**INTEGRATIVE NEUROSCIENCE INITIATIVE ON ALCOHOLISM**
**STRESS**

***"INIA: STRESS, ANXIETY OF ALCOHOL ABUSE"***

# Abstracts and Symposia

**2008 RSA/ISBRA JOINT SCIENTIFIC MEETING**
**June 28 - July 2**
**Washington, D.C.**

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Italy

**CHRONIC STRESS CHANGES THE POTENCY AND EFFICACY OF ETHANOL ON $GABA_A$ RECEPTOR PLASTICITY AND FUNCTION**
**Biggio G[1,2],, Mostallino MC[3], Pisu MG[3], Talani G[1],Carta M[1], Sanna E[1], Serra M[1,2]**
*[1]Department of Experimental Biology; [2]Center of Excellence for the Neurobiology of Dependence; [3]CNR Institute of Neuroscience, Section of Cagliari, University of Cagliari.*

**TOO MANY SUBUNITS SO LITTLE TIME: TICKU'S LEGACY ON ETHANOL MODULATION OF $GABA_A$ RECEPTOR SUBUNIT GENE EXPRESSION**
P. Follesa and G. Biggio
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**ROLE OF BRAIN STEROIDOGENESIS IN THE MODULATION OF GABAergic SYNAPTIC TRANSMISSION BY ETHANOL**
E. Sanna, M. Carta, G. Talani, L. Murru, M.C. Mostallino, M.G. Pisu, M. Serra, and G. Biggio
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**JUVENILE SOCIAL ISOLATION STRESS CHANGES NEURONAL PLASTICITY AND ETHANOL SENSITIVITY**
Serra M, Mostallino MC, Pisu MG, Talani G, Sanna E, Biggio G,
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Maine

**CIRCADIAN RUNNING-WHEEL ACTIVITY DURING WITHDRAWAL FROM CHRONIC INTERMITTENT ETHANOL VAPOR EXPOSURE IN MICE.**
R. W. Logan; J. A. Seggio; A. M. Rosenwasser
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**CHRONIC ETHANOL INTAKE ALTERS CIRCADIAN PACEMAKER FUNCTION IN C57BL/6J MICE.**
A. M. Rosenwasser, R. W. Logan; J. A. Seggio; M. C. Fixaris; J. D. Reed
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Maryland

**VARIATION IN NMDA RECEPTOR EXPRESSION AND SENSITIVITY TO ETHANOL'S BEHAVIORAL EFFECTS IN BXD RECOMBINANT INBRED MICE**

J.L. Brigman, P. Mathur, L. Lu, R.W. Williams, and A Holmes
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**RAJ TICKU, A PIONEER IN THE STUDY OF ETHANOL ACTIONS ON LIGAND-GATED ION CHANNELS**

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**PURIFICATION AND PHOTOAFFINITY LABELING OF THE 5-$HT_{3A}$ RECEPTOR**

D.M. Lovinger; M. Sanghvi; A.K. Hamouda; S. Srivastava; M.I. Davis; R. Morton; G. Luo; D.C. Chiara; T.K. Machu; J.B. Cohen; M.P. Blanton
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**ETHANOL AND ENDOCANNABINOID INTERACTIONS IN THE CONTROL OF GABA RELEASE IN RAT BASOLATERAL AMYGDALA**

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North Carolina

**NEUROSTEROID-LIKE DISCRIMINATIVE STIMULUS EFFECTS OF ETHANOL IN MONKEYS ARE INFLUENCED BY C-3 STEROIDAL STEREOCHEMISTRY AND IONIC CHARGE OF ESTERS**

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**CHRONIC ETHANOL VAPOR EXPOSURE DOWNREGULATES PRESYNAPTIC DOPAMINE FUNCTION IN THE NUCLEUS ACCUMBENS OF MICE**

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**ROLE OF NEUROACTIVE STEROIDS IN ETHANOL/STRESS INTERACTIONS: AN INTRODUCTION**

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**IDENTIFICATION OF NEUROACTIVE STEROID BIOMARKERS OF ALCOHOL CONSUMPTION IN MONKEYS AND MICE**

Patrizia Porcu, Sarah E. Alward, Todd K. O'Buckley, Kathleen A. Grant, Robert W. Williams and A. Leslie Morrow
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**EFFECTS OF ETHANOL ON SERUM LEVELS OF EIGHT GABAERGIC NEUROACTIVE STEROIDS IN RATS MEASURED BY HIGHLY SPECIFIC GC/MS ASSAY.**

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Oklahoma

**REPEATED ETHANOL WITHDRAWAL REDUCES PAIRED-PULSE FACILITATION AND LTP WITHIN AMYGDALA CIRCUITS.**

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Oregon

**POPULATION ANALYSIS OF HPA AXIS FUNCTION IN RHESUS MACAQUES**

B. Ferguson, S. Khouangsathiene, C. Kiley, M. Odagiri, A. Rau, K. Nusser, K. A. Grant
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**SCHEDULED-INDUCED POLYDIPSIA IS ASSOCIATED WITH INCREASED ACTH AND HEAVY DRINKING TYPOLOGIES IN A MONKEY MODEL OF ETHANOL SELF-ADMINISTRATION**

K.A. Grant, K. Nusser, C Kiley, A. Rau, M. Odagiri, S. Gonzales, X. Leng
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**ANTAGONISM OF THE ETHANOL-LIKE EFFECTS OF ZOLPIDEM BY RO15-4513 IN CYNOMOLGUS MONKEYS**

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**$GABA_A$ RECEPTOR SUBTYPES MEDIATING THE ETHANOL-LIKE EFFECTS OF NEUROACTIVE STEROIDS**

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South Carolina

**NEUROSTEROID ALLOPREGNANOLONE INVOLVEMENT IN ETHANOL DEPENDENCE AND DRINKING BEHAVIOR IN C57BL/6J MICE**

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**INFLUENCE OF CHRONIC ETHANOL EXPOSURE AND WITHDRAWAL ON CHOLINERGIC NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS OF MICE**

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Tennessee

**A MULTI-DIMENSIONAL GENETIC ANALYSIS OF ALCOHOLISM AND ADDICTION SUSCEPTIBILITY PHENOTYPES**

E. J. Chesler; S. Duvurru; V. Philip; L. Galloway; T. Ansah; C. Blaha; M. Cook; J. E. Grisel; K. M. Hamre; W. R. Lariviere; G. Mittleman; D. Goldowitz
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**DOPAMINE REGULATION OF SYNAPTIC TRANSMISSION IN THE BNST**

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**REPEATED ALCOHOL EXPOSURE INDUCES HOMEOSTATIC SYNAPTIC ADAPTATIONS IN THE EXTENDED AMYGDALA**

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**CHRONIC ETHANOL EXPOSURE ATTENUATES $\alpha_1$-AR LTD IN THE BED NUCLEUS OF THE STRIA TERMINALIS**

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**THE ONTOLOGICAL DISCOVERY ENVIRONMENT: AN INTERNET RESOURCE FOR INTEGRATION OF PHENOMIC INFORMATION THROUGH GENE-CENTRIC ANALYSES**

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**ACUTE AND CHRONIC ETHANOL EXPOSURE REGULATE NMDA RECEPTOR FUNCTION IN THE VENTRAL BED NUCLEUS OF THE STRIA TERMINALIS**

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Virginia

**FYN TYROSINE KINASE MEDIATES ACUTE ETHANOL-RESPONSIVE MYELIN-ASSOCIATED GENE EXPRESSION IN DBA2/J AND B6;129S7 -** ***Fyn*$^{tm1Sor}$**/J MICE.**

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**EFFECTS OF SOCIAL DEFEAT ON ETHANOL DRINKING AND ANXIETY MEASURES**

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**Italy**

## CHRONIC STRESS CHANGES THE POTENCY AND EFFICACY OF ETHANOL ON $GABA_A$ RECEPTOR PLASTICITY AND FUNCTION

**Biggio G[1,2],, Mostallino MC[3], Pisu MG[3], Talani G[1],Carta M[1], Sanna E[1], Serra M[1,2]**
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Social isolation stress elicits in rats a marked reduction in the cerebrocortical and plasma concentrations of neurosteroids, an effect associated to an increase in the hippocampal level of $\alpha_2$, $\alpha_4$ and $\delta$ subunits immunoreactivity. The amplitude of $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents (mIPSC) recorded from CA1 pyramidal neurons, under the effect of ethanol was greater in hippocampal slices from socials isolated rats than in those from group-housed. Voluntary consumption of ETH during social isolation abolished both the reduction in the concentration of neurosteroids and the potency of ETH on mIPSC recorded from CA1 pyramidal neurons. In contrast, the amplitude of $GABA_A$ receptor-mediated tonic inhibitory currents in granule cells of the dentate gyrus was further increased in socials isolated animals but not in group-housed animals.
Given that voluntary consumption was also associated to a further enhancement in the amount of $\delta$ subunit immunoreactivity through the hippocampus our data suggest that this treatment in social isolated animals may result in an increased number of $GABA_A$ receptors containing both $\alpha_4$ and $\delta$ subunits. To further clarify the effect of social isolation stress on the action of ethanol rats received an intermittent ETH vapour exposure for 8 days a protocol involving multiple withdrawal episodes.
This treatment induced a long-lasting differential increase in the brain concentration of neurosteroids and $GABA_A$ receptor plasticity.

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**TOO MANY SUBUNITS SO LITTLE TIME: TICKU'S LEGACY ON ETHANOL MODULATION OF $GABA_A$ RECEPTOR SUBUNIT GENE EXPRESSION**

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Since the cloning of the many subunits of the $GABA_A$ receptor a plethora of studies were undertaken in order to understand how chronic ethanol exposure as well as ethanol withdrawal could interfere with their gene expression. Ticku's laboratory was one of the first to study this effect of ethanol and for many years until his last publications a great effort was made to elucidate the cellular location, subunit composition and functional role of native $GABA_A$ receptors important in order to develop better drugs.
More work needs to be done to understand how ethanol can interfere with the molecular mechanisms underlying the expression of the various $GABA_A$ receptor subunits and the assembly of subunit combinations to form functional receptors.
In this presentation we will discuss some effects of chronic ethanol exposure and ethanol withdrawal on $GABA_A$ receptor subunit expression and related receptor function obtained by our research group using neuronal cell cultures. We will show that some changes in gene expression and function evident during ethanol withdrawal can be selectively blocked by drugs such as diazepam, flumazenil or γ-hydroxybutyrate, whereas neither gaboxadol nor neuroactive steroids are effective. Moreover, some ethanol withdrawal-induced changes in gene expression seem to be steroids mediated since they can be blocked by the 5α-reductase inhibitor finasteride.

**ROLE OF BRAIN STEROIDOGENESIS IN THE MODULATION OF GABAergic SYNAPTIC TRANSMISSION BY ETHANOL**

E. Sanna, M. Carta, G. Talani, L. Murru, M.C. Mostallino, M.G. Pisu, M. Serra, and G. Biggio
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We have previously shown that in rat hippocampal slices the acute exposure of ethanol (EtOH) results in the stimulation of local synthesis of neurosteroids such as 3α,5α-TH Prog, an effect that is associated to a facilitation of $GABA_A$ receptor function in CA1 pyramidal neurons. More recent results have demonstrated that brain steroidogenesis is also an important mechanism underlying the inhibition of LTP in CA1 pyramidal neurons by EtOH (Izumi et al., 2007). Social isolation is a model of prolonged stress that has been shown to be associated to marked behavioral alterations, a decrease in brain and plasma concentrations of neuroactive steroids, and an abnormal response to acute stressful stimuli as well as to an increased steroidogenic effect induced by the systemic administration of EtOH. We have used the social isolation paradigm both in rats and mice in order to investigate the role of brain steroidogenesis in the actions of EtOH and the interplay with stress. The data obtained demonstrate that social isolation stress is associated to an enhanced sensitivity to the neurosteroidogenic effect of EtOH and to an higher potentiation of $GABA_A$ receptor-mediated mIPSCs in CA1 pyramidal neurons compared to group-housed animals. In addition, we found that social isolation induces a marked increase in extrasynaptic $GABA_A$ receptor-mediated tonic currents recorded in dentate gyrus granule cells, but not in CA1 neurons, an effect that was related to an enhanced expression of α4 and δ subunits. Taken together, these data indicate that a prolonged alteration in neuroactive steroid brain levels during social isolation may lead to marked changes in $GABA_A$ receptor gene expression and function as well as to an hyper-responsiveness to EtOH.
Supported by INIA-Stress (Grant 1 U01 AA016670-01)

**JUVENILE SOCIAL ISOLATION STRESS CHANGES NEURONAL PLASTICITY AND ETHANOL SENSITIVITY**

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Neuroactive steroids play a crucial role in stress, alcohol dependence and withdrawal, and other physiological and pharmacological actions by potentiating or inhibiting neurotransmitters action. Conversely, acute and chronic stress as well as acute and chronic exposure to ethanol are able to modify the content of these hormones in plasma and brain. Juvenile social isolation stress elicits in adult rats a marked reduction in the cerebrocortical and plasma concentrations of neuroactive steroids, an effect associated to alteration in the plasticity of $GABA_A$ receptor, an increased sensitivity to the brain steroidogenic effects of ethanol and a greater capability of this drug to increase the amplitude of GABAA receptor-mediated miniature inhibitory postsynaptic currents (mIPSC) recorded from CA1 pyramidal neurons. Voluntary consumption of ETH during social isolation abolished both the reduction in the concentration of neuroactive steroids and the potency of ETH on mIPSC recorded from CA1 pyramidal neurons. Recent data from our group show that social isolation induces a changes in the expression of the neurotrophic factor BDNF and its precursor, proBDNF in the hippocampus and in the cerebral cortex. Given the functional relationship between steroids and BDNF and the role of GABAergic transmission in modulating the synthesis of BDNF, we are currently evaluating the possible interactions between neuroactive steroids levels and neuronal plasticity on the sensitivity to chronic ethanol during long-term exposure to stress.

**Maine**

## CIRCADIAN RUNNING-WHEEL ACTIVITY DURING WITHDRAWAL FROM CHRONIC INTERMITTENT ETHANOL VAPOR EXPOSURE IN MICE.

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Alcohol withdrawal is associated with affective, neurophysiological, neuroendocrine, and chronobiological disturbances, as well as increased alcohol craving, in both human alcoholics and in animal models of alcohol dependence. In general, these phenomena are potentiated by increased chronicity of prior alcohol exposure and by multiple prior episodes of withdrawal. In several studies, we examined the effects of withdrawal from chronic intermittent ethanol (CIE) vapor exposure on circadian patterns of locomotor (running-wheel) activity in C57BL/6J and C3H mice. Following baseline activity measurements, mice were exposed to a 4-day CIE protocol in which 16 hrs of ethanol vapor exposure alternated with 8 hrs of withdrawal, while control animals were exposed to plain air in an identical environment. During CIE, animals remained in their home cages with access to running wheels, but running-wheel activity was not recorded. Ethanol exposure began at the onset of the dark phase of the daily 12:12 LD cycle, and each exposure period was initiated by an injection of 1.6 g/kg ethanol and 1.0 mmol pyrazole, i.p., to rapidly stabilize blood ethanol concentrations, while controls received pyrazole in saline only. In C57 mice, with low (60-100mg%), to high (150-250mg%), blood ethanol levels, CIE resulted in reductions in locomotor activity, while magnitude and duration of effect seemed to be dose-dependent. Activity levels remained unchanged in controls. In C3H mice, preliminary findings demonstrated a similar reduction of locomotor activity, while drinking activity remained unchanged. Thus the observed reduction in locomotor activity may not be due to a general malaise, such as might accompany compromised health, rather reflecting a specific motivational effect. Analysis of circadian waveforms indicated that reduced activity occurred throughout most of the dark phase of the LD cycle, but that daily activity patterns were otherwise unaltered. These results contrast with those of Kliethermes et al., (2005), who reported little or no effect of withdrawal from three days of (continuous) ethanol vapor exposure on circadian activity patterns recorded by photobeam monitors, rather than running wheels, which are thought to reflect a form of reward-seeking behavior. Subsequent experiments will examine the effects of ethanol dose, mouse strains, repeated CIE cycles, and day- vs. night-time ethanol exposure on circadian activity patterns. Supported by NIAAA AA013893 and by INIA-Stress (K.Grant, OHSU, PI).

**CHRONIC ETHANOL INTAKE ALTERS CIRCADIAN PACEMAKER FUNCTION IN C57BL/6J MICE.**

A. M. Rosenwasser, R. W. Logan; J. A. Seggio; M. C. Fixaris; J. D. Reed
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Recent work from our laboratory showed that chronic ethanol intake alters fundamental properties of the circadian pacemaker, including free-running period and responsiveness to phase-shifting stimuli, in rats and hamsters (Seggio et al., 2007; Rosenwasser et al., 2005a, b). Thus, the present experiments were designed to extend these results to C57BL/6J (B6) inbred mice. In both experiments, male B6 mice were housed individually in running-wheel cages and maintained under either forced ethanol intake (continuous 24-hour access to 10% v/v ethanol solution as the sole drinking fluid) or free-choice ethanol intake (continuous access to both 10% ethanol and tap water in separate bottles), while an additional group consisted of water-only controls. In Experiment 1, free-running circadian period was monitored in all groups under long-term exposure to constant darkness. Relative to water-only controls, free-running period was persistently shortened in the forced-ethanol group but not in the free-choice ethanol group. In Experiment 2, animals were exposed to brief light pulses during either early or late subjective night, followed by 1-2 weeks exposure to constant darkness, in order to assess the effects of ethanol intake on light-induced phase delays and advances. Again, free-choice ethanol failed to alter light-induced phase shifting at either test phase, but forced ethanol attenuated light-induced phase delays and appeared to potentiate phase advances, relative to water-only controls. In both experiments, total weekly fluid intakes were similar in the forced-ethanol and water-only groups, while ethanol intake was about 40% higher in the forced-ethanol group relative to the free-choice ethanol group. Finally, ethanol preference in the free-choice groups was substantially higher under constant darkness (Experiment 1, about 80%) than under light-dark cycles (Experiment 2, about 40%). These results indicate that high levels of chronic ethanol intake alter fundamental properties of the B6 mouse circadian pacemaker. Studies of additional inbred strains and other genetically defined mouse models may help define the mechanisms mediating these effects, which are likely to include ethanol induced alterations in neurotransmission and gene expression within the circadian system. Supported by NIAAA AA013893 and by INIA-Stress (K. Grant, OHSU, PI).

Maryland

# VARIATION IN NMDA RECEPTOR EXPRESSION AND SENSITIVITY TO ETHANOL'S BEHAVIORAL EFFECTS IN BXD RECOMBINANT INBRED MICE

J.L. Brigman, P. Mathur, L. Lu, R.W. Williams, and A Holmes
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Glutamate signaling via N-methyl-D-aspartate receptors (NMDAR) subserves behavioral effects of ethanol (EtOH) and is increasingly implicated in the pathophysiology and treatment of alcohol abuse. At doses that induce behavioral intoxication in vitro, EtOH acts an allosteric inhibitor of NMDAR. The discriminative stimulus effects of uncompetitive NMDAR antagonists such as MK-801 and ketamine generalize to high doses of EtOH in rodent and monkey, and these drugs mimic subjective intoxication in humans. We examined the functional relationship between endogenous brain NMDAR expression and sensitivity to EtOH's acute behavioral effects in a panel of 28 (C57BL/6J x DBA/ 2J) BXD recombinant inbred (RI) lines of mice. Mice were tested for EtOH-induced (rotarod) ataxia, hypothermia and sedation/hypnosis (loss of righting reflex). Behavioral scores were subject to correlational analysis with whole brain, hippocampal and striatal mRNA expression of NMDAR subunits (NR1, NR2A, NR2B), as well as previously published EtOH- related behaviors in BXD RI mice. Results showed significant variation in behavioral responses to EtOH across BXD RI lines. The pattern of differences across BXD RI lines was specific to the behavior assayed, and behaviors cross-correlated poorly: demonstrating dissociable genetic contributions to each ethanol response. Preliminary analyses revealed that whole brain (but not hippocampal or striatal) NMDAR mRNA expression correlated strongly with ethanol- related behaviors in a subunit-specific manner. Ethanol-related behaviors also correlated well with similar, previously published, traits obtained in other laboratories. Collectively, these data show that natural variation in brain NMDAR expression is associated with differences in sensitivity ethanol's acute intoxicating effects. Research supported by the NIAAA-intramural research program and the

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**RAJ TICKU, A PIONEER IN THE STUDY OF ETHANOL ACTIONS ON LIGAND-GATED ION CHANNELS**

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Raj Ticku and his coworkers began their pioneering neurochemical studies of alcohol interactions with the $GABA_A$ receptor/benzodiazepine complex in the early 1980s. Their 1986 Brain Research Bulletin paper was among the first to show that ethanol increases $GABA_A$ receptor function in brain neurons. Subsequently Raj spearheaded important early investigations of chronic ethanol actions on the subunit composition of $GABA_A$ and NMDA-type glutamate receptors that enhanced our understanding of molecular neuroadaptations that contribute to tolerance, dependence and alcohol addiction. Our own investigations of acute alcohol actions on ligand-gated ion channels (LGICs), beginning later in the 1980s, owe a great deal to the early work of Raj and others, as this work established this class of ion channels as likely targets of alcohol actions. Our laboratory and others found that ethanol inhibits the function of NMDA receptors, and potentiates the function of the $5\text{-}HT_3$ receptor. The ensuing wave of ion channel cloning revealed that $5\text{-}HT_3$Rs and $GABA_A$Rs were close molecular cousins while NMDA receptors are members of a different family. This difference has provided an important clue to the differential alcohol sensitivity of these receptors. My discussions with Raj about alcohol and LGICs stimulated our work on the molecular mechanisms underlying ethanol actions at these receptors. In this presentation the latest findings in this area will be discussed, including structure-pharmacology studies of the $5\text{-}HT_3$ receptor and experiments examining NMDA receptors roles in intoxication.

# PURIFICATION AND PHOTOAFFINITY LABELING OF THE 5-$HT_{3A}$ RECEPTOR

D.M. Lovinger; M. Sanghvi; A.K. Hamouda; S. Srivastava; M.I. Davis; R. Morton; G. Luo; D.C. Chiara; T.K. Machu; J.B. Cohen; M.P. Blanton
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The 5-$HT_3$R is a member of the Cys-loop ligand-gated ion channel (LGIC) superfamily, and is known to be a target for acute alcohol actions. Fully functional homopentameric receptors are formed by the A subunit of the receptor, and thus the 5-$HT_3$R is an attractive model protein for structural studies of an ethanol-sensitive cys-loop LGIC. However, to date little information is available on the structure of this receptor. We are interested in expressing and purifying 5-$HT_3$Rs to initiate structural studies. A mouse 5-$HT_{3A}$R containing a C-terminal α-bungarotoxin (αBgTx) pharmatope tag was constructed, and lines of HEK 293 cells stably expressing the tagged receptor were selected. The tagged receptor was fully functional in these cell lines. Cell surface receptors could be tagged with fluorescent αBgTx derivatives. To obtain sufficient quantities of receptor protein for affinity-purification, αBgTx-5-$HT_{3A}$R-HEK cells were cultured either in 140 mm dishes (~1000 dishes) or in a 5 L spinner flask containing microcarrier glass beads (Cytodex-3). Typically, cells were treated with 100 μM serotonin 24 h prior to harvesting resulting in a ~2.5 fold increase in receptor expression. αBgTx-5-$HT_{3A}$Rs ([$^{125}$I]αBgTx Kd ~10 nM) were affinity-purified (αBgTx-derivatized Sepharose 4B affinity column) from detergent (1% CHAPS) solubilized membranes to 30% purity (~2-3 mg protein was obtained). In this first study, the lipid-protein interface of the 5-$HT_{3A}$R was examined by hydrophobic photolabeling with [$^{125}$I]TID. Radiolabled TID photoincorporates into the 5-$HT_{3A}$R and the labeling maps to two proteolytic fragments, designated V8-17K and V8-8K. N-terminal sequencing of each rpHPLC purified fragment revealed that V8-17K starts at $Val^{195}$ and based on its apparent molecular weight extends through the M1- M3 transmembrane segments. V8-8K starts at $Val^{424}$ and contains the M4 segment. Approximately 60% of the total subunit labeling is localized to V8-8K suggesting that the M4 segment has the greatest exposure to lipid. Additional experiments are in progress to further identify lipid-exposed segments/residues in the 5-$HT_{3A}$R and the results will be compared with data from the *Torpedo* nAChR. These findings indicate that partial purification and photoaffinity labeling of the receptor can be successfully carried out. Efforts are underway to improve purification to obtain samples suitable for other biophysical analyses.

**ETHANOL AND ENDOCANNABINOID INTERACTIONS IN THE CONTROL OF GABA RELEASE IN RAT BASOLATERAL AMYGDALA**

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Many of the pharmacological effects of ethanol (EtOH) in the mammalian central nervous system (CNS) may be attributable in interactions with GABAergic synaptic transmission. Pharmacological and behavioral evidence suggest that GABAergic synapses in the basolateral amygdala (BLA) play an important role in the anxiolytic effects of EtOH and alcohol drinking. The endocannabinoids and their target receptor, the CB1 receptor (CB1R), are involved in ethanol consumption. However the mechanisms underlying ethanol-endocannabinoid interactions are still unclear. The goal of this study is to better understand the molecular mechanisms involved in ethanol actions and possible interactions with endocannabinoids in rodent BLA. We performed whole cell patch-clamp recordings in rat BLA coronal slices and evaluated the effect of EtOH 50-80 mM and CB1R agonists (or antagonists) on GABA-mediated spontaneous inhibitory postsynaptic currents (sIPSC) recorded from rat BLA principal neurons and local interneurons. As in previous studies acute EtOH exposure enhanced GABAergic transmission in a concentration dependent manner, and this enhancement appeared to involve increased presynaptic GABA release. EtOH increased both the amplitude and frequency of sIPSCs. Similar EtOH effects were also observed at GABAergic synapses on GABAergic interneurons. The EtOH effect at GABAergic synpases onto principal neurons was prevented by prior perfusion of the CB1 agonist WIN55,212-2 and facilitated by the CB1 antagonist SR141716A. To further evaluate the role of CB1 in EtOH effects we examined CB1-/- and CB1+/+ mice. Preliminary data show that EtOH potentiation of GABA release was decreased in BLA of CB1R KO mice. Our findings indicate that EtOH interacts with CB1 receptors at GABAergic synapses in BLA. Moreover the EtOH-induced increase in GABA release was observed at synapses onto local GABAergic interneurons, suggesting that EtOH could have both disinhibitory and inhibitory effects in this brain region. It remains to be determined if EtOH and CB1 receptors have direct molecular interactions or if the pharmacological interactions are due to the fact that EtOH has effects on CB1-containing axon terminals. This EtOH-CB1 interaction could contribute to the role of endocannabinoids in ethanol drinking and some of effects of ethanol reward.

**North Carolina**

## NEUROSTEROID-LIKE DISCRIMINATIVE STIMULUS EFFECTS OF ETHANOL IN MONKEYS ARE INFLUENCED BY C-3 STEROIDAL STEREOCHEMISTRY AND IONIC CHARGE OF ESTERS

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Positive and negative modulation of $GABA_A$ and NMDA receptors, respectively, contributes to the discriminative stimulus effects of ethanol. Allopregnanolone (3α,5α-P), pregnanolone (3α,5β-P), and androsterone (3α,5α-A) are 3α-hydroxysteroids that positively modulate $GABA_A$ receptors. The 3β-hydroxysteroids epiallopregnanolone (3β,5α-P) and epipregnanolone (3β,5β-P) do not positively modulate $GABA_A$ receptors. A sulfate or hemisuccinate ester at C-3 (e.g., pregnanolone hemisuccinate, 3α,5β-P HS) adds a negative charge to the allosteric modulator that can shift $GABA_A$ modulation from positive to negative or induce positive modulation of NMDA receptors. The current study assessed the substitution of these steroids for ethanol 30 or 60 min after administration in male (n=9) and female (n=8) monkeys (*Macaca fascicularis*) trained to discriminate 1.0 or 2.0 g/kg ethanol (i.g.) with a 30-min pre-treatment interval. The 3α-hydroxysteroids completely substituted for ethanol in ≥ 78% of cases. Androsterone and 3α,5β-P substituted more potently in monkeys trained to discriminate 1.0 g/kg compared to 2.0 g/kg ethanol; 3α,5β-P potency was greater only with a 60-min pre-treatment. Unlike previous findings in rodents, the 3β-hydroxysteroids and 3α,5β-P HS did not substitute for ethanol (≤ 14% of cases). The data indicate that stereochemical transformations and functional group charge of the esters at C-3 influences the ethanol-like discriminative stimulus effects of neuroactive steroids. The data further suggest important species differences in the ability of 3β isomers to produce ethanol-like effects, with the primate in vivo findings being more consistent with the neuropharmacological mechanisms.

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# CHRONIC ETHANOL VAPOR EXPOSURE DOWNREGULATES PRESYNAPTIC DOPAMINE FUNCTION IN THE NUCLEUS ACCUMBENS OF MICE

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Ethanol acutely elevates dopamine levels in the nucleus accumbens and elsewhere in the brain, and chronic exposure induces adaptations aimed at reducing dopamine signaling. We used voltammetry in brain slices to investigate the function of presynaptic dopamine terminals, and found that release was reduced, uptake of released dopamine through its transporter was faster, and inhibitory autoreceptor control of release through D2-type receptors was super-sensitive. These results provide a picture of the nucleus accumbens in the chronic-ethanol exposed mouse as a drastically dopamine-deficient area when ethanol is not present. This is consistent with human PET imaging findings of low dopamine and reduced dopamine responsiveness to ethanol in alcoholics, and provides a potential target for therapeutics to attempt to restore normal dopamine functioning.

# ROLE OF NEUROACTIVE STEROIDS IN ETHANOL/STRESS INTERACTIONS: AN INTRODUCTION

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The production of GABAergic neuroactive steroids, including allopregnanolone and allo-tetrahydrodeoxycorticosterone is a consequence of both acute stress and acute ethanol exposure. Acute, but not chronic ethanol or chronic stress elevates brain levels of these steroids and enhances GABA-A receptor activity. Neuroactive steroids modulate acute anticonvulsant effects, sedation, spatial memory impairment, anxiolytic-like, antidepressant-like and reinforcing properties of ethanol in rodents. Furthermore, these steroids participate in the homeostatic regulation of the HPA axis. Therefore, it is not surprising that neuroactive steroids are involved in ethanol/stress interactions. Nevertheless, the interactions are complex and not well understood. This symposium will address the role of neurosteroids in stress / alcohol interactions. Professor Biggio of the University of Cagliari, Italy will address mechanisms of stress enhancement of alcohol effects on neuroactive steroids and GABA-A receptors. Professor Becker of the Medical University of South Carolina, USA will address neurosteroid involvement in ethanol dependence and drinking behavior. Professor Porcu of the University of North Carolina, USA will describe a neurosteroid biomarker that predicts heavy drinking in monkeys and possibly mice. These presentations will elucidate current theories on the nature of ethanol/stress interactions that may be amenable to therapeutic interventions.

**IDENTIFICATION OF NEUROACTIVE STEROID BIOMARKERS OF ALCOHOL CONSUMPTION IN MONKEYS AND MICE**

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Studies of HPA axis function provide insights into the mechanisms underlying alcoholism risk. We have found that dexamethasone suppression of plasma deoxycorticosterone (DOC) levels in ethanol-naïve cynomolgus monkeys is negatively correlated with subsequent voluntary ethanol intake (Pearson's $r = -0.78$, $p=0.006$). Monkeys that drank the most alcohol exhibited weaker suppression of DOC levels by dexamethasone. The data suggest that DOC sensitivity to dexamethasone may represent a predictive marker of heavy drinking. We have extended this finding to the BXD parental mouse strains, C57BL/6J and DBA/2J that show opposite drinking phenotypes. Mice were injected with three doses of dexamethasone (0.075, 0.1 and 0.13 mg/kg, sc) or saline at 8:00am and were sacrificed 6 hours later. Plasma DOC levels in DBA/2J mice were decreased by 61, 55 and 58%, at 0.075, 0.1 and 0.13 mg/kg, respectively ($p<0.001$). In contrast, plasma DOC levels in C57BL/6J mice were not altered by administration of dexamethasone 0.075 and 0.1 mg/kg; only the dose of 0.13 mg/kg induced a significant decrease (-62%, $p<0.05$). These results suggest that C57BL/6J and DBA/2J mice differ in their sensitivity to dexamethasone suppression of plasma DOC levels in a manner that relates to drinking behavior. Similar to monkeys, the high drinking C57BL/6J mice show a weaker dexamethasone suppression of DOC. We are now investigating dexamethasone suppression of DOC and other neuroactive steroids in different monkeys and BXD mouse strains. These results may identify new biomarkers of alcoholism risk.

# EFFECTS OF ETHANOL ON SERUM LEVELS OF EIGHT GABAERGIC NEUROACTIVE STEROIDS IN RATS MEASURED BY HIGHLY SPECIFIC GC/MS ASSAY.

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Acute ethanol administration increases plasma and brain levels of the progesterone and deoxycorticosterone metabolites 3α-hydroxy,5α-pregnan-20-one (3α,5α-THP) and 3α 21-dihydroxy,5α-pregnan-20-one (3α,5α-THDOC). However, little is known about the effects of ethanol on other GABAergic neuroactive steroids, such as the dehydroepiandrosterone (DHEA) and testosterone metabolites, 3α-hydroxy-5α-androstan-17-one (3α,5α-A) and 3α-hydroxy-5α-androstan-17β-diol (3α,5α-A-Diol). We used a highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) assay to measure serum levels of the 3α,5α- and 3α,5β-reduced GABAergic neuroactive steroids and their precursors pregnenolone and DHEA, following acute ethanol administration. Seven male rats were injected i.p. with 2 g/kg ethanol and were sacrificed 60 minutes later. Nine control rats received an equivalent volume of saline. Ethanol administration selectively increased serum levels of GABAergic neuroactive steroids. Pregnenolone, 3α,5α-THP, 3α,5α-THDOC and 3α,5β-A levels were significantly increased (+589, +191, +291, $p<0.0001$ and +78, $p<0.05$, respectively), compared to control levels. In contrast, ethanol did not alter 3α,5α-A and 3α,5α-A-Diol levels. Basal 3α,5β-THP and DHEA were detected only in 4/9 and 3/9 controls, but levels did not change after ethanol administration. 3α,5β-THDOC and 3α,5β-A-Diol were not detected in serum from both control and ethanol-treated rats. To better validate the biological relevance of this GC/MS assay, we also investigated the effects of pregnenolone administration (50 mg/kg, i.p.) to male rats (n=8). Pregnenolone significantly increased levels of 3α,5α-THP (+1488%, $p=0.0002$), 3α,5α-THDOC (+205%, $p=0.003$), 3α,5α-A (+216, $p=0.0005$), 3α,5α-A-Diol (+190%, $p=0.001$). DHEA levels were also elevated (+1549, $p=0.04$), although basal levels were detected only in 3/9 rats. 3α,5β-THDOC and 3α,5β-A-Diol were increased from undetectable levels to 271 ± 100 and 2.4 ± 0.9, respectively (pg±SEM), but only in 5/8 rats. 3α,5β-THP and 3α,5β-A were not altered by pregnenolone administration. Pregnenolone levels were dramatically increased (greater than 1000 fold) in all rats, but an accurate estimate was not possible using the same GC/MS settings for concurrent analysis. The highly specific GC/MS assay can be used to investigate the physiological and pathological role of neuroactive steroids, develop biomarkers and new therapeutics for neuropsychiatric diseases, including alcoholism.

**Oklahoma**

## REPEATED ETHANOL WITHDRAWAL REDUCES PAIRED-PULSE FACILITATION AND LTP WITHIN AMYGDALA CIRCUITS.

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Withdrawal from chronic ethanol exposure is associated with increased emotionality and behavioral signs of stress. The amygdala plays an important role in the modulation of responses to stress and may contribute to the exacerbation of stress-related withdrawal symptoms that are sometimes observed after repeated withdrawal episodes. The studies reported here examined the effects of chronic alcohol exposure in C57Bl/6J mice on the network properties of the amygdaloid nuclear complex. For these studies, animals received two repeated, 4-day cycles of ethanol exposure that included a priming dose of ethanol (1.6 g/kg, 8% w/v, i.p.), the alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg), and 16 hrs of ethanol-vapor exposure each day followed by a 72 hr period of withdrawal. After the second 72 hr withdrawal period, acute in vitro amygdala slices were studied. Control animals received similar housing, handling and daily pyrazole injections, but were not exposed to ethanol. Studies were conducted using a substrate-imbedded multi-electrode array (Panasonic Med64) that allowed the examination of electrical activity from 64 electrodes across each slice. Individual slices from each animal were examined for the effects of paired-pulse stimulation on response facilitation and also for the development of long-term potentiation (LTP) in response to 5 one-second bursts of electrical stimulation administered to neurons within the basolateral amygdaloid complex (lateral, basolateral, and basomedial nuclei of the amygdala) with recordings obtained within each nucleus and also within the central nuclear group (central lateral and central medial nuclei). Animals exposed to repeated periods of withdrawal from chronic ethanol exhibited significant reductions in paired-pulse facilitation, suggesting reduced pre-synaptic inhibition, and reductions in LTP both locally, within the stimulated nuclei, and in projection sites within the central nuclear group. These findings provide evidence of significant alterations in amygdala circuit function 72 following a period of repeated ethanol exposure and withdrawal and suggest that these effects may contribute to the neuronal hyperexcitability observed during the ethanol withdrawal syndrome.
[Supported in part by NIAAA INIA-Stress]

**Oregon**

## POPULATION ANALYSIS OF HPA AXIS FUNCTION IN RHESUS MACAQUES

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The hypothalamic-pituitary-adrenal (HPA) axis orchestrates the physiological response triggered by acute stress. A blunted HPA axis response has been associated with alcohol abuse and increased risk of relapse among alcoholic men. A study in cynomolgus macaques also suggests that an attenuated HPA axis response is associated with increased ethanol consumption. To explore the determinants of HPA axis function, we initiated a population study in a large, pedigreed rhesus macaque breeding colony. Sixty unrelated, male rhesus, ages 4-8 years old, living in single caged housing were evaluated. Circulating cortisol and ACTH levels were measured 12 hours before and after administration of a low dose of dexamethasone (0.13 mg/kg). This study found the population distributions of cortisol and ACTH suppression values to be strikingly different: there was evidence of strong cortisol suppression ($\geq$75% decrease) in the vast majority of the animals, while the ACTH suppression values were wide ranging and normally distributed in the population. To investigate further the reproducibility of the ACTH suppression, we chose twenty four macaques from either end of the ACTH distribution to retest 6 months after the first challenge. The second set of suppression values were highly correlated with the first set ($r = 0.78$). We also compared the ACTH suppression values of 16 sib-pair rhesus with 32 randomly chosen, unrelated, male monkeys and found that the related pairs were more highly correlated, providing evidence of heritability. We conclude that ACTH suppression is ideally suited for the genetic analysis of factors that contribute to HPA axis function in primates. Further, we will test the hypothesis that ACTH suppression in alcohol naïve animals is predictive of subsequent alcohol consumption in ethanol self-administration protocols.
(Supported by grants AA13510-08 and RR00163)

15346YF

# SCHEDULED-INDUCED POLYDIPSIA IS ASSOCIATED WITH INCREASED ACTH AND HEAVY DRINKING TYPOLOGIES IN A MONKEY MODEL OF ETHANOL SELF-ADMINISTRATION

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Background: We have developed an animal model of alcohol self-administration that employs schedule-induced polydipsia (SIP) to establish reliable ethanol consumption under open access (22 hr/day) conditions with food and water concurrently available. SIP is an adjunctive behavior that is generated by constraining access to an important commodity (e.g., flavored food). The induction schedule is believed to be stressful and ethanol polydipsia generated under these conditions affords the opportunity to investigate the response to stress and risk for chronic, excessive alcohol consumption.
Methods: Adult male cynomolgus monkeys *(Macaca fascicularis)* were induced to drink water and 4% (w/v in water) ethanol by a Fixed-Time 300 sec (FT-300 sec) schedule of banana-flavored pellets. The FT-300 sec schedule was in effect for 120 consecutive sessions, with daily induction doses increasing from 0.0 to 0.5 g/kg to 1.0 g/kg to 1.5 g/kg every 30 days. Prior to, during and following induction, blood samples were drawn for ACTH and cortisol measures 3x/week (morning, afternoon and evening). Following induction, the monkeys were allowed concurrent access to 4% ethanol and water for 22 hrs/day for 12 months.
Results: The FT-300 sec schedule significantly raised circulating ACTH in all monkeys (group mean=30.5 ± 6.7 pg/ml to 60.0 ± 7.4 pg/ml, P<001), but not cortisol (group mean=18.5 ± 3.7 ug/ml to 14.8 ± 4.1 ug/ml). Drinking typographies during the induction of drinking 1.5 g/kg ethanol emerged that were highly predictive of the daily ethanol intake over the next 12 months. Specifically, the ability to ingest 1.5 g/kg without a 5-min lapse in drinking during induction was the strongest predictor (correlation 0.91) of heavy drinking (mean daily intakes >3.0 g/kg for 12 months) characterized by frequent "spree" drinking (intakes >4.0 g/kg/day for 3-4 consecutive days). Blood ethanol during induction were highly correlated with intake and ranged from 100-160 mg% when the monkeys gulped their 1.5 g/kg dose. The proportion of the population that became heavy drinkers was 40%.
Conclusion: This model of ethanol self-administration, highlights the stressful nature of SIP during induction and the importance of accurate measures of alcohol intake for investigating early alcohol drinking typologies that evolve into chronic heavy alcohol consumption in primates. The model may aid in identifying biological risks for establishing harmful alcohol drinking. (Supported by AA13510-08)

**ANTAGONISM OF THE ETHANOL-LIKE EFFECTS OF ZOLPIDEM BY RO15-4513 IN CYNOMOLGUS MONKEYS**

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The discriminative stimulus effects of ethanol are mediated by γ-amino-butyric acid $(GABA)_A$ receptors in non-human primates, but specific $GABA_A$ subtypes and possible individual differences require further study. Zolpidem is a $GABA_A$ receptor positive modulator with high, intermediate and low activity at receptors containing $\alpha_1$, $\alpha_{2/3}$ and $\alpha_5$ subunits, respectively, and is inactive at receptors containing $\alpha_{4/6}$ subunits. The "alcohol antagonist" Ro15-4513 antagonizes the discriminative stimulus effects of ethanol and has the greatest affinity for $\alpha_{4/6}$-containing receptors, lower affinity for $\alpha_5$- and lower, but equal, affinity for $\alpha_1$- and $\alpha_{2/3}$-, -containing $GABA_A$ receptors. In the current study, male ($n$ = 9) and female ($n$ = 8) cynomolgus monkeys (*Macaca fascicularis*) were trained to discriminate 1.0 g/kg ($n$ = 10) or 2.0 g/kg ($n$ = 7) ethanol (i.g.) from water with a 30-min pretreatment interval. Zolpidem (0.017-5.6 mg/kg, i.m.) completely substituted for ethanol (≥ 80% of total session responses on the ethanol-appropriate lever) in 6/7 and 4/10 monkeys trained to discriminate 2.0 and 1.0 g/kg ethanol, respectively. In monkeys that showed substitution, zolpidem dose-response curves were shifted to the right by Ro15-4513 (0.003-0.30 mg/kg, i.m., 5-min pre-treatment) in all monkeys tested, indicating that $GABA_A$ receptors sensitive to Ro15-4513 contribute to the ethanol-like effects of zolpidem. Apparent $pK_B$ and $pA_2$ analyses indicated that Ro15-4513 more potently shifted the zolpidem dose-response function in males (mean ± SEM $pK_B$, 7.29 ± 0.14; females, 7.02 ± 0.14) and in monkeys trained to discriminate 1.0 g/kg ethanol (7.36 ± 0.18; 2.0 g/kg, 7.01 ± 0.10). The substitution data suggest that the discriminative stimulus effects of higher doses of ethanol (2.0 g/kg) are mediated to a greater extent by zolpidem-sensitive receptors compared to lower (1.0 g/kg) doses of ethanol. The $\alpha_1$-specific proprietary antagonist β-carboline-*t*-butyl ester does not antagonize zolpidem substitution for 1.0 g/kg ethanol (i.v.) in squirrel monkeys (Platt et al. 2005 JPET 313: 658-667), suggesting that Ro15-4513 may antagonize the ethanol-like effects of zolpidem by acting at $\alpha_{2/3}$ or $\alpha_5$-containing $GABA_A$ receptors.

# $GABA_A$ RECEPTOR SUBTYPES MEDIATING THE ETHANOL-LIKE EFFECTS OF NEUROACTIVE STEROIDS

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Drug discrimination is a pharmacologically-specific in vivo assay in which some effects of ethanol are trained as a complex discriminative stimulus. More than 20 years of research indicates that only drugs having receptor mechanisms in common with ethanol substitute when systemically-administered as indicated by ethanol-appropriate responding. Structure-activity analyses revealed stereospecificity in the substitution of neuroactive steroids for the discriminative stimulus effects of ethanol. Neuroactive steroids with a –OH group in the 3α position (allopregnanolone, 3α,5α-P and pregnanolone, 3α,5β-P) but not the 3β position (epiallopregnanolone, 3β,5α-P and epipregnanolone, 3β,5β-P) substituted for the discriminative stimulus effects of ethanol. The $GABA_A$ receptor subtypes mediating the substitution of neuroactive steroids for the discriminative stimulus effects of 1.0 and 2.0 g/kg ethanol (i.g., 30-min pre-treatment) were investigated in young adult male (n = 7) and female (n = 7) cynomolgus macaques. Pre-treatment (5 min) with Ro15-4513 (0.003-1.0 mg/kg, i.m.) antagonized the substitution of 3α,5β-P (pregnanolone) for 1.0 (3/3 females, 3/3 males) and 2.0 g/kg (1/2 females, 2/3 males) ethanol. The substitution of 3α,5α-P (allopregnanolone) for ethanol was similarly antagonized by Ro15-4513 (5/8 monkeys tested), but fewer dose-response curves were available for analysis. The data indicate overlap in the $GABA_A$ receptor subtypes by which neuroactive steroids produce ethanol-like discriminative stimulus effects and the receptor subtypes that are sensitive to Ro15-4513. This common $GABA_A$ receptor population may include extrasynaptic receptors containing $\alpha_{4/6}$ and δ subunits. (Support: AA10009 and AA13860)

**South Carolina**

## NEUROSTEROID ALLOPREGNANOLONE INVOLVEMENT IN ETHANOL DEPENDENCE AND DRINKING BEHAVIOR IN C57BL/6J MICE

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We have previously demonstrated that repeated cycles of chronic ethanol exposure and withdrawal experience results in enhanced voluntary ethanol drinking in C57BL/6J mice. Studies were conducted to test the hypothesis that stress associated with repeated cycles of chronic ethanol exposure and withdrawal produces brain regional changes in allopregnanolone levels that relate to enhanced ethanol drinking. Adult male C57BL/6J mice were first trained to drink 15% ethanol in a 2-bottle choice limited access (2 hr/day) procedure. Mice were then exposed to either chronic intermittent ethanol vapor (EtOH) or air (CTL) in inhalation chambers (16 hr/day for 4 days) followed by the opportunity to drink ethanol for 5 days in the limited access paradigm. This procedure was repeated for a second cycle, and EtOH and CTL mice were sacrificed either immediately upon final withdrawal (HR-0), or later times following withdrawal (HR-8 and HR-72), or following 5 or 10 days access to ethanol (5-Day and 10-Day). Brains were removed and cortex, hippocampus, and hypothalamus were dissected and frozen until later assay for allopregnanolone levels by RIA. A separate group of EtOH-naive mice were included for baseline allopregnanolone determinations. At time of sacrifice, blood samples were collected for measuring plasma corticosterone levels by RIA. Preliminary data suggest brain regional and time-dependent changes in allopregnanolone levels in EtOH dependent mice compared to baseline (EtOH-naive) and CTL (non-dependent) groups. Potential relationships between brain allopregnanolone levels and ethanol consumption as well as plasma corticosterone levels are currently being analyzed. Supported by NIAAA grant U01 AA014095.

**INFLUENCE OF CHRONIC ETHANOL EXPOSURE AND WITHDRAWAL ON CHOLINERGIC NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS OF MICE**

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This study examined whether chronic ethanol exposure and withdrawal affects cholinergic neurotransmission in the Nucleus Accumbens (NAC) using *in vivo* microdialysis procedures to measure extracellular acetylcholine (ACh) levels and *ex vivo* preparations to measure acetylcholinesterase (AChE) activity. In Experiment 1, guide cannulae were implanted over the NAC and mice were trained to drink 15% ethanol using a limited access (2 hr/d) 2-bottle choice paradigm. After 4 weeks, mice then received either chronic intermittent ethanol exposure in inhalation chambers (16 hr/d for 4d; EtOH group) or similarly treated in control air chambers (CTL group). Chamber exposure alternated with 5d of limited access drinking for 3 cycles. Ethanol intake was 2.89 ± 0.22 and 4.41 ± 0.66 g/kg for CTL and EtOH groups, respectively, during the 3rd cycle. Microdialysis was conducted after the 3rd cycle and ACh was measured using HPLC with electrochemical detection. Baseline ACh values were 0.29 pmol/sample and 0.16 pmol/sample for CTL and EtOH mice, respectively. Upon systemic challenge with 2 g/kg ethanol, ACh levels in the CTL mice declined to 0.15 pmol/sample and then returned to baseline levels; however, the 2 g/kg ethanol dose did not affect ACh in the EtOH mice. These results indicate that NAC ACh levels are reduced in mice with chronic ethanol exposure and withdrawal experience and, further, that ACh levels in EtOH mice are unaffected after acute challenge with a behaviorally relevant dose of ethanol. In Experiment 2, non-drinking mice were exposed to one cycle of chronic intermittent ethanol exposure (16hr/d for 4d). At 72h following this treatment, NAC tissue from EtOH, CTL, and chamber naïve mice was collected to measure AChE activity using the Ellman method. AChE activity in the NAC was 33% lower in the EtOH group relative to CTL and naïve mice after this short regimen of exposure and withdrawal. These results indicate that AChE activity in the NAC is influenced by chronic ethanol exposure and withdrawal. Collectively, these results indicate that ethanol dependence produces alterations in cholinergic transmission in the NAC. Studies are currently under way to explore the role of such changes in mediating enhanced drinking as a consequence of ethanol dependence.

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**Tennessee**

## A MULTI-DIMENSIONAL GENETIC ANALYSIS OF ALCOHOLISM AND ADDICTION SUSCEPTIBILITY PHENOTYPES

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The propensity toward alcoholism and drug addiction has been related to many predisposing behavioral and physiological preconditions, including novelty seeking, stress response, depression, neurogenesis, anxiety, hyperactivity, sensitivity to drugs of abuse, pain sensitivity, appetitiveness, habituation to environments, withdrawal, and abnormal reward systems. Mouse genetic reference populations have proven to be a powerful resource in identifying the genetic factors underlying alcohol related phenotypes. By performing multi-dimensional phenotyping in these populations, the role of genetic variation in determining vulnerability to addiction to diverse substances can be evaluated. The newly expanded BXD recombinant inbred (RI) lines of mice consist of approximately 80 strains of mice derived from inbreeding the progeny of a cross between C57BL/6 and DBA/2 mice. Members of the INIA-Stress and Tennessee Mouse Genome Consortia have engaged in large scale multi-variate analysis of genetic predisposition to alcohol- and drug-abuse related phenotypes in these lines. 8 mice per sex and strain were distributed for each phenotyping protocol, and over 40 phenotypes have been studied. Protocols for the phenotyping are available online. Alcohol, cocaine, morphine, and MDMA effects have been evaluated, in addition to numerous basal behavioral phenotypes. Supported by NIAAA-NIDA 7R01DA020677-03, DOE ERKP804.

**DOPAMINE REGULATION OF SYNAPTIC TRANSMISSION IN THE BNST**

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Dopaminergic signaling plays a key role in reward-related behavior, with evidence suggesting it undergoes modification following chronic alcohol exposure. A focus of much of this research has been the mesolimbic dopaminergic system, originating in the ventral tegmental area and terminating in the nucleus accumbens. However, recent findings have suggested that dopamine actions in brain regions beyond this canonical pathway, including the bed nucleus of the stria terminalis (BNST), are involved in both acute and chronic aspects of alcohol abuse. Based on these findings, we examined the ability of dopamine to modulate synaptic transmission in the BNST. We find that dopamine enhances glutamatergic transmission in a D1 and D2 dopamine receptor dependent manner. Ongoing experiments will determine the mechanisms underlying this enhancement of function.

**REPEATED ALCOHOL EXPOSURE INDUCES HOMEOSTATIC SYNAPTIC ADAPTATIONS IN THE EXTENDED AMYGDALA**

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Repeated exposure to alcohol leads to alterations in behavior, including increased alcohol drinking behavior. This has been proposed to be due, in part, to changes in neuronal function in the extended amygdala. Previously, we examined the ability of acute alcohol to alter synaptic function in the ventral bed nucleus of the stria terminalis (vBNST), a component of the extended amygdala. We found that acutely applied alcohol inhibits NMDA receptor (NMDAR) function post-synaptically in a NR2B subunit dependent fashion. In this study, we examined the effects of chronic ethanol exposure and withdrawal on synaptic function in the BNST. We found no change in parameters associated with glutamate release, but found evidence for alterations in synaptic NMDAR function. Specifically, we found an increase in the decay time of the evoked NMDAR dependent excitatory post-synaptic current, suggesting an increase in synaptic NR2B-containing NMDAR. In order to determine if there was an alteration in the functional contribution of NR2B-containing NMDAR we examined the ability of the NR2B selective antagonist to inhibit NMDAR EPSCs. Consistent with an increase in NR2B function, we found an increase in inhibition by the NR2B selective antagonist. In order to determine if it was the alcohol exposure or the withdrawal that resulted in this change, we performed a time matched continuous alcohol exposure and found no alteration of function. These findings suggest that chronic ethanol exposure and withdrawal lead to an increase in NMDAR mediated glutamatergic transmission in BNST. When compared with our previous studies these results suggest that alcohol can bidirectly modulate NMDAR function in the vBNST depending on the exposure; with acute application inhibiting NMDAR function in an NR2B dependent fashion and chronic exposure enhancing NR2B function. We hypothesize that the alteration in function due to chronic exposure leads to an increased ability to induce long term forms of plasticity at glutamatergic synapses in BNST, which may play an important role in the progression to alcohol dependence.

**CHRONIC ETHANOL EXPOSURE ATTENUATES $\alpha_1$-AR LTD IN THE BED NUCLEUS OF THE STRIA TERMINALIS**

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It is hypothesized that the pathogenesis of alcoholism develops as a kindling process where the individual cycles through periods of intoxication and withdrawal resulting in altered levels of excitation and inhibition centrally, altered stress responses and changes in affect. Data have highlighted the noradrenergic system as being upregulated in alcoholics (Breese et al. 2005) and blocking $\alpha_1$- (adrenergic receptors) AR in animal models of withdrawal decreases drinking behavior (Walker et al. 2008). Further, anxiety, a predictor for relapse behavior in humans, is enhanced during alcohol withdrawal. Our lab is interested in the role of norepinephrine (NE) in the bed nucleus of the stria terminalis (BNST) a brain region anatomically situated to integrate stress and reward centers. $\alpha_1$-AR activation in this region has been shown to modulate anxiety and HPA axis response after a stressor (Cecchi et al. 2002) and we have recently shown that NE can induce long term depression (LTD) of excitatory synapses via the $\alpha_1$-AR in the BNST (McElligott and Winder, 2008).

Here we expand our findings demonstrating that at least part of the mechanism of $\alpha_1$-AR LTD appears to result in a loss of transmission via calcium permeable AMPA receptors (CP-AMPARs) rendering the synapses insensitive to the CP-AMPAR blocker 1-Naphthylacetyl spermine trihydrochloride (Naspm, a synthetic analogue of Joro Spider Toxin). This suggests that not only does the LTD decrease the excitatory drive of BNST neurons but it limits the calcium availability at these synapses. Further, we have examined the persistence of $\alpha_1$-AR LTD in mice that have experienced chronic ethanol inhalation and withdrawal paradigms. We have found that $\alpha_1$-AR LTD is significantly attenuated in the BNST in animals that have experienced a single withdrawal as compared to their sham controls. Given the role of $\alpha_1$-AR receptor activation in the BNST during stress and its modulation of the HPA axis this is an intriguing result that we are further investigating.

**THE ONTOLOGICAL DISCOVERY ENVIRONMENT: AN INTERNET RESOURCE FOR INTEGRATION OF PHENOMIC INFORMATION THROUGH GENE-CENTRIC ANALYSES**

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The ever increasing array of high-throughput genomic technologies have enabled researchers to rapidly ascribe large sets of genes to alcohol related phenomena in a variety of sequenced organisms. Techniques involving mutant analysis, microarray analysis, and even gene-centric literature analysis have all been used to identify large groups of genes associated with the various behavioral and neurobiological constructs of alcohol effects and alcohol addiction. Integration of these diverse data is performed both within across species via orthologous genes to identify generalizable and robust gene-phenotype associations. The Ontological Discovery Environment is a Web-based tool that has been developed for community submission and integrative analysis of phenotype-centered gene sets, specifically to address questions pertinent to alcohol research. The Ontological Discovery Environment allows submitted gene sets to be compared with existing gene sets for similarity, intersection and overlap. Combinatorial algorithms are applied to the entire database of submitted gene sets to construct a Phenome Integration and Similarity Hierarchy (PhISH) Map. This map of the phenome space is an ontology of the alcohol phenome inferred from empirical data and allows us to confirm our own construction of the relationships among phenotypes and their underlying shared biological processes. The integration of gene-phenotype associations can be used to address a variety of research questions around the biological substrates that are common or unique to sets of alcohol related phenotypes. We have applied this resource to the diverse gene sets related to ongoing studies of stress and alcoholism by the INIA-STRESS consortium along with published literature pertaining to alcohol related studies. These data come from various species and experimental platforms, allowing us to identify the shared biological processes underlying alcohol related phenotypes, pre-disposing phenotypes, stress and behavioral responses to stress.

**ACUTE AND CHRONIC ETHANOL EXPOSURE REGULATE NMDA RECEPTOR FUNCTION IN THE VENTRAL BED NUCLEUS OF THE STRIA TERMINALIS**

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Components of the extended amygdala have been implicated in the mediated anxiety-related behaviors including withdrawal-induced increases in alcohol consumption. The ventral bed nucleus of the stria terminalis (vBNST) is a component of the extended amygdala that has been implicated in the behavioral actions of ethanol. The NMDA receptor (NMDAR) is a major molecular target of ethanol, however, current evidence suggests that ethanol regulation of NMDAR function is widely variable and likely depends on a number of factors. Thus, it is critical to investigate ethanol regulation of NMDAR function at synapses relevant to ethanol regulated behaviors, such as in the vBNST. We find that acutely applied ethanol inhibits NMDAR function in vBNST neurons in a postsynaptic fashion. Further, we demonstrate the functional presence of both NR2A and NR2B containing NMDARs in the vBNST. Pharmacological blockade with an NR2B-selective antagonist rendered synaptically activated NMDARs insensitive to ethanol. In addition to acutely applied ethanol, we also examined the effects of chronic ethanol exposure and withdrawal on glutamatergic signaling in the BNST. We found no change in parameters associated with glutamate release, but found evidence for alterations in synaptic NMDAR function. These findings suggest that chronic ethanol exposure and withdrawal lead to an alteration of NMDAR mediated glutamatergic transmission in BNST, and glutamatergic synapses in BNST may play an important role in the progression to alcohol dependence.

**FYN TYROSINE KINASE MEDIATES ACUTE ETHANOL-RESPONSIVE MYELIN-ASSOCIATED GENE EXPRESSION IN DBA2/J AND B6;129S7- *Fyn*$^{tm1Sor}$/J MICE.**

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Human and animal studies have shown differential gene expression can influence the development of alcohol abuse and alcoholism. But the molecular mechanisms influencing ethanol-induced gene expression remain elusive. Bioinformatics combined with oligonucleotide microarrays have identified ethanol-responsive expression networks, which present several candidate genes for investigating these mechanisms. Two commonly used inbred mouse strains for their divergent behavioral responses to ethanol, DBA2/J (D2) and C57BL/6J (B6), exhibit differing amounts of ethanol induced myelin gene expression. Fyn-deficient mice display a significant reduction in myelin-associated gene expression that parallels a subset of myelin genes induced by ethanol shown previously by other investigators. The present study was aimed at investigating the role of the non-receptor protein tyrosine kinase Fyn mediating increases in myelin-associated gene expression in response to acute ethanol exposure. D2 mice given intraperitoneal ethanol (2 g/kg) injections show a more robust up-regulation of myelin-associated genes that can be partially blocked by the fyn kinase inhibitor PP2. Changes in gene expression are dependent upon both strain (genetics) and ethanol dose as B6 mice exhibit higher basal levels of myelin genes and weaker ethanol-induction. Due to the currently commercially available Fyn-kinase null mutant (B6;129S7- *Fyn*$^{tm1Sor}$/J) we used the B6 related strain B6129SF2/J, to examine dosage effects on myelin gene expression using real-time PCR. Male B6129SF2/J mice presented with either an acute intraperitoneal injection of saline, 2 g/kg of ethanol, or 4 g/kg of ethanol. The highest dose of ethanol produced the greatest induction of myelin-associated gene expression suggesting the presence of a genetic neuroprotective event, which may rely of the relative activation of fyn-kinase. We hypothesize brain regions from B6;129S7- *Fyn*$^{tm1Sor}$/J, fyn-kinase null mutants, exposed to an acute dose of ethanol will not permit myelin-associated gene expression and identify gene networks contributing to behavioral and molecular events presently documented in the literature such as acute tolerance, and preference drinking in two bottle choice paradigms.

**EFFECTS OF SOCIAL DEFEAT ON ETHANOL DRINKING AND ANXIETY MEASURES**

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Human data strongly suggests that stressful life situations increase alcohol abuse and relapse drinking. However, findings in rodent models are inconsistent: increases, decreases or no change in ethanol drinking patterns following a variety of stressors have been reported. Moreover, it has not always been clear if the stress paradigm produces a physiological response that is relevant at the time of ethanol self-administration. Here, we have designed experiments to test the effects of repeated social defeat on ethanol drinking in mice and whether neurochemical and behavioral measures of anxiety are reflected in these changes.

C57BL/6 mice were tested for basal anxiety-like behavior in the light-dark transition model prior to the initiation of drinking studies. Two groups were given voluntary access to 10% (w/v) ethanol and tap water in a 2 bottle choice paradigm while two groups were given access to only water. Following establishment of stable drinking patterns, half the mice were exposed to brief social defeat by an aggressive male or remained in their home cage for 5 consecutive days. Ethanol was continually available in these studies. Separate groups of mice were tested in the light-dark box following defeat and three weeks of ethanol drinking. Corticosterone levels were measured 24 hours following the last social defeat. These measures of anxiety will be used to explore how social stress modifies ethanol drinking.

We have previously shown that social defeat stress may have bidirectional effects on ethanol drinking in C57BL/6 mice. Mice with a predilection for low ethanol preference tend to increase drinking following social stress while high preference mice tend to decrease drinking. We have extended these studies in another inbred strain, 129SvJ, which consumes moderate amounts of ethanol. Interestingly, 129SvJ mice with baseline drinking intake less than 4g/kg in 24 hours increase drinking following social defeat. Mice with greater than 4 g/kg baseline intake either decreased or did not change their intake following social defeat. Together, investigation of social stress modifications on individual variation of ethanol drinking coupled with behavioral and neurochemical anxiety measures will help elucidate role of stress in alcohol abuse.

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