERK and GluR1 in total homogenate and synaptosome-enriched fractions of brain tissue isolated from rats behaviorally described to express a single-trial cocaine CPP. Male rats were conditioned with a single pairing of cocaine (20 mg/kg) or saline (1 ml/kg) in an unbiased CPP apparatus and sacrificed immediately following a 15 min test session. Western blots were used to detect the expression level of total and phosphorylated (activated) GluR1 and ERK in each fraction. Animals conditioned with cocaine that met a statistically verified CPP criterion spent significantly more time in the cocaine-paired chamber (448 ± 35 sec, mean ±SEM) upon test than did control animals (249 ± 30 sec, $p<0.01$) or animals treated with cocaine that did not meet criterion (293 ± 15 sec, $p<0.01$). In total homogenate and synaptosome-enriched fractions isolated from the PFC, we observed no changes in the activation or expression of ERK but decreased expression of GluR1 protein in all rats conditioned with cocaine, regardless of CPP expression ($p<0.05$ vs. control). In the synaptosome-enriched fraction isolated from the hippocampus, a trend ($p=0.07$) towards decreased ERK phosphorylation was seen in rats that expressed a cocaine CPP; no other changes were observed. No differences were observed in the fractions isolated from the amygdala. These data suggest that the molecular neuroadaptations that occur in this model of early cocaine associated memory are subtle. Identifying these neuroadaptations involved in early learning is critical to developing new medications to support abstinence from cocaine taking in the face of environmental triggers.

4

Dopamine Induced Synaptic Plasticity in the Rat Amygdala in Saline- and Cocaine-Treated Animals Undergoing Conditioned Place Preference.

Krishnan Balaji, Genzer Kathy M, Pollandt Sebastian W, Centeno Marjorie, Liu Jie, Gallagher Joel P and Shinnick-Gallagher Patricia.
Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031, USA.

Dopamine projections densely innervate the basolateral amygdala (BLA) and both D1-like and D2-like receptor (DR) types are present in the amygdala and dopamine levels in the amygdala are increased after one month of cocaine withdrawal. Glutamate, like dopamine, plays a major role in cocaine addiction and long-term neuroplastic. Phospholipase D (PLD) an enzyme involved with numerous biological functions including exocytosis, phagocytosis and membrane trafficking is potently activated by glutamate and its activity and expression is regulated by dopamine receptors (DRs). In this study, for the first time, we report a structural and functional association between PLD, metabotropic Glutamate Receptors (mGluRs) and DRs associated with cocaine associated synaptic changes in the amygdala.

Oral Communications

5

Effects of amphetamine derivatives on tissue content of monoaminergic and amino acid neurotransmitters in mice

Kevin S. Murnane[1], Shane A. Perrine[2], William E. Fantegrossi[1,3], Matthew P. Galloway[2], and Leonard L. Howell[1,4]
1. Yerkes National Primate Research Center, Division of Neuroscience, Emory University
2. Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA
3. Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA
4. Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine

Structural derivatives of amphetamine are some of the most commonly abused drugs. Considerable evidence has shown that certain amphetamine derivatives deplete brain levels of monoaminergic neurotransmitters. For example, methamphetamine exposure can lead to persistent reductions in dopamine concentration. However, relatively little work has examined whether this effect extends to amino acid neurotransmitter systems. In this study, mice were noncontingently exposed to dosing regimens of methamphetamine, methylenedioxymethamphetamine (MDMA), or para-chloroamphetamine (PCA). Tissue content of monoamines was determined *ex vivo* using high pressure liquid chromatography whereas tissue content of glutamate and GABA was assessed *ex vivo* via proton magnetic resonance spectroscopy. Consistent with previous studies, significant reductions in tissue content of monoamines were readily apparent. Furthermore, changes in amino acid neurotransmitters were also evident. These studies demonstrate that in mice the long-term neurochemical effects of amphetamine derivative exposure extend beyond monoaminergic systems. Support provided by USPHS grants (DA 00517, RR 00165, DA 020645, RR 020146, and DA 024760).

6

Maternal administration of an anti-(+)-methamphetamine/(+)-amphetamine (METH/AMP) monoclonal antibody (mAb) reduces brain METH and AMP concentrations in pregnant rats.

White, Sarah[1]; Hendrickson, Howard[3], Gentry, W. Brooks[1,2] and Owens, S. Michael[1]
[1]Department of Pharmacology and Toxicology, [2]Department of Anesthesiology, College of Medicine; [3]Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR USA.

Pregnancy is a vulnerable period for exposure to the harmful effects of METH and AMP. A novel approach to offer protection from these drugs during pregnancy is to target METH and AMP peripherally using antibody-based therapy. These studies were designed to test the hypothesis that an anti-METH/AMP mAb could redistribute METH and AMP from susceptible organs, like the maternal and fetal brains. Pregnant rats (1-2 days before delivery) received a 1 mg/kg iv-bolus METH dose followed 30 min later by high-affinity anti-METH/AMP mAb4G9 (K_D for METH=34 nM and for AMP=51 nM) or vehicle treatment. The METH dose was sufficient to produce locomotor effects for ~2-3 hrs in male and non-pregnant female rats. Tissue samples (dam maternal and fetal blood and brain samples) were collected in groups of rats (n=4 per group) at 1, 2 and 5 hrs after METH administration. Analysis of the area under the maternal serum concentration-versus-time curve from 1-5 hr (AUC) showed METH and AMP concentrations increased by approximately 3,200% and 1,600% (respectively) in mAb-treated versus vehicle-treated rats. There was also an increase in METH and AMP serum protein binding from a negligible (~15%) to >95%. Fetal serum protein binding also significantly increased in mAb4G9-treated rats (>30%) relative to controls (~15%); however, fetal serum METH and AMP AUC did not change. Maternal METH and AMP brain concentrations were also significantly reduced at 1 and 2 hr, but by 5 hr METH and AMP concentrations were similar to control. Nonetheless, METH brain AUC decreased by 53% and 25% in mAb-treated maternal and fetal brains relative to vehicle-treated values, and AMP AUC decreased by 32% and 31%, respectively. In conclusion, maternal and fetal protection from acute METH use can be achieved with anti-METH/AMP mAb4G9 therapy. Funded by NIDA grant DA07610.

7

Nicotine withdrawal enhances anxiety-like behavior and expression of stress-related genes in female versus male rats.

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

Previous work in our laboratory has demonstrated that the behavioral effects of nicotine withdrawal are different in male and female rats from different stages of development. However, little is known about the cellular mechanisms that mediate developmental and sex differences during nicotine withdrawal. Thus, this study characterized anxiety-like behavior and gene expression of the stress-related marker, corticotropin releasing hormone (CRH) in male and female adolescent and adult rats. Animals were prepared with subcutaneous pumps that delivered either saline or a dose of nicotine that produces equivalent nicotine blood levels across these age groups. After 14 days, the pumps were removed in order to induce a spontaneous withdrawal syndrome. Twenty-four hours later, rats were tested for overt physical signs of nicotine withdrawal. Next, anxiety-like behavior was assessed using elevated plus maze (EPM) procedures where the amount of time spent in the open and closed arms of the EPM was recorded. Immediately after the behavioral tests, animals were sacrificed and the amygdala and nucleus accumbens were dissected from coronal slices. CRH mRNA expression was measured using quantitative PCR technology. The results revealed that all rats that received nicotine displayed overt physical signs of withdrawal relative to naïve controls. Nicotine-dependent female rats displayed anxiety-like behavior during withdrawal, an effect that was absent in males. Across both brain regions and age groups, nicotine-dependent females displayed an up-regulation of CRH mRNA expression relative to males. Developmental differences were only observed in the amygdala with nicotine-dependent adolescent females displaying an up-regulation of CRH mRNA expression relative to all other groups. These data suggest that nicotine withdrawal produces an up-regulation of stress-related genes that may mediate negative affective states of nicotine withdrawal in an age- and sex-dependent manner. Supported by the National Institute of Drug Abuse Grant R01DA021274.

8

Nicotine withdrawal produces fewer decreases in extracellular dopamine levels in the nucleus accumbens of adolescent versus adult rats.

Natividad, Luis A., Roman, Francisco, Tejeda, Hugo A., Torres, Oscar V., Castañeda Eddie, and O'Dell, Laura E.
Department of Psychology, The University of Texas at El Paso, El Paso, TX USA.

Previous work has demonstrated 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. Previous studies have also shown that dopamine levels in the nucleus accumbens (NAcc) are reduced in adult rats experiencing withdrawal. Thus, this study compared extracellular dopamine levels in the NAcc of adolescent and adult rats experiencing nicotine withdrawal. Animals were prepared with subcutaneous pumps that delivered an equivalent nicotine dose in these age groups. Following 13 days of nicotine exposure, rats were implanted with microdialysis probes in the NAcc and ipsilateral ventral tegmental area (VTA). The next day, dialysate levels were collected following administration of the nicotinic-receptor antagonist mecamylamine to precipitate withdrawal. Mecamylamine produced a maximal average decrease in NAcc dopamine that was lower in adolescent (20%) versus adult (40%) rats. Similar developmental differences were observed with our measures of dopaminergic (DOPAC and HVA) but not serotonergic (5-HIAA) metabolites. A follow up study compared NAcc dopamine levels in adolescent and adult rats receiving intra-VTA administration of bicuculline that reduces gamma-aminobutyric acid (GABA) inhibition of dopamine transmission. The results revealed that blockade of GABA receptors in the VTA produced a 2-fold increase in NAcc dopamine in adult but not adolescent rats. In conclusion, our results provide one potential mechanism involving dopamine that mediates developmental differences to nicotine withdrawal. Furthermore, our results suggest that GABAergic systems are underdeveloped during adolescence and this reduced inhibition of dopamine neurons in the VTA may lead to fewer changes in NAcc dopamine in young animals experiencing withdrawal. This work was supported by NIDA Grants 1F31DA021133 (LAN) and R01DA021274 (LEO) and an NIMH Grant T32MH018882 from the APA- Diversity Program in Neuroscience (LAN)

Oral Communications

9

The effect of ascending and descending schedules of incentives on smoking behavior

Romanowich, Paul J.[1] & Lamb, Richard J.[1]
[1]Department of Psychiatry, University of Texas Health Science Center San Antonio, Texas USA

Contingent incentives can effectively reduce substance abuse in a variety of populations. In smokers, ascending schedules, which begin with a small incentive and progressively increase contingent on meeting a criterion for reduced smoking, have been used to successfully reduce smoking. There is also evidence that descending schedules, where incentives begin at a large amount and decline progressively, are effective at increasing cocaine abstinence in cocaine-dependent adults. The current study examined the differential effects of contingent incentives on smoking behavior that either started with a small incentive and gradually increased (ascending group), or started with a large incentive and gradually decreased (descending group) over 3 consecutive weeks. A consistently higher proportion of participants in the descending incentive condition produced breath CO levels indicative of abstinence. Specifically, participants in the descending incentive condition had more abstinent visits during the first 5 visits of the incentive condition than those in the ascending incentive condition. Both groups showed reduced breath CO levels during a 5 visit post-incentive follow-up period. These results demonstrate both the effectiveness of contingent incentives on smoking behavior and the need for further testing on the differential effects of contingent schedules of incentives.

10

Binge ethanol during adolescence increases sweetened voluntary ethanol, but not saccharin,
intake in young adulthood in male and female rats.

Maldonado, Antoniette M., M.A., and Kirstein, Cheryl L., Ph.D.
Cognitive and Neurosciences, Dept. of Psychology, University of South Florida, Tampa, FL, USA.

Binge alcohol consumption during adolescence is a rising concern in the United States. In animalmodels, sex differences in the consequences of binge ethanol (EtOH) exposure have been observed. The present set of experiments investigated the impact of repeated binge EtOH exposure during adolescence on voluntary sweetened EtOH (Exp. 1) or saccharin (Exp. 2) intake in adulthood in male and female rats. In both experiments, adolescent rats were exposed to water or EtOH (1.5, 3.0 or 5.0 g/kg/ig) on postnatal day (PND) 28-31, 35-38, and 42-45. All rats underwent an abstinence period from PND 46 to PND 59. On PND 60-69, rats were assessed for voluntary sweetened EtOH (Exp. 1) or saccharin (Exp. 2) intake using a limited access two-bottle choice paradigm. In Exp. 1, females consumed more EtOH than males in control rats. Binge EtOH during adolescence enhanced voluntary EtOH intake (g/kg) and preference for EtOH in adulthood in both males and females. However, when equated for innate sex differences in EtOH intake, males exposed to EtOH during adolescence showed a greater relative increase in EtOH intake in adulthood as compared to their female counterparts. In Exp. 2, repeated binge adolescent exposure to water or EtOH did not alter voluntary saccharin consumption in adulthood between EtOH-treated and water-treated rats, regardless of sex. Together, the results of the present set of experiments indicate the increased EtOH intake observed in young adulthood following adolescent binge exposure to EtOH were not merely due to greater consumption of a palatable solution, but specifically to the EtOH solution. Therefore, it is not merely enhanced responsivity to natural reinforcers mediating the enhanced EtOH intake observed in adulthood in male and female rats following adolescent binge EtOH exposure. The present results further validate the importance of examining long-term consequences of adolescent EtOH exposure and sex differences in these effects on subsequent propensity for greater EtOH intake in adulthood, which could result in a number of EtOH-induced problems.

11

Mitochondria as a Primary Target for Liver and Neuronal Lesions During Chronic Ethanol Feeding in Cynomolgus Macaque Monkeys

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Mitochondria appear to be the primary target in the development and maturation of neurodegenerative diseases. Moreover, a growing body of evidence indicates that chronic alcoholism appears to be linked to dementia. However, there is no study of how mitochondrial damage occurs during chronic alcoholism. The aim of this investigation is to determine the effect of chronic alcohol feeding under mitochondrial morphology in adult Cynomolgus macaques fed 20% ethanol over two years. We investigated frontal cortex, cerebellum, and liver tissue using routine transmission electron microscopy (TEM). Ultrathin sections from the liver, cerebellum, and frontal cortex obtained from the control animal fed with water did not show any visible ultrastructural change in the morphology of mitochondria. Contrary to these observations, animals fed with 20% ethanol in the mild drinker group showed significant damage in the mitochondria in hepatocytes, a smaller degree of lesions in the mitochondria in cerebellum tissue, and severe damage in the neuronal mitochondria in the frontal cortex. After 2 years of feeding, the heavy drinker group showed that all of liver tissue had become cirrhotic and there appeared to be electron dense small mitochondria which indicated a hypoxic condition in liver. Neurons from the frontal cortex of heavy drinking animals had severely damaged mitochondria, which were mostly localized in the cell body. Our study, for the first time, demonstrates that mitochondrial pathology appears to be a primary target during alcoholism and most likely this pathway plays a key role for alcohol dependent dementia.

12

Agonism of the endocannabinoid system modulates binge-like alcohol intake in male C57BL/6J mice: involvement of the posterior ventral tegmental area.

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Recent studies have shown that the endocannabinoid system is involved in the behavioral and physiological effects of alcohol (ethanol). For example, enhancement of the endocannabinoid system through pharmacological and/or genetic manipulations increases ethanol seeking behaviors whereas inhibition of this system reduces them (Colombo *et al.*, 2007; Colombo *et al.*, 2005). Despite the increasing evidence for this system's involvement in ethanol seeking behaviors, its role in modulating binge-like intake and/or the mechanism by which it may exert these effects remains poorly understood. Using a newly developed animal model of binge drinking, dubbed 'Drinking In the Dark' (DID), we recently reported that facilitation of the endocannabinoid system with the synthetic cannabinoid agonist WIN 55-212,2 (WIN) effectively modulates binge-like ethanol intake in male C57BL/6J mice (Linsenbardt *et al*., 2008). Based on the results of these systemic (i.p.) manipulations and evidence in support of the involvement of sub regions of the Ventral Tegmental Area (VTA) in governing self-administration of ethanol (Rodd-Henricks *et al.*, 2000) as well as binge-like intake using the DID model (Moore & Boehm, *in press*), the current study extended these findings to evaluate the role of the endocannabinoid system within the posterior sub region of the VTA (pVTA) using site-specific microinjections. Intra-pVTA microinjections elicited significantly higher intake in the 90 minute bin at the two lowest doses (0.5 and 1 µg/mouse) and significantly lower intakes in the 30 minute bin at the highest dose (5 µg/mouse). Importantly, a follow-up study revealed that alterations in ethanol consumption observed at the highest WIN dose may have been influenced by competing locomotor activity. The present data are consistent with previous research in that agonism of the endocannabinoid system increases ethanol intake in ethanol preferring rodents and implicate the pVTA in the modulation of drinking to intoxication. Additionally, dose dependent locomotor alterations emphasize the importance of directly assessing possible competing behaviors when evaluating drug effects on voluntary consumption.**Acknowledgments:** This work was supported by a grant from Integrative Neuroscience Initiative on Alcoholism (INIA) – West**,** NIAAA # AA015434, and the Center for Development and Behavioral Neuroscience

Oral Communications

13

The behavioral and neurochemical effects produced by kappa-opioid receptor stimulation are diminished in nicotine-dependent adolescent versus adult rats.

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This study examined the role of kappa-opioid receptors (KORs) in mediating the behavioral and neurochemical effects of nicotine withdrawal in adolescent and adult rats. The affective properties of nicotine withdrawal were compared using conditioned place aversion (CPA). Rats were first tested for their preference for either of 2 distinct compartments of our conditioning apparatus. The next day, rats underwent sham surgeries or were prepared with subcutaneous pumps that delivered an equivalent dose of nicotine in these age groups for 14 days. Six days after pump implantation, rats received various doses of the KOR agonist U50,488 in their initially preferred compartment. The next day, they received saline in their initially non-preferred compartment. This 2-day procedure was repeated over 8 consecutive days. Following conditioning, rats were re-tested for their preference. The results revealed that KOR stimulation produced CPA in nicotine-dependent adult but not adolescent rats.

The neurochemical effects of nicotine withdrawal were assessed using in vivo microdialysis to compare dopamine levels in the nucleus accumbens (NAcc) of adolescent and adult rats. Rats were prepared with pumps containing the same nicotine doses that were used in the behavioral studies. Thirteen days after pump implantation, rats were prepared with dialysis probes in the NAcc. The next day, dialysate samples were collected every 10 min during a 1 hr baseline period and then for 4 additional hrs following administration of a dose of U50,488 that produced developmental differences in the CPA tests. The results revealed that U50,488 produced a decrease in NAcc dopamine in adults (67%) that was lower in adolescents (37%). Our results suggest that adolescent rats are less sensitive to the modulatory role of KORs in mediating nicotine withdrawal compared to adult rats. (This research was supported by DA021274)

14

Differences in D2 receptor availability and behavioral flexibility as a function of trait impulsivity in monkeys

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Introduction: Individual variation in behavioral impulsivity, and proposed underlying cognitive endophenotypes, are thought to represent vulnerability factors for substance abuse, but the molecular basis of impulsivity remains unknown. Stimulant dependent individuals exhibit lower D2-like receptor availability in the striatum and low midbrain D2-like receptor binding has been shown to relate to impulsivity novelty-seeking, as well as drug abuse vulnerability. These results suggest impulsivity may depend upon D2-like receptor function. This study examined the association of D1 and D2 receptor availability with impulsivity in non-human primates.
Methods: 10 vervet monkeys were selected from their birth cohort based on an impulsivity score derived from the Intruder Challenge Task (5 high impulsive, 5 low impulsive). Monkeys received a single PET scanning session with [^{11}C]NNC-112, to assess D1 receptor availability, and [^{18}F] Fallypride to assess D2 receptor availability. Activity was extracted using ROIs drawn on corresponding MRIs. Behavioral flexibility was assessed in these individuals using a reversal learning task.
Results: High impulsive monkeys had significantly greater normalized [^{18}F]fallypride activity in the caudate and ventral striatum, suggestive of increased D2-like receptor levels. Cortical activity is still under investigation, although preliminary results suggest increased [^{18}F]fallypride activity in the dorsal lateral prefrontal cortex. Further, high impulsive monkeys required more trials to reach criterion during the initial discrimination learning as well as during the reversal learning phase.
Conclusion: This study demonstrates dopaminergic correlates of individual variation in an ethologically-valid measure of impulsivity. Deficits in reversal learning mirror those displayed in monkeys chronically exposed to cocaine and further demonstrate a relationship between cognitive control and impulsivity. These results reveal further support for a relationship between the dopamine system, impulsivity and cognitive control and suggests that alterations in the dopaminergic system may result in poor cognitive control contributing to the impulsive phenotype.

15

Alpha-2A adrenoceptors play a role in behavioral flexibility in mice, but are not required for improvements caused by atomoxetine

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Behavioral inflexibility is thought to be a key component of the acquisition and maintenance of substance dependence disorders. Recent studies from our lab indicate that atomoxetine, and other inhibitors of noradrenaline reuptake, enhance behavioral flexibility in mice, rats and monkeys. Nevertheless, the mechanisms by which increased catecholamine signaling produce this effect are unknown. Given that alpha-2A receptors have been implicated in working memory performance, this subtype may be of important in other prefrontal cortex-dependent cognitive processes; to further characterize the role of this receptor in cognitive control, we studied operant serial reversal learning in homozygous alpha-2A null mutants, heterozygotes and wild-type littermate mice. Animals were initially trained to a performance criterion on a simple rule (e.g., respond to the left aperture to get reward). On the following day, the spatial discrimination was reversed, and mice performed under this condition over consecutive days until the same criterion was met. In a second study, saline or atomoxetine (1.0 mg/kg, s.c.) was administered 30 minutes before reversal sessions. While there was no significant effect of genotype on trials required to reach criterion during acquisition of the initial aperture-reward association, homozygotes required significantly more trials than wild-types to complete the first reversal, and heterozygotes exhibited an intermediate impairment. The second study replicated these results for homozygotes and wild-type mice, and demonstrated that atomoxetine reduces trials to reach criterion in both genotypes. These results reveal an alpha-2A gene-effect that is specific to the contingency reversal. Additionally, they indicate that atomoxetine improves behavioral flexibility in this task, but that its effects are not fully dependent on the alpha-2A receptor. Atomoxetine's effects, in fact, appear to be larger in homozygote mutants than in wild-type animals, which could be the result of either lower baseline performance or neuroadaptations triggered by the mutation; further work is needed to disentangle these possible interpretations.

16

Salt addiction: the role of age

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Adult rats submitted to repeated episodes of water deprivation (WD) increase their daily average hypertonic sodium chloride solution ingestion. This behavioral sensitization may be analogous to addiction, with salt inducing the addictive state as psychoactive drugs do. In this work, we investigated the effects of repeated episodes of WD on the enhancement of daily sodium intake in late adolescent (2 months old, mo), young adult (4 mo), and older adult (6 mo) rats. At the beginning of the experiment (n = 7-9/group), male Sprague-Dawley-Holtzman rats, all age groups, had water and 0.3 M NaCl measured daily for 6 weeks. The average fluid intake in the 1st week was considered basal intake. All age groups were submitted to 5 WD episodes, with 7-day intervals between all episodes. At the same time, fluid intake was measured in non-deprived controls from each age group of rats. Daily 0.3 M NaCl intake was not affected by repeated WD in 6 mo rats. Similar results were observed in each control group. Compared to basal intake, repeated episodes of WD increased daily 0.3 M NaCl intake in 2 mo rats from the 2nd to the 6th week (2.7±0.5, 4.8±1.0, 4.8±0.7, 6.3±1.6, 6.9±2.1, and 6.9±2.1 ml/100 g of body weight (bw), 1st to the 6th week respectively) and 4 mo rats from the 3rd to the 6th week (1.8±0.5, 3.0±0.9, 3.5±0.7, 4.8±1.1, 5.1±1.2, and 4.4±1.2 ml/100 g bw, 1st to the 6th week respectively). Daily water intake was not altered by repeated WD in any of the groups. These data show that repeated exposure to WD during late adolescence results in an early enhancement of daily salt intake compared to adults. These results are consistent with studies demonstrating that exposure to drugs of abuse, such as nicotine and amphetamine, during adolescence is associated with increased behavioral sensitization. In addition, the results show that early life experience with repeated WD like high-sodium diet, dietary sodium restriction, or multiple sodium depletions produces long-term changes in ingestive behavior.

Oral Communications

17

Chronic reduction of cocaine action in neostriatum by transduced hydrolase—A gene therapy approach.

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We used a rat model to examine whether gene transfer of a cocaine hydrolase derived from human butyrylcholinesterase (hBChE) can reduce drug actions in brain reward centers. Initially, 10^{10} plaque-forming units of a standard, early region 1 (E1)-deleted adenoviral vector encoding a quadruple mutant hBChE was injected into the tail veins of 5 rats. Four to 7 days later plasma cocaine hydrolase activity rose 25,000-fold. During this period, in a protocol that typically induces FosB expression in the caudate nucleus, these rats and "unprotected" controls given only empty vector or saline were given repeated twice-daily injections of cocaine (30 mg/kg, i.p.). FosB immunohistochemistry of striatal sections from the unprotected rats on day 8 showed many intensely FosB-reactive nuclei, while rats pretreated with active vector showed the same low levels of FosB-stained nuclei as rats never exposed to cocaine. Western blots confirmed this result. In contrast protection against cocaine-elicited FosB induction was more localized when hydrolase vector was injected directly into the ventral striatum, which generated high transgene expression in many neurons. Equally good results were obtained with systemic and local injections of a more efficient helper-dependent adenoviral vector. This advanced hdAV vector transduced hydrolase for impressively long periods of time: at least two months at high levels, with continued expression up to one year. Behavioral tests must be carried out to determine if such effects lead to lasting reductions of drug-seeking behavior.

18

Cross-sensitization of rotational behavior and exocytotic-like dopamine release evoked by electrical stimulation in rats displaying amphetamine-induced sensitization.

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The neural basis of stimulant drug addiction is believed to involve neurochemical sensitization, a strengthening of mesotelencephalic dopamine (DA) release. In the addicted human brain, DA systems are also activated by drug-paired environmental cues in the absence of drug. In rats, similar mesotelencephalic DA neurons respond to drug-conditioned cues by producing conditioned DA overflow. Cross-sensitization involving conditioned DA overflow most likely involves neurotransmission via exocytosis, which is different from the carrier-mediated mechanism by amphetamine to produce DA overflow at the DA transporter. To test the working hypothesis that exocytosis is augmented in rats sensitized to amphetamine (AMPH), we used electrical stimulation of the medial forebrain bundle to produce neostriatal DA release that mimics normal exocytosis. First, parametric studies were conducted to demonstrate that electrical stimulation of DA release, measured by in vivo intracerebral microdialysis (IVMCD), is intensity- and frequency-dependent, and calcium-mediated in rats also displaying electrically stimulated rotational behavior (ESRB). In an independent set of rats, following implantation of bipolar electrodes as before and verifying reliable ESRB across 5 days, subjects subsequently received daily injections of AMPH (1.5 mg/kg, ip) or saline (1.0 ml/kg) for 7 days. Behavioral sensitization was verified as an increase in AMPH-evoked stereotyped behavior from Day 1 to Day 7. Seven days after the last injection, animals were tested for electrically evoked DA overflow and ESRB using IVMCD procedures. Rats behaviorally sensitized to AMPH displayed greater electrically evoked DA overflow and ESRB relative to drug-naïve controls, thus demonstrating cross-sensitization of exocytotic-like DA release and concomitantly evoked behavior. The present results support the idea that presynaptic mechanisms of exocytosis become cross-sensitized and likely mediate conditioned behavioral responses that sustain stimulant drug addiction.

19

Phosphatidylethanol levels in red blood cells as a marker of ethanol consumption.

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Upon the consumption of alcohol, a chemical reaction occurs in red blood cells, catalyzed by phosphalipase D, to produce the formation of phosphatidylethanol (PEth). PEth then becomes incorporated into the RBC membrane and remains there until metabolized. A lab test for PEth levels in RBCs is not yet available in clinical pathology labs, but has been partially characterized in research labs, mostly in Europe. Markers for the detection of ethanol consumption have been used in a variety of medical and other settings, e.g., for the assessment of drinking levels in treatment studies, general medical practice, insurance industry, etc. Indirect markers such as serum GGT, serum %CDT, and mean corpuscular volume have been measured as toxic side effects of ethanol on the liver and blood, but these tests lack acceptable sensitivity and lower limit of detection. However, ethanol is metabolized directly to compounds such as ethyl glucuronide (EtG), ethyl sulfate (EtS), fatty acid ethyl esters (FAEE), and phosphatidylethanol (PEth). These direct markers appear to be more accurate than indirect markers to estimate alcohol consumption. Recent advances in analytical technology have allowed the quantification of direct markers, which account for less than 1% of the disposition of ingested ethanol. PEth appears to be a sensitive, specific, and reliable marker of recent alcohol intake. It is nonvolatile, lipid-soluble, and very stable upon storage (-80 C). The level of PEth in red blood cells is detectable after a single episode of drinking and remains detectable for up to two weeks after the last drink, depending on the amount consumed. The half life of PEth in RBCs is 4.0 ± 0.7 days. PEth levels correlate better with levels of consumption than any other marker. Recent studies suggest that sensitivity and specificity of PEth for estimation of alcohol consumption approach 100%. Finally, induction of phospholipase D appears to occur in RBCs of alcoholics, resulting in higher levels of PEth in RBCs of alcoholics than in healthy control subjects. It does not appear that drugs, age, smoking, body mass index or cirrhosis affect PEth levels, but such interferences are not well-studied. These recent studies suggest that PEth is a sensitive and specific marker for recent ethanol consumption and will be very useful for the estimation of alcohol consumption in the future.

20

Regulation of activity of the dopamine and glycine transporters by phosphorylation and ubiquitination.

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The dopamine transporter belongs to a large family of neurotransmitter transporters, the SLC6 family, that includes the norepinephrine, serotonin, glycine, and GABA transporters. It is well described that the activity of the dopamine transporter (DAT), as many other members, is tightly regulated by Protein Kinase C (PKC) in the brain and heterologous expression systems. To date, two post-translational modifications that affect the activity of DAT have been described in response to PKC activation, ubiquitination and phosphorylation. Transporter modifications happened at the plasma membrane following by DAT internalization and subsequent degradation in the lysosomes. In previous studies, we provided evidences that PKC-dependent ubiquitination of N-terminal lysine residues was the signal for transporter internalization and downregulation. In the other hand, it has been suggested that DAT phosphorylation, a process that can be induced by amphetamines, is important for dopamine efflux and reversal of the transporter. In this study, we analyzed the effects of PKC activation on DAT activity and phosphorylation of the wild type, and a DAT mutant in which ubiquitination and endocytosis was abolished by substitution of N-terminal lysines. We found that incubation of PAE cells stably expressing human DAT wild type or mutant with phorbol ester (1 μM) dramatically reduced DAT uptake activity by 50% from both cell lines. In addition, metabolic labeling with (^{32}P)-ortho-phosphate following by activation of PKC by phorbol ester resulted in phosphorylation of wild type and mutant DAT, suggesting that phosphorylation may be responsible for the reduction of DAT activity. Site-directed mutagenesis analysis is underway to analyze the role of specific phosphorylation sites on dopamine uptake and efflux. Similar findings have been obtained for the glycine transporter 1, enhanced ubiquitination and phosphorylation, and a reduction of uptake activity after PKC activation. Taken together, these data provide evidence for a role of transporter ubiquitination in internalization and phosphorylation in regulation of uptake activity.

Oral Communications

21

The triple reuptake inhibitors DOV216,303 and JZAD-IV-22 differ in their discriminative stimulus and locomotor stimulant properties despite similar potency at three monoamine transporters.

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DOV 216,303 and JZAD-IV-22 exhibit high potencies for monoamine (serotonin, norepinephrine and dopamine) transporter inhibition. Although these compounds are of interest primarily as potential antidepressants, inhibition of all three monoamine transporters, similar to cocaine, raises the possibility that these compounds may be liable for abuse in humans. Drug discrimination is a behavioral assay which can be performed in both animals and humans. The concordance of preclinical and clinical drug discrimination data indicates the highly translatable nature of the assay. Locomotor sensitization is a preclinical assay with high predictive validity for abuse liability in humans. In the present experiments, the discriminative stimulus and locomotor stimulant properties of DOV 216,303 and JZAD-IV-22 were evaluated in rodents. d-amphetamine and the selective dopamine reuptake inhibitor GBR12909 produced dose-dependent increases in cocaine-appropriate responding and fully substituted for cocaine, while morphine and nicotine failed to produce cocaine-appropriate responding. Repeated cocaine or d-amphetamine resulted in locomotor sensitization. DOV 216,303 and JZAD-IV-22 partially substituted for cocaine at doses that substantially decreased response rates. Interestingly, despite comparable efficacy in the forced swim test and similar IC_{50} values for all three monoamine transporters, JZAD-IV-22 (DAT/SERT/NET: 110nM/16nM/150nM) exhibited substantially less substitution for cocaine compared to DOV216,303 (DAT/SERT/NET: 81nM/27nM/49nM). Further, repeated DOV216,303, but not JZAD-IV-22, resulted in locomotor sensitization. These results may suggest that the abuse liability profiles of DOV 216,303 and JZAD-IV-22 are substantially different. Ongoing neurochemical and target occupancy studies seek explanations for the different abuse liability profiles of DOV216,303 and JZAD-IV-22.

22

Therapeutics to prevent methamphetamine neurotoxicity

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Sigma receptor antagonists attenuate the locomotor activity and convulsive effects seen with the stimulants methamphetamine and cocaine. The introduction of fluorine atoms was aimed to prevent rapid metabolism to inactive metabolites, and resulted in the development of novel sigma receptors antagonists with greater potency against cocaine. An extension of these studies demonstrated that sigma receptors antagonists prevent the neurotoxicity of methamphetamine both in vivo and in vitro. Thus, sigma receptor antagonists have the potential to act as antidotes to the toxic effects of stimulants, thereby improving the health of patients in treatment programs. DA-19634 (AC); DA-13978 (RR)

Abstracts

Poster Communications

1

Amphetamine-conditioned place preference is positively correlated with mGluR-dependent facilitation of burst-induced Ca^{2+} signals in VTA dopamine neurons

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The mesolimbic dopamine (DA) system plays a critical role in reward-based reinforcement learning and the development of drug addiction. Drugs of abuse may influence synaptic plasticity in DA neurons themselves as well as plasticity in their target structures. Glutamatergic inputs trigger phasic bursts of spikes in DA neurons primarily via activation of NMDA receptors. Our laboratory has recently found that long-term potentiation of NMDA receptor-mediated transmission onto DA neurons can be induced in a manner dependent on burst-induced Ca^{2+} signals. Activation of metabotropic glutamate receptors (mGluRs) facilitates these burst-induced Ca^{2+} signals via generation of inositol 1,4,5-triphosphate (IP_3) in DA neurons. Therefore, we examined how mGluR-dependent regulation of burst-induced Ca^{2+} signals is affected by repeated psychostimulant exposure *in vivo*. Rats received daily i.p. injections of saline or amphetamine (5 mg/kg) for 7 days. Midbrain slices were prepared 1 day after the final injection and electrophysiological recordings were made from DA neurons in the ventral tegmental area. A burst of 5 unclamped spikes was evoked in voltage clamp and the resulting Ca^{2+} signal was assessed by measuring the tail current through Ca^{2+}-sensitive SK channels (I_{burst}). Bath perfusion of an mGluR agonist DHPG (1 μM) increased I_{burst} by 13.8 ± 4.6% in saline-treated rats (n = 9). This effect of DHPG was dramatically augmented in amphetamine-treated rats (46.7 ± 10.8%, n = 10, $p < 0.01$). The amplitude of I_{burst} itself was not significantly different. We next examined the change in IP_3-evoked Ca^{2+} response by performing flash photolysis of caged IP_3 (100 μM) and measuring the resulting SK-mediated current (I_{IP3}). The capacitance (μF) of the capacitor feeding current to the flash lamp determined the concentration of IP_3 released. The capacitance producing half-maximal I_{IP3} was significantly smaller in amphetamine-treated rats (210 ± 22 μF, n = 8) compared to saline-treated ones (325 ± 42 μF, n = 9, $p < 0.05$), suggesting increased IP_3 sensitivity after amphetamine exposure. The maximal I_{IP3} amplitude was not significantly affected by amphetamine treatment. Finally, we conditioned rats to an amphetamine-paired environment for 7 days using a conditioned place preference (CPP) paradigm and found that the magnitude of CPP was positively

2

Alcohol inhibits serotonin clearance by a serotonin transporter independent mechanism: Are organic cation transporters the answer?

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Emotional and behavioral responses to alcohol have been attributed to ethanol increasing extracellular serotonin (5HT) in brain. This could be ascribed to blockade of the serotonin transporter (SERT) by alcohol. However, we showed that ethanol potently inhibits 5HT clearance in hippocampus of SERT-knockout (-/-) mice suggesting the involvement of another transport mechanism (Daws et al., 2006). A growing literature supports a role for the organic cation transporter 3 (OCT3) in mediating 5HT neurotransmission. Consistent with such a role, we found that OCT3 expression and function is increased in SERT-/- and heterozygous (+/-) mice, that have 50% fewer SERTs than wildtype (+/+) mice. Moreover, this corresponded to greater inhibition of 5HT clearance after blockade of OCT3 with corticosterone in +/- and -/- mice (Baganz et al., 2007, SfN Program no. 248.19). The remarkable similarity in the effects of corticosterone and ethanol on 5HT clearance among SERT genotypes led us to reason that OCT3 could be a site of action for ethanol. To test this hypothesis we locally applied ethanol into hippocampus in combination with selective blockers of OCT3 (corticosterone) or SERT (fluvoxamine). As expected, in +/+ but not SERT-deficient mice, co-administration of ethanol and fluvoxamine inhibited 5HT clearance more robustly than either drug alone. Importantly, in no SERT genotype did corticosterone given with ethanol produce a greater net inhibition of 5HT clearance compared to either drug given separately, a finding consistent with corticosterone and ethanol having a common action at OCT3. Collectively, these data suggest that OCT3 mediates, at least in part, the effect of ethanol to inhibit 5HT clearance. These findings are intriguing given evidence that humans expressing fewer SERTs are predisposed to alcoholism and are resistant to SSRI treatment. OCT3 may therefore be a novel target for treatment of alcoholism and addiction-related disorders, especially for individuals who respond poorly to currently available therapies.

3

Training dose alters the potency but not the substitution profile in rats discriminating pregnanolone

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The behavioral effects of neuroactive steroids are thought to be mediated by positive modulation of GABAa receptors; however, other receptors may also be involved. Drug discrimination is a pharmacologically selective method to explore potential mechanisms of action of drugs. The purpose of this study was to explore the discriminative stimulus effects of different doses of pregnanolone and to examine the role of GABAa receptors in the behavioral effects of pregnanolone. Two groups of male Sprague-Dawley rats (n=14) were trained to discriminate pregnanolone while responding under a fixed ratio 10 schedule of food presentation. One group started at a training dose of 3.2 mg/kg (i.p.), which gradually decreased to 1.33 mg/kg, and the other group started at a training dose of 3.2 mg/kg, which gradually increased to 7.5 mg/kg. Substitution tests were conducted with the benzodiazepines flunitrazepam and midazolam, and the opioid agonist morphine. In both groups of rats, cumulative doses of pregnanolone, flunitrazepam and midazolam produced greater than 80% pregnanolone-lever responding; in contrast, cumulative doses of morphine produced no pregnanolone-lever responding up to rate-decreasing doses. Doses of pregnanolone, flunitrazepam and midazolam needed to produce greater than 80% pregnanolone-lever responding were smaller in rats discriminating 1.33 mg/kg of pregnanolone as compared to rats discriminating 7.5 mg/kg. These findings suggest that substitution profiles are qualitatively similar regardless of training doses, indicating a predominant role of GABAa receptors in the discriminative stimulus effects of pregnanolone. Although these data do not exclude the possibility of receptors other than GABAa receptors being involved in the discriminative stimulus effects of pregnanolone, the likelihood is reduced.

4

Food restriction differentially modifies the behavioral effects of the dopamine receptor agonist quinpirole in rats

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In order to better understand how nutrition impacts the effects of drugs acting on DA systems (e.g., cocaine), the current study compared the effects of food restriction on the discriminative stimulus and yawning effects of quinpirole. Six free-feeding male Sprague Dawley rats responded under a schedule of shock avoidance and discriminated between saline and 0.032 mg/kg quinpirole in multiple cycle sessions. Each cycle consisted of 10 trials and began with a 10-min timeout followed by illumination of a house light that signaled the delivery of a shock stimulus every 10 s; a response on the injection-appropriate (correct) lever or the passage of 30 s turned off the house light, ended the trial, and initiated a 30-s timeout. Saline and 0.0032 mg/kg quinpirole occasioned responding predominantly on the saline-associated lever, whereas larger doses of quinpirole (.01 and .032 mg/kg) increased responding on the quinpirole-associated lever. In the same rats, quinpirole induced yawning, yielding an inverted-U shaped dose-response curve. During food-restriction, (10 g/day for 7 days), quinpirole failed to induce any yawning whereas the quinpirole discrimination dose-response curve was not different from the dose-response curve determined when rats were free-feeding. Since food restriction differentially modifies behavioral effects of quinpirole, these behavioral effects might be mediated by different DA receptors. Although it is generally believed that D2 receptors mediate the discriminative stimulus effects of quinpirole, most of those data were obtained in food-restricted rats. That quinpirole discrimination in free-feeding rats might be mediated by D3 receptors is supported by the finding that the non-selective D2/D3 antagonist raclopride, but not the selective D2 antagonist L-741,626, shifted the quinpirole discrimination dose-response curve to the right in free-feeding rats. These findings suggest that the relative contribution of D2 and D3 receptors to the behavioral effects of drugs acting on DA systems is impacted by diet. CPF is supported by a Senior Scientist Award (DA17918).

Poster Communications

5

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 (24), 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 learning may shed light on the cellular mechanisms of opioid stimulation that lead to long lasting synaptic plasticity. (Supported by the Ewing Halsell Endowment, The Texas Consortium in Behavioral Neuroscience T32-MH065728 and the Alfred P. Sloan Founda-

6

The rewarding effects of alcohol are enhanced in female versus male rats

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Epidemiological studies have demonstrated that there are major differences in alcohol intake and dependence among women and men in a clinical setting. However, the mechanisms that mediate sex differences to alcohol abuse are not well understood. Pre-clinical rodent studies have shown that female rats consume more ethanol and show less symptoms of withdrawal relative to male rats. The goal of this study was to compare the rewarding effects of ethanol in adult female and male rats using conditioned place preference (CPP) procedures. Briefly, male and female Wistar rats were tested for their initial preference for either of 2 distinct compartments of our conditioning apparatus. Six days later, conditioning was conducted over 10 days where separate groups of rats (n=8-14) received a systemic injection of ethanol (0, 0.5, 1.0 and 2.0 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, rats were given free access to both compartments and the amount of time spent on each side was recorded. A CPP was operationally defined as a significant increase in the amount of time spent in the initially non-preferred side after conditioning with repeated ethanol injections relative to control rats that received saline during conditioning.
In general, the results revealed that the rewarding effects of ethanol are enhanced in female versus male rats. Ethanol induced a CPP in both male and female rats in an inverted U-shaped dose-response manner, with the intermediate dose producing rewarding effects and the high dose producing aversive effects. Overall, the dose-response curve was shifted upwards in female rats, suggesting that the rewarding effects of ethanol are enhanced in female versus male rats. Also, a high dose of ethanol produced aversive in male rats, but this effect was not observed in female rats. Our findings suggest that the rewarding effects of ethanol are enhanced, whereas the aversive effects of are reduced in female versus male rats. These findings have implications for understanding enhanced vulnerability to alcoholism in females. (Supported by the NIH/NIGMS/Bridges to the Baccalaureate Program (2R25GM049011; AM) and the Minority Access to Research Careers Program (BSB).

7

Biochemical Characterization of AMPA Glutamate Receptors in the Hippocampus: Study of Morphine-induced Neuroadaptations

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Repeated administration of morphine causes long-lasting effects, both at the behavioral and molecular level. These neuroadaptations include changes in α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) function, which is important for glutamanergic neurotransmission. Previous studies in our laboratory have found that 12 hours after a behaviorally-sensitizing morphine administration paradigm there is an increased expression of AMPARs lacking glutamate receptor 2 (GluR2-lacking) in the hippocampus, an area important for learning and memory. This increase in GluR2-lacking receptors suggests that morphine treatment leads to changes in AMPARs composition. The current study focuses on determining changes in localization of the different AMPAR subunits (GluR1-3) at the synapse and in the composition of the receptors in the hippocampus that may be responsible for the observed morphine-induced neuroadaptations. We have adapted receptor crosslinking to examine changes in AMPAR surface expression and quantitative co-immunoprecipitation has been applied to examine changes in AMPAR composition. Our hypothesis is that repeated morphine administration alters the expression and composition of AMPA glutamate receptors and that these effects persist overtime and could be responsible for long-term behavioral sensitization induced by repeated morphine administration. This study elucidates key mechanisms underlying neuroadaptive changes in the hippocampus and behavioral responses that occur upon repeated morphine exposure.

8

RGS9-2 affects synaptic plasticity and calcium signaling.

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Dopaminergic transmission plays a pivotal role in the modulation of striatal function within the motor system. Lack of dopamine release from the substantia nigra underlies Parkinson's disease that is typically treated with dopamine receptor agonists. On the other hand, side effects of neuroleptic therapy employing dopamine D2 receptor (D2R) antagonists include dyskinesias.
Regulator of G protein signaling 9, isoform 2 (RGS9-2) is a GTPase-accelerating protein that modulates the intracellular signaling pathway of D2R and is specifically co-expressed with D2R in striatum. Previous studies have shown that RGS9-2 knock out mice display enhanced dyskinesia after treatment with D2R agonists and it seems that RGS9-2 plays a more important role in striatal signaling that previously recognized. Its molecular role in dopaminergic control of striatal function is still unresolved.
In a screening approach, we subjected striatal tissue of RGS9-2 knock out mice to genome-wide expression profiling using Affymetrix microarray technology. Expression levels of selected genes were subsequently quantified by real-time PCR. The expression data revealed a significant downregulation of genes involved in long term depression, long term potentiation and calcium signaling. These alterations were also found in protein expression levels using western blot analysis. In addition western blot analysis revealed abnormal phosphorylation of ERK1, ERK2 and DARPP32, a key signaling molecule integrating dopaminergic and glutamatergic signals, in RGS9-2 knock out mice. These finding suggest that RGS9-2 may not only modulate D2R signal transduction but also influence glutamate receptor signaling and neuronal plasticity.

Poster Communications

9

Novel positive allosteric modulators of $GABA_B$ receptors: in vivo activity.
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$GABA_B$ receptors are potential targets for novel treatments of psychiatric disorders. Positive allosteric modulators at these receptors could have fewer side effects than agonists such as baclofen. However, it is largely unknown if positive $GABA_B$ modulators identified in vitro also act as positive $GABA_B$ modulators in vivo. As part of an effort to characterize the in vivo activity of $GABA_B$ modulators, we studied their effects on body temperature. The compounds were administered i.p. to C57BL/6J mice and body temperature was assessed at different times after the injection. We examined the dose- and time-dependency of the hypothermic effects of baclofen, gamma-hydroxybutyrate (GHB), and the positive $GABA_B$ modulators CGP7930 and GS39783. Baclofen, CGP7930, and GS39783 were examined also in the presence of the $GABA_B$ antagonist CGP35348. Results obtained to date show that baclofen, GHB, CGP7930, and GS39783 all produced hypothermia, but differed in potency (i.e., baclofen > GHB > GS39783 > CGP7930) and onset of action (time to peak effect: 60, 30, 120, and 120 min for baclofen, GHB, GS39783, and CGP7930, respectively). CGP35348 appeared to shift the baclofen dose-response curve 3-fold to the right.
The positive allosteric $GABA_B$ modulators produced hypothermia, like baclofen. We are currently studying whether these hypothermic effects are also mediated by $GABA_B$ receptors. Future studies will examine if the modulators can enhance baclofen-induced hypothermia at doses that are inactive when given alone. A detailed characterization of the in vivo effects of $GABA_B$ receptor modulators will help to assess whether such compounds have fewer side effects than direct acting $GABA_B$ receptor agonists. Supported by U.S. Public Health Service Grant DA15692

10

Discriminative Stimulus Effects and Receptor Binding of Dimethyltryptamine.
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Dimethyltryptamine (DMT) is a short-acting hallucinogen that has been used recreationally for many centuries, yet still little is known about the discriminative stimulus effects and mechanisms of action. The subjective effects of DMT are qualitatively different than classical hallucinogens. DMT binds at the sigma 1 receptor and to the $5\text{-}HT_{2A}$ receptor, the site of action for most classical hallucinogens. Drug discrimination (DD) and radioligand displacement binding (RDB) were used to assess the possible mechanisms of action. In DD rats were trained to discriminate DMT from saline and tested for similarity to LSD, DOM, (+)-methamphetamine, and (±)-MDMA. The cross-substitution of DMT was also tested. A 10 μM concentration of DMT was used for RDB in HEK293 cell lines expressing $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$, sigma 1, and D_4 receptors, and in Xenopus oocytes expressing mouse $5\text{-}HT_{3A}$ receptors. LSD, DOM, and MDMA all fully substituted in DMT-trained rats, whereas DMT fully substituted only in DOM-trained rats. Methamphetamine did not share discriminative stimulus effects with DMT. DMT bound to the $5\text{-}HT_{3A}$ receptor with a K_i = 4.72 μM. The 10 μM concentration of DMT had substantial inhibition of radioligand binding at the $5\text{-}HT_{2A}$ (7%) and $5\text{-}HT_{2C}$ (10%) receptors and sigma 1 (45%) receptor, with less inhibition at the $5\text{-}HT_{1A}$ (73%) and D_4 (64%) receptors. DMT produces discriminative stimulus effects most similar to those of DOM, with some similarity to the discriminative stimulus effects of LSD and MDMA. Like DOM and LSD, DMT seems to produce predominately hallucinogenic-like discriminative stimulus effects and minimal psychostimulant effects. DMT not only binds at $5\text{-}HT_{2A}$ receptors, but to $5\text{-}HT_{2C}$, $5\text{-}HT_{3A}$, and sigma 1 receptors, one or more of which may contribute to the different subjective effects of DMT.

11

Motor Response to Acute Ethanol Exposure in Adolescent Rats
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Alcohol use starts during adolescence for many people. Those who use alcohol during adolescence may be more likely to abuse alcohol later in life when compared to those who initiate alcohol consumption as adults. Ongoing research examines patterns of alcohol abuse development employing adolescent animal models. The present study examines the impact of acute ethanol exposure on locomotor activity during early adolescence in male rats. Eighteen male Sprague-Dawley rats (post-natal day 30) were placed in a circular open field for 50 minutes to habituate animals to the novel environment. Rats were acutely administered ethanol (0.75 or 1.5 g/kg/ip; 17% v/v) or an isovolumetric administration of saline. Rats were immediately returned to the open field for an additional 60 minutes to assess ethanol-induced locomotor activity. The experiment is currently being conducted and results will be discussed. Based on previous research, it is expected that rats will express stimulatory effects of ethanol at the lower dose and sedative effects at the higher dose. This study used moderate doses that have not been used previously

12

A modified attentional set-shifting test may enable assessment of perseverative errors in rodents.
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Executive functions (EFs) are cognitive processes necessary for complex behavior. Current definitions of EFs include processes of attentional selection, behavioral inhibition and decision-making. Studies of EFs in humans with the Wisconsin Card Sorting Test (WCST) have shown that set-shifting ability and behavioral inhibition are impaired in populations with brain diseases that affect the frontal lobes (e.g. schizophrenia, Alzheimer's disease), and in some drug addicts (e.g. alcoholics, opioid abusers). The most prominent changes in performance in these populations are increased perseverative errors. Birrell & Brown (2000) developed an attentional set-shifting task (ASST) to measure EF in rodents. In this procedure, rats are trained to dig in cups for food. The cups are presented as pairs different in both digging medium and odor, and on each trial only one stimulus (medium or odor) is paired with food. The rats learn to select the baited cup by either medium or odor. The test session contains a series of discriminations: compound discrimination (CD), intradimensional (ID) shift, extradimensional (ED) shifts and one reversal following each stage. We modified the ASST to measure the perseverative errors captured by the WCST. Modifications include addition of a third cup, changed stages (CD, ID1, ED1, ID2, and ED2), use of the same exemplars in all stages, and collection of measures other than trials to criterion. Measures include total errors, perseverative errors and non-perseverative errors, categories achieved, failure to main set, and omission. Trials to criterion in the ASST and the modified set-shifting and perseveration test (SSPT) were similar in CD, ID1, and ED1. In SSPT, however, rats could not achieve criterion in ID2, even after 50 trials. This failure in ID2, which was expected to be easier than the ED shift in ASST, may result from the S+ to S- and S- to S+ reversals incorporated in the set-shifting stages of SSPT and WCST. We were able to differentiate error types (e.g. perseverative errors) in all stages except CD. Further studies regarding the sensitivity of SSPT to pharmacological manipulation are warranted. *Supported by TTU Research Enhancement Funds.*

Poster Communications

13

Functional Selectivity of Agonists and Inverse Agonists.

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Functional selectivity is a term that has typically been used to describe the ability of agonists to differentially regulate cellular signal transduction pathways coupled to a single receptor. In fact, some of the original names for this phenomenon include "agonist-directed trafficking of receptor stimulus" and "biased agonism" which emphasize the applicability to agonists. However, multi-state receptor models predict that inverse agonists also should also display selectivity toward signaling mechanisms which would be a consequence of relative differential affinities of a drug for the various active and inactive receptor conformations. We have tested the effects of a series of agonists and inverse agonists across a range of cellular responses coupled to the $5HT_{2C}$ receptor. Our data support the contention that functional selectivity extends beyond the actins of agonists and should be considered a "ligand" phenomenon.

Therapeutic benefit is often ascribed to the degree of selectivity of a drug and adverse effects are generally attributes to actions at non-target receptors. Functional selectivity of ligands means that ligands have more selectivity than that afforded by differential affinity for different receptor subtypes. Ligands can selectively regulate the activity of each of multiple signaling pathways coupled to a single receptor subtype. Therefore, it may be possible to improve the therapeutic / adverse effect ratio by developing drugs that have greater selectivity for signaling pathways that lead to beneficial effects while minimizing activity toward pathways that may lead to adverse effects.

14

Nicotine, varenicline, and cytisine decrease operant responding through mecamylamine sensitive receptors in mice

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Cigarette smoking is a leading preventable cause of death in the United States and there is a compelling need to develop effective pharmacotherapies to promote smoking cessation. In addition to nicotine, other nicotine acetylcholine receptor agonists have been used to promote abstinence in cigarette smokers, yet relatively little is known about the in vivo pharmacology of non-nicotine pharmacotherapies. This study compared the time course and nicotine receptor mechanism(s) underlying the behavioral effects of nicotine, varenicline and cytisine. Male C57BL/6J mice (n=7) responded on a fixed ratio 30 schedule of food delivery and received the nicotine agonists alone and in combination with a nicotine antagonist (mecamylamine). The agonists dose-dependently reduced responding; nicotine was more potent (i.e., maximally reduced responding at 1.78 mg/kg) than varenicline and cytisine, which were equipotent (i.e., each maximally reduced responding at 5.6 mg/kg). The agonists had a similar time course of activity, including a rapid onset (less than 5 min) and relatively short duration of action (40 min). Mecamylamine, while having no effect alone, dose-dependently and surmountably antagonized the rate-decreasing effects of nicotine, varenicline, and cytisine. The potency of mecamylamine was similar for antagonizing the rate-decreasing effects of the three nicotine agonists. These results suggest that nicotine, varenicline, and cytisine decrease fixed ratio responding for food through mecamylamine-sensitive receptors. These results further suggest that any difference in the clinical effectiveness between nicotine and the other agonists is not due to a difference in their time course or receptor site(s) of action. Supported by United States Public Health Service grants DA19222 and DA25267.

15

Effects of pregnanolone, flunitrazepam, and ketamine on response rates before and during chronic pregnanolone treatment in rats.

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Chronic treatment with benzodiazepines alters $GABA_A$ and NMDA receptors leading to the development of tolerance and dependence. Because neuroactive steroids are positive modulators of $GABA_A$ receptors and have additional actions at NMDA receptors, there is reason to believe that chronic treatment could have similar effects. Male Sprague-Dawley rats (n=7) were treated daily with pregnanolone to assess the development of tolerance and dependence. Rats responded under a FR10 schedule for food reinforcement; dose-effect curves were determined for pregnanolone, flunitrazepam, and ketamine. Daily treatment began at 5.6 mg/kg/day (i.p.) pregnanolone and increased over 2 months to 25.6 mg/kg/day. Dose-effect curves were determined under two conditions: either 1 hour following pregnanolone treatment or 25 hours after the last pregnanolone treatment. Acutely, pregnanolone had maximal rate increasing effects at a dose of 5.6 mg/kg, and larger doses decreased rates in a dose-dependent manner. Flunitrazepam and ketamine had rate decreasing effects only, and doses of 3.2 and 5.6 mg/kg respectively, eliminated responding. The potency of pregnanolone and flunitrazepam remained the same during treatment as compared to potency before treatment, but there was a 3-fold shift to the right in the ketamine dose effect curve during chronic treatment. After 5 months of daily treatment, tolerance to the rate increasing effects of pregnanolone developed. Dependence, which would be evident by a disruption in response rates following a temporary suspension in treatment, did not develop during chronic pregnanolone treatment. Under these chronic treatment conditions, modest tolerance to pregnanolone developed although there was no evidence for dependence. Taken together with changes in sensitivity to ketamine, these results suggest that adaptations at both $GABA_A$ and NMDA receptors occurred during treatment.

16

Effects of amphetamine derivatives on passive avoidance learning and delayed matching to position performance in mice.

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Chronic exposure to the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) elicits behavioral effects consistent with decrements in memory in human users as well as laboratory rats and monkeys, but few such studies have been performed in the mouse. We tested separate groups of mice in a passive avoidance procedure or under a delayed matching to position procedure after administration of 4 discrete injections (one injection administered every 2 hours) of MDMA (10 or 20 mg/kg), methamphetamine (METH; 10 or 20 mg/kg), parachloroamphetamine (PCA; 5, 7.5 or 10 mg/kg), or saline vehicle. The highest dose of each amphetamine derivative produced approximately 50% lethality 24 hours after the final injection, decreased body weight, and induced a transient hyperthermic effect. In saline control mice, a single pairing of a 0.3 mA electrical stimulus with the dark side of a shuttle box increased step-through latency upon subsequent tests, but mice treated with either of the doses of METH, or the highest dose of PCA, exhibited disrupted passive avoidance learning upon subsequent trials. Neither dose of MDMA altered passive avoidance learning in the mouse. Prior to drug administration in the matching to position task, mice exhibited delay-dependent performance, achieving close to perfect performance at short delays (1 and 2 sec) and working at chance performance at the longest delay (11 sec). Each of the amphetamine derivatives suppressed responding under the delayed matching to position schedule on the first few days after the dosing regimen, but when behavior returned to pre-drug baseline (within 2 to 5 days), no specific effects consistent with memory decrements were observed. These data suggest that amphetamine derivatives may alter long- but not short-term memory in the mouse.

Poster Communications

17

Differential attenuation of the discriminative stimulus effects of benzodiazepines and neuroactive steroids by flumazenil or pentylenetetrazole in diazepam-treated rhesus monkeys

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Despite their similar behavioral effects, the potency of positive $GABA_A$ receptor modulators acting at benzodiazepine or neuroactive steroid sites is differentially modified by chronic diazepam treatment. Changes in $GABA_A$ receptors that account for these differences are not known. One behavioral approach that can begin to elucidate these changes is to determine whether chronic treatment alters the nature of interactions between drugs acting at the same or different sites. Two groups of four monkeys were used: one group received 5.6 mg/kg of diazepam daily and discriminated 0.1 mg/kg of flumazenil, a neutral modulator acting at benzodiazepine sites, and the other untreated group discriminated 0.178 mg/kg of the benzodiazepine midazolam. The training dose of flumazenil or 32 mg/kg of pentylenetetrazole (a negative modulator that does not act at benzodiazepine or neuroactive steroid sites) produced >80% flumazenil-lever responding, and the effects of each were reversed by positive modulators acting at benzodiazepine or neuroactive steroid sites (midazolam and pregnanolone, respectively). When a larger dose of flumazenil was administered, a larger dose of midazolam was needed to reverse flumazenil-lever responding; however, increasing the dose of flumazenil did not change the dose of pregnanolone needed to produce the same effect. When a larger dose of pentylenetetrazole was administered, larger doses of both positive modulators were needed to reverse flumazenil-lever responding. In untreated monkeys discriminating midazolam, flumazenil antagonized the effects of midazolam and not those of pregnanolone while pentylenetetrazole antagonized the effects of both positive modulators. Thus, chronic diazepam treatment does not alter the qualitative nature of interactions between positive and negative $GABA_A$ receptor modulators acting at the same or different sites, suggesting that the sites are not fundamentally changed by this treatment. Supported by USPHS grant DA09157 and DA17918 (CPF).

18

Acute administration of mirtazapine alters cue-induced reinstatement of methamphetamine-seeking in rats following self-administration

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Methamphetamine (METH) is a potent psychostimulant, abuse of which can lead to compulsive drug-seeking. Mirtazapine (Mirt) is an FDA-approved antidepressant which antagonizes serotonin (5-HT2A/C and 5-HT3), norepinephrine (α2), and histamine (H1) receptors. The present study evaluated the ability of Mirt to alter cue-induced reinstatement of METH-seeking behavior. Rats were trained in an operant chamber equipped with an "active" and an "inactive" lever. Depressing the active lever resulted in METH infusion, illumination of a light above the lever followed by an in-house light, indicating a time-out period (20 sec) in which additional METH was unavailable; inactive lever responses had no consequence. After behavior stabilized on an FR5 schedule, rats were tested for cue-induced reinstatement of METH-seeking (i.e., cue-reactivity; CR). CR testing (cues presented on an FR1 schedule; 1 hr) consisted of measuring the number of lever presses in the absence of reinforcement (no METH infused). A 15min pretreatment of 0.5, 1.0, 5.0 mg/kg Mirt or vehicle was administered in randomized order using a within-subjects repeated measures design. Twenty-four hr after CR testing, rats self-administered for 2 consecutive days and were tested for CR 24hr later. This pattern of testing was repeated until all doses and vehicle were tested. Low dose Mirt (0.5 mg/kg) increased METH-seeking behavior whereas 5.0 mg/kg Mirt decreased METH-seeking within the first 15min of CR testing. To determine if Mirt influenced motor performance, which may influence the ability to depress the lever, rats were trained to perform on a rotarod. Testing occurred at 15min intervals after injection of 0.5, 1.0, 5.0 mg/kg Mirt or vehicle; in randomized order. Mirt had no effect on the ability to perform rotarod task. These studies demonstrate that Mirt may be an efficacious treatment to attenuate METH-seeking behavior and prevent relapse in the abstinent METH addict without resulting in gross motor impairment or impeding the ability to conduct motivated behavior. Supported by USPHSG DA015760 to TCN.

19

ALCOHOL BINDING SITE(S) IN THE C1 DOMAIN OF PKC EPSILON AND MUNC 13.1

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Protein Kinase C (PKC) plays a central role in signal transduction and has been proposed to be a target of alcohols. Knockouts of PKC epsilon showed significant decrease in alcohol consumption compared to wild type mice. Based on the studies on the effect of alcohols on PKC alpha activity and the alcohol binding data in its C1 domain, we hypothesized that similar alcohol binding site could be present in the C1 domain in PKC epsilon and structurally similar C1 domain of MUNC 13.1, a protein in the brain responsible for neurotransmitter release. To test this, we studied the interaction of alcohols with the C1B subdomain of PKC□epsilon and the C1 domain of MUNC 13.1 by fluorescence, photolabeling and mass spectrometry. Ethanol, Butanol and octanol increase the affinity of a fluorescent phorbol ester Sapintoxin D to PKC□C□□ in a concentration dependent manner with EC50s 74 mM, 8 mM and 0.36 mM respectively indicating the presence of an allosteric alcohol binding site. The alcohols were found to quench the intrinsic fluorescence of MUNC 13.1 C1. To identify the alcohol binding residue(s) the proteins were photolabeled by photoactive azialcohols, 3-azibutanol and 3-azioctanol. After photolabeling the protein was reduced with DTT, alkylated with iodoacetamide, digested with trypsin/chymotrypsin and subjected to electrospray ionization mass spectrometric analysis. While 3-azioctanol labeled Tyr-238, 3-azibutanol labeled His-236 of PKC□C1B. In the case of MUNC 13.1 C1, both azibutanol and azioctanol labeled the Glu-582. Homology modeling indicated the presence of a grove consisting of His-236 and Tyr-238 in PKC□C1B and Glu-582 in MUNC 13.1 C1 which could be the putative alcohol binding sites. The present results indicate that alcohols interact with the C1 domains of PKC epsilon and MUNC 13.1 and that His-236, Tyr-238 and Glu-582 are important in alcohol binding. Supported by 1 R21 AA16140-01

20

Role of flexibility in the function of TGF-β3

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The TGF-β isoforms (β1, β2, and β3) share 71–79% sequence identity, have similar overall structures, and signal through a pair of homologous receptors, known as T□R-I and T□R-II. They nevertheless induce distinct biological activities *in vivo*, as suggested by the distinct phenotypes of the isoform-specific null mice and by differences in response induced by the addition of purified TGF-β isoforms in tissue explant assays. We hypothesize that these distinct activities arise as a consequence of structural differences among the TGF-β isoforms, the most significant of which is that only TGF-β3 has a disordered palm region, which effects the relative configuration of its monomers. To examine this, we generated a chimeric protein by swapping residues in the homodimer interface of TGF-β1 into TGF-β3. NMR chemical shift assignment and analysis indicates that this chimeric protein adopts a "closed" conformation, characteristic of TGF-β1, not TGF-β3. PDGF induced dermal fibroblast cell migration, which has been shown to be uniquely inhibited by TGF-β3, but not by TGF-β1, shows that the chimeric protein has poor inhibitory potential, like that of TGF-β1, not TGF-β3. These results suggest that the residues in the homodimer interface of TGF-β3 underlie its distinct homodimer arrangement and biological activity.

Poster Communications

21

CM156, a novel sigma receptor antagonist, attenuates the neurotoxic effects of methamphetamine in mice

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With an increased potential for life threatening abuse, methamphetamine (METH) has become a drug of significant importance and study. Methamphetamine is one of many psychostimulant drugs that interact with sigma receptors. We recently developed the selective sigma receptor antagonist, CM156, which exhibits high affinity for sigma-1/2 receptors and negligible interactions for 50 non-sigma targets. In the present study, we determined the effects of CM156 on METH-induced neurotoxicity by measuring dopamine (DA) and 5-HT levels in the striatum, striatal dopamine transporter (DAT) expression, and body temperature. Male, Swiss Webster mice were injected (i.p.) four times at two hour intervals with one of the following treatments: Saline/Saline, Saline/METH (1.25, 2.5, 5, or 10 mg/kg), CM156 (5, 10, or 20 mg/kg)/METH (5 or 10 mg/kg), or CM156 (5, 10, or 20 mg/kg)/Saline. Pretreatment with CM156 significantly prevented METH-induced striatal DA and 5-HT depletions while attenuating hyperthermia. Furthermore, pretreatment with CM156 reduced the striatal DAT deficits produced by METH. Collectively, the results demonstrate that CM156 prevents a variety of METH-induced effects. Further studies are underway to evaluate sigma receptors as potential therapeutic targets for mitigating the actions of psychostimulant drugs and to determine the cellular mechanisms through which they convey their protective effects. (Supported by NIDA)

22

SN79, A Novel Sigma (σ) - 2 Receptor Antagonist, Attenuates Cocaine-Induced Behaviors In Mice

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Cocaine is highly addictive and its abuse is involved in more emergency room visits than any other illicit drug. Unfortunately, there is no effective pharmacotherapy to treat cocaine abuse. Cocaine interacts with both sigma (σ)-1 and 2 receptors. The role of σ-1 receptors in attenuating cocaine-induced behaviors has been established using pharmacological antagonists and antisense oligonucleotides in earlier studies. In contrast, the role of σ-2 receptors in the treatment of cocaine abuse is unclear due to the lack of σ-2 selective compounds. In the present study, radioligand and behavioral studies were conducted to characterize SN79, a putative σ-2 selective compound. Radioligand binding studies showed that SN79 has nanomolar affinity for σ-2 receptors and negligible affinity for σ-1 receptors and over 40 other non-σ sites. In behavioral studies, pretreatment of male, Swiss Webster mice with SN79 (0.1-10 mg/kg, i.p.) significantly attenuated the effects produced by a convulsive (70 mg/kg, i.p.) or locomotor stimulant (20 mg/kg, i.p.) dose of cocaine. SN79 also significantly blocked the development and expression of the sensitized response to subchronic treatment with cocaine. Alone, SN79 had sedative effects at acute higher doses, which disappeared upon repeated exposure. These results point towards the viability of targeting σ-2 receptors in the development of an effective anti-cocaine therapy, and also establish SN79 as the most σ-2 selective compound to date. (Supported by NIDA)

23

Effects of modafinil: studies on cocaine self-administration, neurochemistry, and activity in rhesus macaques.

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Modafinil is currently used as a treatment for excessive daytime sleepiness. Increasing evidence suggests modafinil affects the dopamine transporter. Therefore, we examined the effects of modafinil (10 mg/kg; i.v.) on daytime locomotor activity, cocaine self-administration, reinstatement of cocaine-maintained behavior, and extracellular dopamine levels. Locomotor activity was measured in the home cage using Actiwatch telemetry, with four days of baseline recording preceding a morning injection. Administration of modafinil did not significantly increase daytime locomotor activity. Monkeys self-administered cocaine under a second order, fixed ratio 20 schedule; pretreatment with modafinil did not have a significant effect on cocaine self-administration. Cocaine maintained responding was then extinguished and extinction level responding maintained for a minimum of two days before priming with modafinil. Modafinil was able to significantly reinstate extinguished cocaine-maintained behavior. Finally, dopamine levels following modafinil administration were measured using *in vivo* microdialysis followed by sample analysis using high performance liquid chromatography. Modafinil significantly increased dopamine overflow within the caudate nucleus approximately 20 minutes after injection. Modafinil did not appear to have behavioral stimulant effects at 10 mg/kg, but at this dose does appear to have dopaminergic effects as measured by *in vivo* neurochemistry and cocaine reinstatement assays.

24

Effects of intranasal *d*-amphetamine and methamphetamine administration in humans

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Data from previous studies investigating low, oral doses of methamphetamine and *d*-amphetamine in humans have indicated that the acute behavioral and physiological effects of the two stimulants are similar. However, there is a dearth of empirical data comparing the two drugs employing a route of administration and dose levels commonly associated with abuse. This ongoing outpatient, within-participant, double-blind study examines the effects of larger doses of intranasal methamphetamine and *d*-amphetamine on physiological and behavioral measures. To date, four non-treatment seeking methamphetamine users completed the study, which is comprised of five 2-day blocks of sessions. On the first day of each block, participants completed a sample trial, where they were given one methamphetamine or *d*-amphetamine dose (0, 12, 50 mg/70 kg) and a monetary reinforcer ($20). Amphetamines plasma levels, cardiovascular, subjective, and psychomotor performance effects were assessed before drug administration and repeatedly thereafter. On the second day, participants completed one choice trial, where they had the opportunity to work for the sampled reinforcers (drug or money). Both methamphetamine and *d*-amphetamine produced dose-dependent increases on cardiovascular and 'positive' subjective-effect measures. Under all drug conditions, participants chose more money than drug (95% versus 5%). There were no significant differences between methamphetamine and *d*-amphetamine on any measure. These preliminary data are consistent with previous findings investigating oral doses and suggest that these two amphetamines produce similar acute effects in humans. Supported by DA019559 and DA023883-02.

Poster Communications

25

Regulation of serotonin 5-HT2C receptor (5-HT_{2C}R) in models of addiction: Measurement of 5-HT_{2C}R protein expression at cortical synaptic membranes.

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Acute systemic administration of 5-HT_{2C}R agonists and antagonists reduce and enhance, respectively, cocaine-evoked hyperactivity in rats, an effect that is lost during withdrawal from repeated cocaine exposure. Because cortex is an important site of action for 5-HT_{2C}R control of cocaine-induced behaviors, we hypothesized that a molecular mechanism underlying this regulation is translocation of 5-HT_{2C}R from cortical synapses. However, study of 5-HT_{2C}R by Western blot analysis is complicated by posttranslational modifications and alternative splicing of 5-HT_{2C}R that affect its electrophoretic migration. We assessed 5-HT_{2C}R expression in postsynaptic density (PSD), an electron-dense specialized structure of the submembrane cytoskeleton involved in receiving and transducing synaptic signals. Motor cortex from Sprague-Dawley rats (n=3) was pooled and fractionated into synaptosomal, synaptic junction and PSD compartments. Purity of each fraction was validated with appropriate antibodies (ABs). Five anti-5-HT_{2C}R ABs were employed to determine best conditions to measure 5-HT_{2C}R in PSD fraction: mouse monoclonals PH (C-terminus; Pharmingen) and D-12 [C-terminus, Santa Cruz (SC)]; goat polyclonals C-20 (C-terminus; SC) and N-19 (N-terminus, SC); and rabbit polyclonal CH (N-terminus; Chemicon) ABs. Anti-5-HT_{2C}R ABs detected similar and distinct bands of MW 20, 41, 46, 50, and 55 kDa in PSD fraction. Future studies involve enzymes that remove posttranslational modifications to determine identity of the observed multiple bands. We have demonstrated 5-HT_{2C}R expression in cortical PSD; this observation paves the way for quantitative analyses of regulation of 5-HT_{2C}R protein at synapse following cocaine exposure. NIDA DA006511, DA020087, Peter F. McManus Charitable Trust.

26

Discriminative stimulus effects of DOM in rhesus monkeys: interactions between 5-HT_{1A} and 5-HT_{2A} receptor agonists

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Many drugs acting on serotonin (5-HT) systems, including 5-HT releasers and reuptake inhibitors, indirectly activate multiple 5-HT receptor subtypes; however, the impact of the interactions between these receptors is not fully understood. In rodents, activity at 5-HT_{1A} receptors enhances actions at 5-HT_{2A} receptors, although the generality of this finding is unknown. This study examined interactions between 5-HT receptor agonists in rhesus monkeys discriminating between saline and 0.32 mg/kg of the 5-HT_{2A} receptor agonist DOM. The selective 5-HT_{1A} receptor agonists 8-OH-DPAT and F13714 shifted the DOM dose response curve to the right and this effect was reversed by the 5-HT_{1A} receptor antagonist WAY100635. The non-selective 5-HT_{1A} receptor agonist buspirone had no effect while the 5-HT_{2A} receptor agonists 2C-T-7 and dipropyltryptamine shifted the DOM dose response curve to the left. Thus, agonism at 5-HT_{1A} receptors attenuates the discriminative stimulus effects of a drug acting at 5-HT_{2A} receptors. That this interaction in monkeys may be opposite to what has been observed in rodents underscores the need to examine these interactions under a broader range of conditions and in multiple species. CPF is supported by a Senior Scientist Award (DA17918).

27

Effects of physostigmine on the conditioned hyperactivity and the behavioral sensitization to morphine in rats

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Environmental stimuli paired with injections of many drugs of abuse can acquire the ability to evoke conditioned hyperactivity. We were interested in developing a rodent model of conditioned hyperactivity which was elicited by environmental stimuli and examining the effects of physostigmine on the conditioned hyperactivity and the behavioral sensitization to morphine. Rats were randomly assigned into six groups (n=10-12). During conditioning training, one group of animals (physostigmine-morphine group) received physostigmine (0.1mg/kg, i.p.) and other five groups received saline and subsequently animals were put into locomotion measurement chambers (paired with an odor stimulus) for 20 minutes. Then, animals in morphine paired group1, morphine paired group 2 and physostigmine-morphine group received morphine (5mg/kg, i.p.) whilst other animals (control group, morphine unpaired group and physostigmine group) received saline. Animals were put into locomotion test chambers again for two hours. The same schedule was repeated daily for 10 consecutive days.

Rats in morphine paired group1 and in physostigmine-morphine group exhibited significant conditioned hyperactivity to environmental cue compared with rats in morphine unpaired group or in saline group on day 8 or 25 after training. Rats in morphine-paired group 2 also showed conditioned hyperactivity on day 8 after training, but it was inhibited by physostigmine pretreatment (0.1mg/kg, i.p., 10 minutes prior to test) on day 25. We further examined the expression of the behavioral sensitization challenged by morphine (5mg/kg, i.p.) for 2 hours after test of conditioned hyperactivity on day 25 and the results are as follows: rats in morphine paired group 1 increased significantly the locomotor activity while animals in morphine paired group 2 and in morphine unpaired group did not, compared with rats in saline group; the locomotor activity of rats in physostigmine-morphine group was higher than that of rats not only in saline group but also in morphine paired group 1.

The present results show that Pavlovian conditioned responses to environmental cues directly contribute to morphine-induced behavioral sensitization and physostigmine could block the expressions of conditioned hyperactivity and of behavioral sensitization but enhance the development of behavioral sensitization induced by morphine.

28

Using Conformational Constraint to Develop Potent Drug Abuse Therapies

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Substance abuse disorders result in significant personal and economic impact of many Americans. Currently, 9.3% of the US population suffers from a substance abuse disorder. Substance abuse contributes to the spread of HIV-1, Hepatitis B and C, as well as drug-resistant tuberculosis. A growing amount of evidence suggests that kappa opioid (KOP) receptors are involved in the control of several abuse related effects of central nervous system (CNS) stimulants. KOP receptor agonists modulate the activity of dopamine neurons and decrease self-administration of cocaine in non-human primates. KOP receptor antagonists have the potential to be utilized as opioid abuse therapies and in the treatment of stress-induced reinstatement (a model of drug relapse). Salvinorin A (SVA) is the first non-nitrogenous scaffold having high affinity and efficacy at KOP receptors. In addition to a unique structure, SVA induces hallucinations through a mechanism different from classical hallucinogens. Past structure-activity relationship (SAR) studies have shown that modification of the C-2 acetate to a methoxy alkyl ether leads to increased potency at KOP receptors. However, the reason for the increase in potency remains unclear. Further SAR studies were conducted to investigate these findings. Here, we report our efforts towards the synthesis and evaluation of conformationally constrained analogues of SVA as potential drug abuse therapies.

Poster Communications

29

Compartmental distribution of mammalian IDP2, an enzymatic source of NADPH

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The mammalian non-mitochondrial isozyme of NADP+-dependent isocitrate dehydrogenase is found both in the cytosol and in peroxisomes. We seek to understand the mechanism and the functional significance of this dual subcellular compartmental distribution using yeast and mammalian systems. Mammalian IDP2 is hypothesized to have combined functions of its yeast counterparts (peroxisomal IDP3 and cytosol IDP2, respectively) in supporting beta-oxidation of fatty acids in peroxisomes and in providing NADPH/alpha-ketoglutarate in the cytosol for macromolecular biosynthesis and antioxidant reactions. Thus, various environmental conditions may impact the organellar distribution of mammalian IDP2. We kinetically characterized mammalian IDP2 to ascertain any unique properties that might contribute to function in different cellular compartments. As compared with mammalian mitochondrial IDP1, and with yeast isozymes (mitochondrial IDP1, cytosolic IDP2 and peroxisomal IDP3), mouse IDP2 was found to exhibit a much broader pH optimum for activity (for the decarboxylation reaction), indicating a possible adaptation to pH variations in different subcellular compartments. For both mammalian enzymes, affinities for isocitrate were ~40-fold greater than affinities for alpha-ketoglutarate, suggesting that catalysis is predominantly in the decarboxylation direction under normal cellular conditions. To study factors affecting the subcellular distribution of mammalian IDP2, mouse IDP2 was expressed in yeast cells using a tetracycline-inducible system, and differential centrifugation was conducted to separate cytosolic and organellar compartments. Western blots assays showed that the mammalian enzyme is localized both in the cytosol and in peroxisomes in yeast cells. Complementation tests also indicated that the mammalian enzyme is functional in both cellular compartments.

30

The NET effect: Insulin's Impact on Modulating NE Clearance

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Norepinephrine (NE) transporters (NET's) are crucial in terminating NE neurotransmission by high affinity uptake of NE released into the extracellular fluid. NETs are also the site of action of psychotherapeutic drugs such as antidepressants. Diabetic patients have higher rates of depression than the general population (Musselman, 2003) yet the role by which insulin impacts the central nervous system is not entirely understood. Previous studies have demonstrated that a decrease in circulating insulin can have a profound impact on monoamine clearance. Dopamine clearance is reduced (Owens et al, 2005), while serotonin clearance is enhanced (Owens et al, unpublished), however the effect of insulin on NE uptake is unknown. Using *in vivo* high-speed chronoamperomety we measured the rate of NE clearance from the dentate gyrus in mice rendered hypoinsulinemic by a single injection of streptozotocin (STZ; 200mg/kg; i.p) or control mice, which received a single injection of saline (i.p.). Recordings were made seven days after the injection of STZ or saline. NE clearance was significantly faster in STZ-treated than their vehicle treated counterparts. This finding suggests that NET and SERT may be under similar regulatory control by insulin. Future studies will focus on determining the underlying mechanisms as they stand to bear important insights into tailoring treatments for diseases such as depression, particularly in individuals with dysregulated insulin signaling.

31

ENT1 regulates motivational effects of ethanol and CREB-mediated neuroadaptation through glutamate-neurogranin signaling in the striatum.

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Chronic increase of glutamate levels is implicated in psychiatric disorders, including alcoholism. Here, we have elucidated the role of glutamate signaling in ethanol intoxication and motivational ethanol consumption, as well as paradoxical hypo-CREB activity in the striatum using ENT1$^{-/-}$ and CRE-lacZ/ENT1$^{-/-}$ bi-transgenic mice. We demonstrated that the diminished aversive effects of ethanol might be associated with increased voluntary ethanol self-seeking behavior without affecting ethanol reward in ENT1$^{-/-}$ mice. Microdialysis-HPLC analysis showed higher levels of glutamate in response to ethanol in the dorsal striatum, but not in the ventral striatum of ENT1$^{-/-}$ mice. Using a proteomic method, we identified that the neurogranin could mediate elevated glutamate signaling, thereby promoting Ca^{2+}-calmodulin dependent kinase II activity, which may reduce CREB activity. Notably, we found that baseline CRE-driven β-galactosidase expression levels were decreased in CRE-lacZ/ENT1$^{-/-}$ bi-transgenic mice compared to CRE-lacZ/ENT1$^{+/+}$ mice. Furthermore ethanol reduced β-galactosidase levels in CRE-lacZ mice. Thus, the deficiency of ENT1 mimics the model of an ethanol exposed state, which is associated with increased tolerance to ethanol. Together, our findings demonstrate that regulation of glutamate signaling via neurogranin and CREB-mediated neuroadaptation by ENT1 may contribute to susceptibility of developing alcoholism.

32

Benzodiazepine receptor ligands, binding sites, and anxious zebrafish behavior in novel environments.

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Zebrafish (*Danio rerio*) neurochemical and behavioral assays can be useful tools in neuroscience research as well as in toxicology and pharmaceutical screening. To determine their similarity to mammalian $GABA_A$ receptors, we characterized zebrafish $GABA_A$ receptor binding in whole brain homogenates with [^{3}H] flunitrazepam in saturation and displacement binding assays: Their K_D is 1.5 ± 0.4 nM and Bmax is 125 ± 50 fmol/mg protein. [^{3}H] flunitrazepam was displaced from zebrafish brain homogenates by the benzodiazepine receptor ligands diazepam (K_i = 23 ± 4 nM), and chlordiazepoxide (K_i =143 ± 55 nM). The norepinephrine reupake inhibitor desipramine, with low affinity for mammalian $GABA_A$, failed to displace [^{3}H] flunitrazepam binding in fish brain at physiologically relevant concentrations (K_i =10,096 ± 2534 nM). Hence zebrafish brain $GABA_A$ receptors exhibit a similar pharmacological profile to those in mammalian brains. We then examined the behavioral effects of 1 week dietary exposure to the benzodiazepine receptor agonist diazepam and inverse agonist FG-7142, and a 3-min acute bath exposure to agonist chlordiazepoxide (CDE) on the behavior of adult zebrafish (N=5-9) in two novel environments: A dive tank in which naïve zebrafish initially dwell in the bottom 1/3 of the tank, and an aquatic light/dark plus maze in which naïve fish seek black arms, avoiding white arms at first. Behavior was observed for 5 min per test. Paradoxically, zebrafish administered 10 and 50 mg/d diazepam or 10 mg/d FG-7142 in diet spent more time in the dive tank bottom ($p<0.05$), and tended to make fewer entries into white arms ($p=0.06$) and arms overall ($p=0.06$) in the light/dark maze than controls at the 50 mg/d doses. A 1 mg/d dose of diazepam had no significant effect on these behaviors. However, a 3-min, 25 mg/L CDE exposure resulted in zebrafish entering white-arms more frequently and spending more time in them ($p<0.05$). Hence diazepam and FG-7142, appear angiogenic, while CDE had anxiolytic effects on zebrafish behavior in novel environments at these selected doses.

Poster Communications

33

The role of socio-economic factors in drug addiction

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Drug addiction has been a societal problem for a very long time. We all know the side effects of drugs / controlled substances in our system and majority of the time we have been focusing chemical aspect of drug effects on the body without trying to analyze first of all how the person came to be in contact with controlled drugs / substances. And so with these in mind, I have been investigating the factors that influence an individual to take drugs / controlled substances.
Emotional factors play an important role in drug addiction. Many individuals come from a home where there is high level of stress maybe the parents fight all the time or maybe it is a home where there is so much sibling rivalry. Those individuals most of the time end up seeking ways to overcome those stress and uncomfortable situation and most of the time they end up been introduced to drugs that depress the nervous system such as Benzodiazepines, solvents. This type of drug specializes in depressing the nervous system thereby creating the effect of tension relieve and anxiety and it also promotes or seems to promote relaxation which is an added sweetener to the individual. Now at the initial stage the drug / substance creates this wonderful world that seems so perfect with no feeling of stress or any kind of burden, the world now seems so relaxed. As the individual starts taking these controlled drugs frequently / higher doses they start to develop tolerance for it and before they realize it they become physically dependent on it. And in that stage they are what we call 'DRUG ADDICTS'. I will explain the other factors to drug addiction in my presentation.

34

Varenicline appears to produce differential effects on ethanol intake in dependent and non-dependent rats

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Varenicline (Chantix®) is a partial nicotinic α4 β2 receptor agonist that has clinical efficacy in reducing tobacco use. The goal of this study was to begin to address the issue of the effects of varenicline on oral ethanol intake in rats. Briefly, male Wistar rats were first trained to self-administer 10% ethanol 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/12 off each day) and maintained at an average blood alcohol level of 200-350 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) 6 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 preliminary results revealed that varenicline reduced ethanol intake in non-dependent rats and a strong trend towards increased ethanol intake in dependent rats. Our findings suggest that the effects of varenicline may be different in dependent versus non-dependent rats, and may enhance the rewarding effects of ethanol in dependent populations. (Supported by the NIH/NIGMS/Bridges to the Baccalaureate Program (2R25GM049011; AM) and Minority Access to Research Careers Program (BSB).

35

Enhancement in the sodium appetite: the role of age

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An age-related decline is observed in the average amount of hypertonic sodium chloride solution ingested in response to sodium depletion. Adult rats submitted to repeated episodes of water deprivation (WD) increase sodium appetite in a 2 h sodium appetite test (SAT). In this work, we investigated the effects of repeated episodes of WD on the enhancement of sodium appetite in late adolescent (2 months old, mo), young adult (4 mo), and older adult (6 mo) rats. At the beginning of the experiment (n = 7-9/group), male Sprague-Dawley-Holtzman rats, all age groups, were submitted to 5 episodes of WD and SAT. At the same time, non-deprived controls from each age group of rats were submitted to SAT. After 36 h of fluid deprivation with only food available, water was given for 2 h (rehydration). Then, 0.3 M NaCl was made available and ingestion of water and 0.3 M NaCl were recorded after SAT. Sodium appetite of deprived 6 mo rats and all age non-deprived rats was not enhanced. Compared to the 1st SAT, repeated episodes of WD increased sodium appetite in 2 mo rats in the 2nd, 3rd, and 4th SAT (2.6±0.4, 5.2±0.6, 5.0±0.8, 5.2±0.8, and 3.9±0.8 ml/100 g bw, 1st to 5th SAT respectively) and in 4 mo rats in the 3nd and 4th SAT (3.6±0.4, 4.9±0.6, 5.5±0.4, 5.0±0.5, and 4.5±0.5 ml/100 g bw, 1st to 5th SAT respectively). Water intake during rehydration and SAT was not altered by repeated WD in all groups. These data show that repeated exposure to WD during late adolescence results in an early enhancement of sodium appetite. Thus we concluded that the long-term changes in the sodium appetite are age dependent.

36

Interactions between delta- (DOR) and kappa- (KOR) opioid receptors *in vitro* and *in vivo*.

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Peripherally acting opioids produce analgesia under conditions of tissue injury or inflammation. The DOR is expressed on peripheral sensory neurons in culture and is nonfunctional under basal conditions; However, after brief treatment ("priming"; 15 min) with inflammatory mediators such as bradykinin (BK), it becomes competent to inhibit adenylyl cyclase (AC) activity and neuropeptide release. DOR have also been shown to function as oligomers with KOR both in vitro and in vivo. Here we report that occupancy of KOR with a selective antagonist, nor-BNI (3nM, 100 x Ki), differentially altered the potency of DOR agonists, DADLE and DPDPE, to inhibit PGE2-stimulated AC in BK-primed cultures derived from rat trigeminal ganglion (5-6 days in culture). Concentration curves for DPDPE were shifted to the left 10- fold whereas curves for DADLE were shifted to the right 20-fold (DPDPE EC50 was 0.24 nM vs 0.06 nM and for DADLE was 0.058 nM vs 1.19 nM ($p < 0.05$) without or with nor-BNI, respectively). In addition, the DOR-KOR heterodimer-selective agonist, 6'GNTI, also produced a BK priming-dependent inhibition of PGE2-stimulated AC. Consistent with these in vitro findings, peripheral opioid receptors were nonfunctional under basal nociceptive testing conditions and required priming with inflammatory mediators, such as BK, for functional competence to produce inhibition of thermal allodynia in response to peripheral application of PGE2 *in vivo*. Further, we found a profound enhancement of the DPDPE effect upon occupancy of KOR with nor-BNI. Administration of the DOR-KOR heterodimer-selective agonist, 6'GNTI, also produced BK priming-dependent reversal of thermal allodynia. We hypothesize that DOR-KOR interactions regulate nociceptor function and participate in opioid-mediated analgesia in inflamed tissue.
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Poster Communications

37

The effect of acute caffeine administration on behavior in C57BL/6J and DBA/2J mice

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Caffeine is the most widely used drug in the world, and produces both stimulatory and anxiogenic effects in mice. We sought to characterize the range of effects of caffeine on various behaviors in two strains of mice that vary in anxiety-related and motor behaviors, C57BL/6J (B6) and DBA/2J (D2). Caffeine was administered (I.P.) to male and female adult B6 and D2 mice 1 h prior to the measurement of behavior in an activity chamber, elevated plus maze, and rotorod. Doses used were 15, 33, 66, 100, and 160 mg/kg. In the activity chamber, total distance traveled varied as a function of caffeine dose relative to vehicle controls. Low doses were far more stimulatory to D2 mice, while high doses produced greater inhibition in B6 mice. On the rotorod, 100 and 160 mg/kg caffeine significantly decreased the mean time and speed of B6, whereas all concentrations increased the time and speed for D2 mice. For the plus maze, concentration-dependent effects of caffeine were found for number of beam breaks in open and closed arms in both strains. We also investigated oral self-administration of caffeine in these strains. A 2-bottle drinking study and 2-bottle fading study were performed with male and female B6 and D2 mice. The 2-bottle test consisted of 48 h water, followed by 48 h each of 0.1%, 0.2%, 0.4%, 0.8%, 1.6%, and 3.2% caffeine solution. The fading study was conducting similarly, but the caffeine solutions were presented in reverse order, the 12-hour light cycle was reversed, and the mice were given 2 hours each day to ingest the solutions. B6 mice consumed more 0.8%, 1.6%, and 3.2% caffeine. Collectively, these data indicate strain-dependent effects of caffeine on behavior, and suggest an opportunity for further genetic analysis of these effects.

38

Inhibition of intracellular Ca (Ca_i^{++}) release by synthetic 5-HT_{2A}R antagonists with chemical tethers at various sites in CHO cells transfected with the serotonin (5-HT) 2A receptor (5-HT_{2A}R). Patricia K Seitz,[1,2] Matthew J Shashack,[1,2] Cheryl S Watson,[2,3] Scott R Gilbertson,[1,2] and Kathryn A Cunningham,[1,2] [1]Department of Pharmacology, [2]Center for Addiction Research, [3]Department of Biochemistry/Molecular Biology, UTMB, Galveston TX 77555

The 5-HT_{2A}R and 5-HT_{2C}R play important modulatory roles (stimulatory and inhibitory, respectively) in modulating behavioral effects of cocaine. There is evidence in cell culture models that the receptors hetero-dimerize. Synthetic bivalent ligands have been used in several receptor systems, sometimes with increased potency and fewer side effects relative to combinations of single ligands. We propose that a bivalent 5-HT_{2A}R antagonist/5-HT_{2C}R agonist may have utility to suppress cocaine-seeking and/or -taking, with potential to serve as an abstinence enhancer in humans. To determine the optimal site to attach chemical tethers, we synthesized M100907, a selective 5-HT_{2A}R antagonist and four derivatives: an alcohol (for linker attachment) substituted for the F on the aromatic ring (#107); 4-carbon alkane (#80) or 2-methoxy-ethyl ester (#255) attached to the phenol residue in lieu of F; and 2-methoxy-ethyl ester in lieu of the methoxy residue (#256). Following preincubation with putative antagonist, we tested the activity of each to inhibit release of Ca_i^{++} induced by 1μM 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) in CHO cells transfected with 5-HT_{2A}R and loaded with Ca_i^{++}-sensitive dye. None of the compounds (10^{-10} to 10^{-4} M) exhibited agonist activity. Stimulation of DOI-induced Ca_i^{++} release was inhibited by 3 of the modified compounds with estimated ED_{50} values: M100907=30 nM; #107=90 nM; #255=9000 nM; #256=120 nM. Compound #80 did not reduce the Ca_i^{++} signal to baseline levels, even at 10^{-4} M.

39

The synthesis of serotonin (5-HT) 2A receptor (5-HT_{2A}R) antagonist/ 2C (5-HT_{2C}R) agonist bivalent ligands

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Previous studies indicate that the 5-HT_{2A}R and 5-HT_{2C}R play a role in cocaine induced behavior modification through either stimulation or inhibition respectively. Studies have shown the 5-HT_{2A}R and 5-HT_{2C}R hetero-dimerize and the 5-HT_{2C} receptor homo-dimerize in-vitro. In other biological systems, bivalent ligands have been shown to possess increased affinity and potency as well as decreased side effects when compared to administration of the monomers. We propose that administration of a heterodimeric 5-HT_{2A} R antagonist/5-HT_{2C}R agonist will exhibit improved ability to control cocaine induced behavior modifications compared to the concurrent administration of a 5-HT_{2A} R antagonist and a 5-HT_{2C}R agonist. The 5-HT_{2A} R antagonist M-100907 and the 5-HT_{2C}R agonist WAY-470 will be utilized as the monomers for this study. The first step in the successful synthesis of dimers is the elucidation of the optimal binding site on each monomer. The optimal binding site is determined by synthesizing analogs of each monomer and attaching a tether. We have synthesized M-100907 and 4 derivatives as well as WAY-470 and three derivatives. Biological screening of M-100907 and its derivatives shows the activity of these compounds is more dependent on substituent composition than site of attachment. WAY-470 and derivatives have not yet been tested.

40

Response perseveration in adolescent and adult rats in a reversal learning task

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Perseverative behavior is the tendency to repeatedly engage in a behavioral response despite the increased likelihood of negative consequences following the response. In behavioral laboratory tasks individuals with substance use disorders demonstrate a greater propensity for perseverative responding relative to control subjects with no substance abuse history. It is possible that individuals with high trait levels of perseverative responding are more likely to become substance abusers, and that perseverative patterns of behavior contribute to escalation of drug use and relapse to drug taking. However, it is impossible to determine from human studies if excess perseverative behavior is a cause or consequence of repeated substance use. Perseverative responding has been studied in animals using typical operant chamber tasks. However, these tasks require several weeks of training in order to measure perseverative behaviors. The purpose of this study was to attempt to validate a new animal model of perseverative behavior that does not require lengthy training. The study employed a spatial reversal learning task. In a single 2-3 hour session rats were trained locate a food reinforcer hidden in one of four piles of clean cage bedding in a square arena. After rats reliably located the food reward, the location of the reward was moved; rats were required to abandon their initial search strategy and locate the food in the new location. Because adolescent rats have poorer behavioral control, both adult and adolescent male rats were tested in the task. It was hypothesized that adolescent and adult rats would take similar amounts of time to complete the initial acquisition of the task, but that adolescent rats would require more trials to complete the reversal phase of the task, and that adolescent rats would make more errors (continue to search the formerly reinforcing location) relative to adult rats. Results indicated that adolescent rats required more trials to complete the reversal, and had a different pattern of errors during the reversal relative to the adult rats. This preliminary study suggests the spatial reversal learning task may be suitable for measuring perseverative behavior in rats, and may provide a more efficient animal model for assessing individual differences in perseveration as a vulnerability factor to the behavioral effects of drugs of abuse.

Poster Communications

41

Dissecting the Role of Phosphorylation on the Glycine Transporter 1 by Protein Kinase C

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The amino acid glycine is involved in excitatory and inhibitory neurotransmission and its action is modulated by the activity of glycine transporter 1 (GlyT1). GlyT1 is a glycoprotein containing 12 transmembrane helix domains that maintain the protein embedded in the plasma membrane. The neurotransmitter glycine is transported from synaptic cleft back into the cytoplasm of the presynaptic neuron in a Na/Cl^- dependent fashion. Over the years, it has been shown that post-translational modifications (PTMs) modulate the activity of several transporters, such as the dopamine transporter at different cell models. Phosphorylation by protein kinase C (PKC) is a PTM that can be easily stimulated by the PKC activator phorbol 12-myristate 13-acetate (PMA). In this study, we demonstrate that glycine uptake mediated by GlyT1 in stably transfected porcine aortic endothelial cells (PAE) can be decreased in a significant level after pretreatment of cells with PMA, but not when bisindolylmaleimide (a specific PKC inhibitor) is also present. Our results demonstrate that GlyT1 is phosphorylated in a PKC-dependent fashion. The PKCs isoenzymes are grouped into three groups according with their requirements for full activation (conventional, novel and atypical). In order to elucidate which isoenzymes are involved on GlyT1 phosphorylation, we conducted a mechanistic study using specific inhibitors available for PKA, CaMKII, conventional PKC (α β, γ, as well as δ, ζ), novel PKC (θ−δ) and PKC−α−β1. Our results show that PKA, CaMKII, CaMKIII and novel PKC are not involved in the regulation of GlyT1 activity. However, glycine uptake was only diminished when a specific inhibitor of PKC−α−β1 was employed. Our data suggest that PKC−α and/or PKC−β1 are responsible for the reduction of Gly uptake and more likely phosphorylation of GlyT1. Western blotting experiments are underway to determine if PKC−α and PKC−β1 are present in PAE cells.

42

Association of genes encoding alcohol metabolizing enzymes with Alcohol Dependence: First Indian study

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Background: The importance of genetic influences and ethnic differences in the sensitivity to ethanol are well recognized. Linkage and association studies have identified several chromosomal regions and a few genes (ADH_2, $ALDH_2$, ADH_3) that influence the risk for developing alcohol abuse and dependence. The G to A substitution that generates the deficient variant $ALDH_2$* 487Lys & high activity variant encoded by ADH1B*47His has been reported in East Asian populations at high frequencies. Functional polymorphism in ADH1B, ADH1C CYP2E1 and ALDH2 genes are considered most important among several genetic determinants of alcohol dependence (AD), a complex disorder. There is no report on the widely studied Arg47His and Glu487Lys polymorphisms from either Indian alcohol dependents or subjects with alcohol related disorders. Aims: We, for the first time, report allelic and genotypic frequencies of Arg47His and Glu487Lys SNPs in Indian alcohol dependent subjects along with subjects of alcoholic pancreatitis and compare this prevalence with reported values for different alcoholic populations, worldwide. Methodology: 174 alcohol-dependent subjects were tested for the distribution of genotypes using PCR-RFLP analysis. Genotypic and allelic frequencies were calculated for both genes and critically analyzed by appropriate statistical tests. Results obtained from genetic analysis were correlated with clinical parameters using T test or Mann Whitney's U test.
Results: ADH1B gene polymorphism was found to be largely monomorphic with minor allele frequency (ADH1B*2) =0.0014 showing only Arg47 as the prevalent allele (ADH2-1/2-1).
For ADH1C, patients with alcohol induced pancreatitis (AP) were found to have Val allele.
Three patients with alcoholic pancreatitis (AP) & one with non alcoholic pancreatitis (NAP) were found to be heterozygous for G1259Cmuatation in CYP2E1 while none of the controls carried the mutated allele. For the ALDH2 Glu487Lys SNP, genotypic frequencies were 0.73 (2*1/*1), 0.16 (2*1/*2) and 0.11 (2*2/*2), with minor allele frequency (ALDH2*2) = 0.19. Various clinical parameters were found to be significantly associated with ALDH2 polymorphism. Conclusions: The highlight of the study is a clear association of ALDH2

43

Chronic cannabinoid administration decreases the number of circulating CD4 cells and down-regulates chemokine receptor expression by CD4+ cells in rhesus macaques.

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Marijuana continues to be the most common illicit drug used in the United States. Drug abuse is associated with increased risky behaviors which can lead to HIV infection. Δ9-tetrahydrocannabinol (Δ9-THC) is the primary psychoactive component in marijuana. Cannabinoid receptors are expressed in the central nervous system as well as in cells of the immune system. We hypothesized that chronic Δ9-THC use would impact immune function, potentially affecting host response to immunodeficiency virus infection. The aim of this study was to examine the effects of chronic Δ9-THC administration (0.32mg/kg i.m., 2 X daily, starting 30 d pre-infection) on the early changes in circulating lymphocyte subpopulations in young adult male rhesus macaques prior to inoculation with simian immunodeficiency virus (SIVmac251; 100 $TCID_{50}$/ml, i.v.). Total number of lymphocytes, T-lymphocyte subpopulations, and co-receptor including CCR5 and CXCR4 expression on these cells were determined. Chronic (30 day) THC administration did not alter total number of lymphocytes, but resulted in lower percent of CD4+ lymphocytes and significantly decreased expression of the CCR5, CXCR4 (0.91% vs. 2.81%, $p<0.05$) by CD4+ cells as compared to their time-matched vehicle-treated controls. These results suggest that chronic THC modulates host immune status, particularly decreases CD4+ cells and co-receptor expression by CD4+ cells, which may alter host response to SIV infection. Supported NIDA-020419-01A1

44

Cocaine-seeking after cocaine self-administration is associated with plasticity of the serotonin 5-HT_{2C} receptor (5-HT_{2C}R) in prefrontal cortex

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The 5-HT_{2C}R in the prefrontal cortex (PFC) has been identified as an important mediator of the behavioral effects of psychostimulants such as cocaine. The purpose of these experiments was to determine the time-dependent neuroadaptive changes in the 5-HT_{2C}R following periods of forced abstinence (FA) from chronic cocaine self-administration. Rats were trained to self-administer cocaine under a fixed ratio (FR) 5 schedule of reinforcement, while yoked animals passively received saline according to the active animal's pattern of administration for a period of 15 days. Subsequently, the response to conditioned cues at 1 and 30 days of FA from cocaine self-administration was assessed, along with 5-HT_{2C}R expression in the PFC at 1 and 30 days (Western blot). Results indicate that cocaine-seeking was significantly elevated on Day 1 of withdrawal and further elevated by Day 30. The expression of 5-HT_{2C}R protein levels in the prefrontal cortex of the cocaine group were significantly elevated on Day 1 and reduced below baseline at Day 30, suggesting that higher 5-HT_{2C}R expression in the PFC is associated with lower cocaine-seeking. These results indicate a correlation between chronic cocaine administration and 5-HT_{2C}R expression in the PFC.

Poster Communications

45

Exploring resistance to change by pharmacological disruption: Reinforcement magnitude.

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Behavioral momentum theory proposes that operant behavior is the product of two separable processes: rate of occurrence and resistance to change. Generally speaking, operant situations providing more densely spaced or greater magnitudes of reinforcement should be more resistant to disruption, irrespective of response rate. Attempts to disrupt ongoing behavior by manipulating the availability of food or deprivation level generally have supported the predictions of behavioral momentum. Tests with pharmacological disruptors, however, have yielded mixed results. The present experiment was an attempt to investigate further the resistance to change of operant behavior by pharmacological disruption. Pigeons were trained to key peck on a multiple fixed-ratio thirty schedule of food presentation, where different components programmed 2-, 4-, or 8-s access. Resistance to change in response to chlordiazepoxide, cocaine, clonidine, haloperidol, morphine, or ethanol administration was evaluated. Generally, resistance to change by drug administration was not modulated by reinforcement magnitude. Clonidine was the only exception, and even there effects were modest. Pre-feeding and extinction tests replicated previous work, indicating that our procedure was sensitive to more common disruptors. The results give additional support to the notion that pharmacological disruptors may not behave in the manner predicted by behavioral momentum theory.

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