

**INTEGRATIVE NEUROSCIENCE INITIATIVE ON ALCOHOLISM  
STRESS**

*“INIA: STRESS, ANXIETY AND ALCOHOL ABUSE”*



**Abstracts**

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**Maryland – Lovinger, Wand and McCaul Labs**

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SEX AND AGE OF CHRONIC ETHANOL DRINKING AFFECTS ETHANOL INTAKE AND SYNAPTIC TRANSMISSION ONTO MEDIUM SPINY NEURONS IN THE STRIATUM OF THE RHESUS MONKEY

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PRENATAL ALCOHOL EXPOSURE ENHANCES L- AND R-TYPE CALCIUM CHANNEL CURRENTS IN NEONATAL INFERIOR COLLICULUS NEURONS

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PRENATAL ETHANOL EXPOSURE DISRUPTS ENDOCANNABINOID MODULATION OF DORSAL STRIATAL CIRCUITS GOVERNING HABIT FORMATION

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EVALUATION OF ETHANOL RESPONSES IN S296A A1 GLYR KNOCKIN MICE

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GLUTAMATE TRANSPORTER EXPRESSION IN NUCLEUS ACCUMBENS AND DORSAL STRIATUM AFTER CHRONIC INTERMITTENT ETHANOL EXPOSURE

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NOVEL MU-OPIOID RECEPTOR SELECTIVE ANTAGONIST NAQ SELECTIVELY REDUCES CONSUMPTION OF HIGH-CONCENTRATION ETHANOL IN MICE

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P = Poster

S = Symposium



**Maryland – Lovinger, Wand and McCaul Labs**

P0377

SEX AND AGE OF CHRONIC ETHANOL DRINKING AFFECTS ETHANOL INTAKE AND SYNAPTIC TRANSMISSION ONTO MEDIUM SPINY NEURONS IN THE STRIATUM OF THE RHESUS MONKEY

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As many as 1 in 12 adults abuse alcohol or are alcohol dependent. Studies suggest that alcohol-related problems and prevalence of binge or excessive drinking are highest among young adults (18–29 years). Similarly, the risk of alcohol dependence increases the younger the onset of drinking to intoxication. There are also differences in alcohol abuse and its effects due to gender. In general more men than women are alcohol dependent. However due to differences in drinking patterns as well as the effects of alcohol due to gender, women are at a greater risk to develop alcohol related problems.

Here we examine the effect of age of onset and gender on daily EtOH intake, pattern of drinking and the accompanying changes in synaptic transmission in MSNs of putamen, a brain region that we have previously discovered shows decreased GABAergic transmission that correlates with BEC/intake in chronic EtOH drinking monkeys. To examine age-of-onset effects on EtOH consumption and changes in synaptic transmission, young adult (age at drinking onset 5–6 years) and adult (age of onset drinking onset 7–11 years) male rhesus monkeys were trained to orally self-administer EtOH under “open access” for approximately one year. To examine potential sex differences, these young adult males were compared to age-matched females. Younger male monkeys had average daily intakes between 2.7 – 4.1 g/kg (yielding BECs 45.8–142.3 mg/dl) whereas older males averaged from 0.3 – 2.6 g/kg (yielding BECs 0–96.1 mg/dl). Young females, on the other hand, averaged between 4.1 – 5.6 g/kg (yielding BECs 47.0–99.4 mg/dl). This suggests that younger individuals drank more than older yielding higher BECs. What is interesting is that females drank more than their male counterparts yet had similar BECs. Our data suggests that this may be in part due to the pattern of drinking that differed between the sexes of rhesus monkeys. After necropsy, acute slices containing the dorsal striatum were obtained. Whole-cell patch clamp electrophysiology examined GABAergic miniature inhibitory postsynaptic currents (mIPSC) in putamen MSNs. Chronic EtOH drinking was associated with decreased mIPSC frequency in the putamen that correlated with EtOH intake. We are currently examining the interaction between gender and pattern of drinking with alterations in GABAergic transmission. The observed changes in striatal physiology may shed light on the increased risk for habitual EtOH drinking in younger onset and women drinkers.

P0385

PRENATAL ALCOHOL EXPOSURE ENHANCES L- AND R-TYPE CALCIUM CHANNEL CURRENTS IN NEONATAL INFERIOR COLLICULUS NEURONS

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Alcohol consumption during pregnancy is one of the leading preventable causes of birth defect and neurobehavioral disorders in humans. In the most severe cases, these abnormalities make up a pattern of malformations termed fetal alcohol syndrome (FAS). This

syndrome is associated with high prevalence of generalized tonic-clonic seizures and hearing disorders in neonates and children. Thus, alcohol exposure in utero is a potential factor for the etiology and mechanisms of enhanced seizure susceptibility in neonates with FAS. Evidence indicates that rodent subjected to gestational alcohol exposure exhibit altered auditory brainstem responses, which lasted into adulthood. The inferior colliculus (IC), the generator of auditory brainstem responses wave V, plays a critical role in the initiation of tonic-clonic seizures following alcohol withdrawal. Thus, alcohol-induced changes in IC neuronal excitability may contribute to the mechanisms of enhanced seizure susceptibility associated with FAS. Here, we examined the effects of prenatal alcohol exposure on firing and voltage-activated  $Ca^{2+}$  channel (Cav) currents in dissociated IC neurons obtained from neonatal rat offspring (P3–P7). Pregnant rats received a single dose of 5 g/kg body weight ethanol (95%) as a 30% (v/v) solution in Isomil by gastric intubation at gestational day 18. Control animals received only Isomil. IC neurons were recorded in whole cell patch clamp configuration for current- and voltage-clamp experiments. Cav currents were measured using 5 mM  $Ca^{2+}$ . Quantification shows that prenatal alcohol exposure elicits the occurrence of >2 action potentials (APs) evoked by depolarizing current injections. Such elevated responses were not seen in control IC neurons which only exhibited a single AP. Increasing extracellular (from 2 mM to 5 mM)  $Ca^{2+}$  concentration reversibly enhanced the number of APs in control IC neurons. Quantification also shows that Cav current density was significantly elevated in IC neurons following prenatal alcohol exposure. Pharmacological analysis reveals that Cav1.2/Cav1.3 and Cav2.3 contribute to the enhanced current density in neonatal IC neurons following prenatal alcohol exposure. These findings suggested that altered Cav1.2/Cav1.3 and Cav2.3 signaling may play important roles in the pathophysiology of FAS-associated diseases of neuronal excitability. Supported by NIH grants AA020073 (PN) and the NIAAA Division of Intramural Clinical and Biological Research (DML).

P0387

#### PRENATAL ETHANOL EXPOSURE DISRUPTS ENDOCANNABINOID MODULATION OF DORSAL STRIATAL CIRCUITS GOVERNING HABIT FORMATION

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Prenatal ethanol (EtOH) exposure-induced changes in brain development are associated with impairments in learning and memory-related and activity-related behaviors governed by dorsal striatal regions in the basal ganglia. To investigate early life EtOH exposure effects on dorsal striatum function, we exposed mice to EtOH (yielding a BEC of ~80mg/dl) from embryonic day 0.5 through postnatal day 10, and assessed the ability to learn and execute dorsalstriatum dependent self-initiated actions in adulthood. Using a novel variant of an instrumental task, mice were trained to shift between performing the same action for the same reward in a goal-directed versus habitual manner. We found that while goal-directed actions were intact, prenatal EtOH exposure disrupted the ability to perform habitual actions. Since goal-directed and habitual actions depend upon dorsal medial (DMS) and dorsal lateral (DLS) striatum respectively, we then used in-vivo recordings of DMS and DLS neurons to examine activity changes. We observed that early life EtOH exposure disrupted shifts in circuit activity during learning and execution of habitual actions corresponding to impaired habit formation. Since habitual actions depend on cannabinoid type 1 (CB1) receptors that are known to modulate DMS and DLS synaptic transmission and plasticity, we hypothesized that endocannabinoid modulation of dorsal striatal synaptic transmission may be altered following early life EtOH

exposure. Indeed, early life EtOH exposure resulted in altered endocannabinoid modulation of DLS GABAergic transmission in adult mice. The CB1 agonist induced depression of mIPSC frequency seen in control mice was lost, while a CB1 receptor antagonist increased mIPSC frequency, and effect not seen in controls. These findings suggest that early life EtOH exposure increased ambient endocannabinoid levels. Immunohistochemistry suggested only slightly higher CB1 receptor expression, while binding studies showed a similar level of CB1 receptor binding. Altogether, these results suggest that early life EtOH exposure induces developmental adaptations in dorsal striatum resulting in increased endocannabinoid modulation of GABAergic synapses, leading to impaired habit formation. We are currently testing whether we can mimic this finding by increasing endogenous endocannabinoid levels, and whether we can rescue the EtOH-induced deficit in habit formation by altering CB1 receptor or endocannabinoid levels in the DLS.

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#### EVALUATION OF ETHANOL RESPONSES IN S296A A1 GLYR KNOCKIN MICE

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Accumulating evidence has suggested that strychnine-sensitive glycine receptors (GlyRs) are one of the primary targets of alcohol action in the nervous system. Ethanol can allosterically potentiate glycine-activated current (IGly) in various native neurons and in heterologous cell lines expressing recombinant  $\alpha 1$  subunit-containing GlyRs. Although the precise molecular mechanism of ethanol-induced allosteric modulation of GlyRs remains elusive, a number of residues in the transmembrane (TM) domains of the  $\alpha 1$  GlyR are found to be critical for the sensitivity of GlyRs to ethanol-induced potentiation. One of them is the serine residue at 296 in the TM3. A previous study has shown that the S296A mutation can reduce the magnitude of ethanol potentiation of the  $\alpha 1$  GlyR expressed in HEK 293 cells (Yevenes et al, J. Biol. Chem. 285(39): 30203–3021, 2010). To determine if S296 contributes to ethanol-induced behaviors, we constructed S296A knockin mice by gene targeting. There were no significant differences in body weight, food consumption, locomotor activity, startle response and rotarod performance between homozygous S296A mice and their wild type littermates. We next compared genotypes following acute ethanol exposure. Ethanol (1.2 g/kg, i.p.) significantly reduced the time to fall from a rotarod, body temperature, and locomotor activity in wild type mice. Parallel experiments in homozygous knockin mice indicate that these ethanol-induced changes were similar in compared to wild type littermates. Further examination of responses to ethanol in the S296 mice is in progress. Supported by the NIAAA intramural research program and AA017875.

P0711

#### ACUTE ETHANOL EFFECTS ON ENDOCANNABINOID-MEDIATED PLASTICITY OF DORSOLATERAL STRIATUM INHIBITORY MICROCIRCUITRY

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The purpose of this study is to elucidate the circuit dynamics underlying ethanol-induced disinhibition of the dorsolateral striatum, a basal ganglia substructure necessary for habit learning. The principal cell type of the striatum is the medium spiny neuron (MSN). These

GABAergic neurons project to downstream basal ganglia sites via the direct and indirect pathways. MSN output is inhibited by GABAergic innervation arising from other MSNs and from striatal fast-spiking interneurons (FSIs). When direct and indirect pathway MSNs are voltage clamped at a depolarized “up state” these cells express a form of endocannabinoid-mediated long-term depression (eCB-LTD) selectively of the GABAergic input arising from MSN collaterals. However, when direct, but not indirect, pathway MSNs are clamped at a hyperpolarized “down state” these cells express a different form of eCB-LTD that involves both MSN and FSI inputs. Here, we hypothesize that ethanol interacts with this inhibitory microcircuit eCB signaling system to enhance the disinhibition of the direct pathway, a neural circuit operation thought to encode action reinforcement. To test this, we examine acute ethanol effects on FSI-MSN and MSN-MSN GABAergic transmission, and on up and down state forms of eCB-LTD using whole-cell patch clamp electrophysiological recordings combined with optogenetics in the adult mouse striatal slice preparation. Bath application of ethanol (50 mM) depresses both MSN-MSN and FSI-MSN GABAergic transmission evoked by selective light-induced activation of channelrhodopsin that is virally expressed in presynaptic MSNs or FSIs. We also find that in the presence of ethanol, up state eCB-LTD is completely lost, but down state eCB-LTD is preserved. We conclude that ethanol disinhibits dorsolateral striatum MSNs, and that further disinhibition of the direct pathway may occur through selective retention of the down state form of eCB-LTD. These results may provide mechanistic insight into the reinforcing properties of ethanol.

P0150

#### ASSOCIATION OF SMOKING WITH MU-OPIOID RECEPTOR AVAILABILITY IN ALCOHOLDEPENDENT SUBJECTS

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Persons with a history of alcohol dependence are more likely to use tobacco and to meet criteria for nicotine dependence when compared to social drinkers or nondrinkers. The high levels of co-morbidity of nicotine and alcohol use and dependence are thought to be related to complex interactions between nicotinic, opioid and dopamine receptors, which are co-localized in several brain regions including the nucleus accumbens and amygdala. The current study examined whether individual differences in regional mu-opioid receptor (MOR) binding were associated with tobacco use, nicotine dependence, and levels of nicotine craving in alcohol dependent subjects (n=25). Alcohol dependent subjects completed an inpatient protocol, which included medically supervised alcohol withdrawal, monitored alcohol abstinence, nicotine maintenance using transdermal nicotine patches (21 mg), and Positron Emission Tomography (PET) imaging using the MOR-selective ligand [11C]-carfentanil. There was an inverse relationship between nicotine use and nicotine dependence and binding potential (BPND) in alcohol dependent subjects. Subjects who regularly smoked a higher number of cigarettes per day had significantly lower BPND in mesolimbic regions including the amygdala (p=0.0001), globus pallidus (p=0.0019), thalamus (p=0.0078) and insula (p=0.016). Likewise, higher scores for the Fagerstrom Nicotine Dependence Test were associated with significantly lower BPND across mesolimbic regions with largest differences in the amygdala (p=0.0001). Lower BPND in amygdala was highly associated with higher nicotine craving (p=0.0001). These data suggest that intensity of cigarette smoking and severity of nicotine dependence decreased BPND across multiple brain regions in alcohol dependent subjects. These data are compelling given the high co-morbidity of alcohol and nicotine use and dependence. Supported by NIH AA11872, AA11855, AA12303 and K24 DA000412.

P0506

#### WHAT IS STRESSFUL FOR HIV-INFECTED WOMEN? CHRONIC GLOBAL STRESS AND TRAUMA-RELATED STRESS AMONG HAZARDOUS/HEAVY DRINKERS IN HIV PRIMARY CARE

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Purpose: Global stress and specific trauma-related stress may independently influence alcohol consumption and alcohol-related problems among HIV-infected women. We examined the effects of both types of stress and their combined relationship to hazardous/heavy drinking and drinking related consequences.

Methods: Women were recruited from an urban HIV primary care clinic as part of a randomized trial of a brief alcohol intervention. We used the Perceived Stress Scale (PSS), a global measure of stress appraisal and the Impact of Events Scale-Revised (IESR), a measure of symptoms of posttraumatic stress disorder related to their most severe problem. AUDIT-C and Short Index of Problems (SIP) were used as alcohol outcomes. All logistic regressions were adjusted for age, education, race, employment status, living arrangements and drug use, including marijuana, opioids, cocaine, and benzodiazepines.

Results: HIV-infected women (n=387) were: median age 46 years, single 50%, 86% African American, 83% unemployed, 40% hazardous/heavy drinkers and 51% using drugs. Women

with higher PSS scores were more likely to screen positive screen ( $\neq 3$ ) on the AUDIT-C (OR=1.04; 95%CI, 1.01–1.07,  $p=.01$ ); whereas no association between IESR scores and hazardous drinking was detected. Among hazardous/ heavy drinkers ( $n=153$ ), IESR scores were associated with higher SIP scores (OR=1.04; 95%CI, 1.01–1.07,  $p=.006$ ), whereas no association was found between PSS and SIP scores. In multivariable regression, higher IESR scores were associated with higher SIP scores (OR=1.11; 95%CI, 1.0–1.24,  $p=.05$ ); neither the PSS nor the interaction between PSS and IESR was significant.

Conclusions: Different types of stress mediate alcohol use and alcohol-related problems among HIV-infected women. Chronic stress is associated with hazardous/heavy alcohol use among HIV-infected women. Trauma-induced stress does not appear to directly affect alcohol consumption. Among hazardous/heavy alcohol users, however, trauma specific stress, but not global stress, is associated with reporting alcohol-related problems.

P0603

#### BRIEF ALCOHOL INTERVENTION AMONG HAZARDOUS AND HEAVY DRINKING HIV INFECTED WOMEN

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Objective: Hazardous and heavy alcohol use is associated with worse HIV treatment outcomes and increased mortality among women with HIV. We examined the effectiveness of brief alcohol intervention among HIV infected women.

Methods: We performed a randomized trial of hazardous and heavy drinking women receiving care in the Johns Hopkins HIV Clinic. Women were randomized to either a 2-session brief alcohol intervention with 2 booster phone calls or control. Ninety-day alcohol use, measured using the Time Line Follow Back, was measured at baseline and 3, 6, 12 month follow-up visits. Our main outcomes included 90-day frequency of alcohol use (%days alcohol was consumed), number of standard drinks per drinking day, and % binge drinking days, defined as days when  $>3$  standard drinks were consumed. Changes in outcome from baseline for visits 2–4 were analyzed with general linear mixed effects regression models for three factors with interactions. The factors included treatment group, visit (longitudinal), and a high/low alcohol group designation based on the overall median of the baseline outcome across all subjects. Treatment group differences at each visit were calculated from these models.

Results: 153 women were randomized to intervention ( $n=76$ ) and control ( $n=77$ ). At baseline, their median age was 46, 86% were African American, 70% used illicit drugs. Overall, the median % drinking days was 24, the median %binge drinking days was 14%, and the median drinks per drinking day was 7.7. The two groups did not differ significantly in their baseline characteristics. The, 90-day frequency of alcohol use decreased significantly at 3 (decreased 13%,  $p=0.045$ ) and 6 months (decreased 20%,  $p=0.001$ ) among women in the high frequency group, but was not sustained at 12 months. Drinks per drinking day also decreased significantly among women with higher levels of daily drinking at 3 months (decrease in 5 drinks/occasion,  $p=0.008$ ), but this effect was not sustained at 6 or 12 months. We found no difference in the %binge drinking days at intervention follow-up in either group.

Conclusions: Brief alcohol intervention resulted in decreased frequency of alcohol use among HIV-infected women at 6 months with higher frequency of use, but not at one year. Additional booster sessions may be necessary for a sustained effect.

P0647

#### GABRG1 MARKERS MODERATE AMPHETAMINE-INDUCED STRIATAL DOPAMINE RELEASE

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Risk of alcohol dependence (AD) has a substantial genetic component. Alcohol exerts many of its effects via interactions with gamma-aminobutyric acid (GABA) receptors, which populate limbic and reward-related areas and help regulate mesolimbic dopamine (DA) neurotransmission. AD is associated with markers in the GABAA c-1 receptor subunit gene (GABRG1) and the intergenic region 5'GABRG1-3'GABRA2. However, research has yet to show an association between these polymorphisms and human brain's reward system function. The rewarding effects of most drugs of abuse are via enhancement of mesocorticolimbic DA neurotransmission. PET imaging has demonstrated that amphetamine (AMPH) increases mesolimbic DA concentrations. Given that the mesolimbic system is a mediator of drug reward, it is important to understand the genetic factors that modulate its function. Therefore, we determined if DA responses to AMPH is related to variation in GABA receptor subunit genes.

Methods: 86 healthy social drinkers [57% males, 70% Caucasian, 22.9 yrs (SD=3.2)] completed two positron emission tomography (PET) scans with high-specific activity [<sup>11</sup>C] raclopride. The first scan was preceded by intravenous (i.v.) saline and the second by AMPH. The major outcomes were: Baseline global striatal DA binding potential (BP) following i.v. saline and AMPH-induced striatal DA release. Genotype effects were analyzed using linear regression analysis model, controlling for gender. Ancestry was estimated by the Structure software and was adjusted for in the association analyses. We applied a Bonferroni correction for Type I error.

Results: Baseline global striatal DA BP: carriers of the minor allele for a single nucleotide polymorphisms (SNP) in the GABRG1 region demonstrated on average significantly higher baseline BP (rs993677 p=0.010) compared to those homozygous for the common allele. AMPH-induced striatal DA release: carriers of the minor allele for SNPs in the GABRG1 gene and intergenic region 5'GABRG1-3'GABRA2 showed on average lower DA release compared to those homozygous for the common allele (rs1497577 p=0.002; rs488447 p=0.010; rs479277 p=0.007).

Conclusion: Our results suggest that the proposed variations modulate AMPH induced striatal DA release and thus may affect the rewarding effects of drugs of abuse, including alcohol. Consistently, carriers of the minor allele for SNP rs1497577 (GABRG1) have been associated with AD. [Supported by NIH grants AA017466 (M Uhart), AA10158 (GS Wand)]

S104

#### DELTA-OPIOID RECEPTOR AVAILABILITY AND DRINKING IN ALCOHOL-DEPENDENT SUBJECTS

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The current study examined relationships between alcohol drinking and d-opioid receptor (DOR) availability in alcohol dependent subjects. Subjects were studied during early alcohol abstinence, after stabilization on 50 mg naltrexone and during a 3-month outpatient naltrexone clinical trial. Subjects completed PET scans with the DOR selective ligand [<sup>11</sup>C]methylnaltrindole

and the  $\mu$ -opioid receptor (MOR) selective ligand [11C]carfentanil. Alcohol drinking levels were determined via Time Line Follow Back over the 90 days prior to the study and during the outpatient clinical trial. After 4 days of alcohol abstinence, there was substantial individual variability in DOR availability across 15 brain regions. Greater drinking intensity (mean drinks/drinking day) prior to the study positively predicted DOR availability in early abstinence in the caudate. There was near maximal occupancy of MOR by naltrexone in all subjects and across all brain regions. In contrast, subjects only averaged approximately 40% occupancy of DOR following stabilization on naltrexone 50 mg, and there was considerable inter-subject variability. Across multiple brain regions, one of the strongest predictors of the degree of naltrexone occupancy was DOR availability during early alcohol abstinence. Subjects with lower naltrexone occupancy of DOR in hippocampus tended to drink at higher levels during outpatient naltrexone treatment. These preliminary findings suggest that subjects whose DOR availability is high during early abstinence for whatever reason, including recent drinking history, show lower naltrexone occupancy of DOR, and may experience worse naltrexone treatment response. These associations may reflect a predisposition to heavy drinking (i.e., elevated DOR density or higher affinity may drive greater alcohol consumption and reward) or the effects of alcohol toxicity on the opioid system (i.e., chronic heavy drinking increases DOR density or alters affinity). Finally, these findings highlight that the current FDA recommended naltrexone dose does not adequately block DOR in most alcohol dependent subjects. This failure to achieve therapeutic levels of DOR blockade may help to explain the mild to moderate efficacy and considerable across-subject variability of naltrexone treatment outcomes. Supported by NIAAA grants AA11872, AA11855, and AA12303.



### **North Carolina – Kash, McCool, and Morrow Labs**

P0372

#### STRESS AND ALCOHOL INDUCED PLASTICITY OF OPIOID SIGNALING IN THE EXTENDED AMYGDALA

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The bed nucleus of the stria terminalis (BNST) orchestrates physiological outcomes related to stress and anxiety. The BNST is a heterogenous structure with multiple cell types that receive glutamatergic inputs from the hippocampus, prefrontal cortex, paraventricular thalamus and basolateral amygdala and GABAergic inputs from the central nucleus of the amygdala. Kappa opioid receptors (KOR) have been previously shown to inhibit GABAergic signaling in the BNST, but their effect on glutamatergic transmission remains largely unexplored. The current study identified KOR-mediated modulation of glutamatergic signaling, and assessed plasticity in KOR-mediated effects on both GABAergic and glutamatergic transmission in the BNST following stress and alcohol exposure.

Whole-cell patch clamp experiments were conducted in brain slices containing the BNST from exposed mice 24 hours following manipulations. In the BNST, application of both the endogenous KOR agonist dynorphin, as well as the synthetic agonist U69,593 inhibited evoked excitatory post-synaptic currents (eEPSCs) and was blocked by pre-application of norBNI. These results indicate KORs play a role in regulating glutamatergic signaling in the BNST. Ongoing studies are exploring the signaling mechanisms underlying this KOR mediated inhibition, as well as the locus of action. We then examined the impact of repeated restraint stress and binge-like alcohol exposure on KOR modulation of synaptic transmission. We found that stress, but not binge-like alcohol exposure, impaired KOR modulation of GABA transmission. Future studies will examine plasticity in this KOR mediated inhibition of glutamatergic signaling following stress and alcohol exposure.

P0410

#### ROLE OF SEROTONIN 2C RECEPTOR SIGNALING IN THE BNST IN ETHANOL-INDUCED ANXIETY-LIKE BEHAVIOR

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Anxiety is a co-symptom of ethanol withdrawal that is associated with relapse and ethanol dependence. Several lines of evidence suggest that the serotonin 2c receptor (5HT<sub>2c</sub>R) plays a key role in anxiety and may be altered following ethanol exposure. Preliminary data from our lab suggests that chronic intermittent ethanol (CIE) increases both 5HT<sub>2c</sub>R mRNA and anxiety-like behavior in mice. Furthermore, the 5HT<sub>2c/b</sub> agonist mCPP increases anxiety in detoxified alcoholics (Krystal, 1994). The mechanism of 5HT<sub>2c</sub>R-induced anxiogenesis is unknown, but some evidence suggests that CRF neurons in the bed nucleus of striaterminalis (BNST) are involved. Our goal was to determine if 1) 5HT<sub>2c</sub>R antagonists reduce CIE-induced anxiety and 2) 5HT<sub>2c</sub>R modulate CRF neurons in the BNST. For CIE studies, male DBA/2J mice pretreated with pyrazole (1 mmol/kg, i.p.) were exposed to ethanol vapor or room air for 5 days, 16 hr/day. After withdrawal (24 hr and 1 week), mice received the 5HT<sub>2c</sub>R antagonist SB242,084 (3 mg/kg, i.p.) 1 hour before the social interaction test. We found that SB 242,084 significantly attenuated anxiety-like behavior in the social interaction test in CIE mice, but not in controls. Using ex vivo electrophysiology, we studied the effects of mCPP (20 IM) on CRF

neurons in the BNST in a CRF reporter mouse. The effect of mCPP (5 mg/kg, i.p.) on neuronal activity (i.e. FOS-IR) in the BNST was examined in DBA/2J mice using immunohistochemistry (IHC). We found that mCPP depolarized CRF neurons in the BNST, an effect which was completely blocked by SB 242,084. Likewise, mCPP increased FOS-IR in the oval nucleus of the BNST, which contains a high density of CRF neurons. The anxiolytic effect of SB 242,084 in CIE, but not control mice, indicates that 5HT<sub>2c</sub>R signaling may be altered following CIE exposure. This is supported by data from our lab showing that 5HT<sub>2c</sub>R mRNA is upregulated following CIE, and we are following up on this finding with Western blots. Upregulation of 5HT<sub>2c</sub>R following CIE, together with electrophysiological data showing that 5HT<sub>2c</sub>R modulate CRF neurons in the BNST, may partially explain the anxiety in CIE mice and the anxiolytic effects of 5HT<sub>2c</sub>R antagonists. Studies are underway to determine if mCPP increases FOS-IR in CRF neurons in the BNST using the CRF reporter line. Taken together, our findings suggest that 5HT<sub>2c</sub>R actions on CRF systems may represent a novel molecular target for intervention of anxiety associated with alcohol abuse.

P0415

#### CHRONIC CORTICOSTERONE INDUCES NEUROADAPTIVE CHANGES IN THE ACCUMBENS AND IMPACTS SENSITIVITY TO THE INTEROCEPTIVE EFFECTS OF ALCOHOL VIA MGLUR5

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Metabotropic glutamate receptors-subtype 5 (mGluR5) in the nucleus accumbens (NA) functionally regulate sensitivity to the interoceptive/subjective effects of alcohol and alcohol self-administration in rats. We have shown that chronic exposure to the stress hormone corticosterone (CORT) in the drinking water results in reduced sensitivity to the interoceptive effects of alcohol and increased alcohol self-administration. We sought to investigate whether CORT exposure alters the expression of mGluR5 in the NA. Long Evans rats were given CORT in the drinking water (0.3 mg/ml) or water for 7 days. On Day 7, rats were sacrificed and brains processed for mGluR5 immunoreactivity (IR). A significant decrease in mGluR5 IR in the NA (core and shell) was observed in CORT-exposed rats, while no change in mGluR5 IR was found in the caudate putamen (positive control region). To confirm specificity of the change to mGluR5, mGluR2/3 IR in the NA was examined and no change was observed. In addition, IR of phosphorylated extracellular regulated kinase (pERK1/2), a downstream target of mGluR5, showed a parallel decrease to mGluR5 in the NA. Next, we assessed whether CORT exposure altered glutamate synaptic function in the NA and found significant elevations in spontaneous excitatory post-synaptic current (sEPSC) frequency, but no change in amplitude or AMPA/NMDA ratio, suggesting increased glutamate release within this brain region. To begin to examine whether mGluR5 plays a functional role in the decreased sensitivity to the interoceptive effects of alcohol following CORT, we assessed whether pharmacological manipulation of mGluR5 could restore sensitivity to alcohol. Briefly, rats trained to discriminate alcohol from water (1 g/kg, IG) underwent CORT exposure. On Day 7, rats were injected with the mGluR5 positive allosteric modulator CDPPB (10mg/kg, IP) prior to alcohol (1 g/kg, IG). CDPPB restored sensitivity to alcohol (1 g/kg), suggesting the functional involvement of this receptor in modulating sensitivity to alcohol following CORT. Together these data show specific neuroadaptive changes in mGluR5 and synaptic function in the NA following chronic CORT exposure which may contribute to decreased sensitivity to alcohol,

and possibly to increased alcohol self-administration. Further, manipulation of mGluR5 may be a viable target for restoring sensitivity to alcohol following a stressful episode, which may have therapeutic value. Supported by the ABMRF and AA019682.

P0710

ALCOHOL AND OPIOID MODULATION OF GABAergic TRANSMISSION IN  
DOPAMINERGIC CELLS IN THE PERIAQUEDUCTAL GRAY (VPAG)

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The ventral periaqueductal gray (vPAG), a region strongly implicated in negative affective disorder and ethanol withdrawal, is interesting due to its cell type heterogeneity, including a sub-population of dopamine neurons. Several studies have shown that dopamine neurons in the vPAG are required for opioid-mediated antinociception and locomotor sensitization. However, to date, there have been no studies of how drugs of abuse can alter these neurons. Our initial studies focused on the ability of acute alcohol to modulate functions in these neurons. We found, surprisingly, that acute ethanol had no impact on GABAergic transmission. This finding suggests that the dopamine neurons located in the PAG are differentially altered by alcohol when compared to dopamine neurons in the ventral tegmental area. Ongoing studies in the lab are examining the impact of chronic alcohol vapor exposure. Previous studies have found that alcohol exposure leads to an upregulation of dynorphin, the endogenous kappa opioid receptor (KOR) agonist, in the PAG. KORs have been linked to anxiety, depression and withdrawal, thus we hypothesize that upregulation of this system in the PAG may play a role in the negative affective state during alcohol withdrawal. We first sought to evaluate how this might impact dopamine neuron functions in the PAG. We found that activation of KOR lead to a reduction of GABAergic transmission in the PAG, similar to what was observed in previous studies in the BNST. We next examined the signaling mechanisms of KOR activation-induced attenuation of GABAergic input. Upon KOR activation, several downstream signaling pathways, including the ERK1/2 and MAPK p38 pathways, are activated. Studies have suggested a link between both the rewarding properties and withdrawal of alcohol to ERK signaling in other brain regions. Preliminary data in miniature inhibitory postsynaptic currents (mIPSC) frequency and amplitude suggests that KOR activation-induced attenuation of GABAergic input on the vPAG dopamine neurons are mediated via the ERK1/2 pathway, but not the p38 pathway. Ongoing studies investigate the effects of chronic ethanol exposure on kappa opioid functions by using ethanol vapor chambers. Selective recording from eGFP+ neurons of these transgenic mice will enable understanding of the role of vPAG dopamine neurons in drug abuse, abstinence, craving, relapse, and series of behaviors.

P1015

RECEPTOR-SPECIFIC EFFECTS OF BINGE-LIKE ETHANOL DRINKING ON NPY  
MODULATION OF SYNAPTIC TRANSMISSION IN THE EXTENDED AMYGDALA

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Endogenous anti-stress systems, including neuropeptide Y (NPY) signaling, protect against

the deleterious effects of alcohol by acting in key brain regions involved in the regulation of emotional and drinking behaviors, including the bed nucleus of the stria terminalis (BNST) in the extended amygdala. We hypothesized that repeated cycles of binge alcohol drinking and withdrawal cause persistent adaptations in the NPY system in the BNST that contribute to negative behavioral consequences, such as increased alcohol drinking. Because the antidrinking effects of NPY are thought to occur via activation of the NPY Y1 receptor (Y1R), while activation of the NPY Y2 receptor (Y2R) leads to increased ethanol intake, the prodrinking effects of chronic alcohol exposure may be due to dysregulation of NPY signaling via alterations of Y1R and/or Y2R. We recently showed that NPY reduces GABAergic transmission in the dorsolateral BNST (dLBNST) via activation of presynaptic Y2R. Therefore, the goals of the current study were to 1) determine the role of Y1R in NPY modulation of inhibition of the dLBNST, 2) examine the receptor-specific effects of binge-like ethanol drinking on NPY signaling, and 3) establish a direct relationship between NPY in the BNST and alcohol drinking in male C57BL/6J mice. When we examined the role of Y1R in NPY's modulation of inhibition in the dLBNST, we found that Y1R activation locally and presynaptically increases GABA release. We also found that mice exposed to three cycles, but not one cycle, of binge-like ethanol drinking had reduced Y2R-mediated NPY modulation of inhibitory transmission in the dLBNST. In addition, both Y1R and Y2R protein expression were increased in the dLBNST after three cycles of drinking. Our results suggest that Y1R and Y2R functionally oppose one another in the BNST, possibly via direct interaction at presynaptic terminals of NPY neurons, and that three cycles of binge drinking and withdrawal may lead to increased alcohol drinking via alterations in the expression of NPY and its receptors, as well as functional dysregulation of Y2R-mediated NPY signaling in the dLBNST. Ongoing experiments are examining the signaling pathway(s) by which Y1R presynaptically decreases GABA release and the effects of binge-like ethanol drinking on Y1R-mediated modulation of inhibition in the dLBNST. We are also currently examining the effects of local pharmacological manipulation of Y1R and Y2R in the BNST on binge-like ethanol drinking.

P0399

SIMULTANEOUS ANALYSIS OF APPETITIVE AND CONSUMATORY SELFADMINISTRATION BEHAVIORS IN RATS FOLLOWING PHYSICAL DEPENDENCE TO ETHANOL

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The transition from alcohol use to dependence and abuse is theorized to occur via a cycle of preoccupation/anticipation, binge/intoxication, and withdrawal/negative affect. Operant self-administration

is a highly validated and frequently utilized animal model for the binge/intoxication stage of this addiction cycle. Previous work with physically dependent animals has focused primarily on the appetitive/seeking aspect of ethanol operant self-administration. In contrast, measurement of the consummatory behaviors has been relatively unexplored. In the current study, we have used the Samson Sipper Model of operant self-administration to evaluate the impact of physical dependence on both appetitive and consummatory behavior. Daily ethanol seeking and consumption by Long-Evans rats was measured with six hour sessions consisting of a FR1 schedule reinforced with an eight-second presentation of a sipper tube. Sessions included both an ethanol-reinforced lever which delivered a sipper tube containing 10% ethanol in water and a "water only"-reinforced lever. Following a baseline period, we induced ethanol dependence using a ten day chronic intermittent vapor exposure; animals were subsequently placed back in the daily operant sessions 72hr after withdrawal from the ethanol vapor. In a preliminary study, we found that dependence increased both the total number of ethanol-reinforced lever presses and the average number of consecutive ethanol-reinforced lever presses. Water reinforced responding was not significantly altered by ethanol dependence. Chronic intermittent ethanol significantly increased both the total number licks but not the number of licks per reinforced lever-press. This suggests that the eightsecond access period during each reinforced presentation may have been too brief to measure any dependence-related changes in consumption. However, total ethanol intake significantly increased from  $0.4\text{g/kg/session}$  to  $1.4\text{g/kg/session}$  after the CIE exposure. In roughly half the animals, only  $70\%$  of the ethanol-reinforced lever presses resulted in consumption of the ethanol solution; and this was increased to  $90\%$  by physical dependence. In summary, this preliminary study has shown that physical dependence can increase both appetitive and consummatory behaviors. The model may permit extensive investigation of the mechanisms controlling consumption following physical dependence. This work was supported by NIH grant #AA014445.

P0698

ALLOPREGNANOLONE IMMUNOHISTOCHEMICAL STAINING IS REGIONALLY ALTERED DURING WITHDRAWAL FROM CHRONIC INTERMITTENT ETHANOL EXPOSURE IN C57BL/6J MICE

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The GABAergic neuroactive steroid allopregnanolone ((3 $\alpha$ ,5 $\alpha$ )-3-hydroxypregnan-20-one) has been studied during ethanol withdrawal in humans, rats and mice. Previous work has shown that acute ethanol (2g/kg) did not alter serum levels of GABAergic neuroactive steroids in male C57BL/6J mice. Immunohistochemical detection of allopregnanolone allows brain region specific analysis of the effects of chronic intermittent ethanol (CIE) exposure and withdrawal. Given CIE exposure increases subsequent voluntary ethanol drinking, we examined brain regions known to influence this behavior. Adult male C57BL/6J mice were exposed to the CIE model of ethanol dependence. Briefly, after establishing stable baseline drinking using a limited access (2 hr/day) 2-bottle choice (15% ethanol vs. water) paradigm, mice received four cycles of chronic intermittent exposure (16 hr/day x 4 days) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers. Exposure cycles 1–3 were followed by a week of daily limited access drinking. All mice were sacrificed and perfused at 8 hr following the final exposure cycle. Free floating brain sections (40 microns; 3–4 sections/region) were immunostained and analyzed for each animal. Data were transformed and expressed as a percent of CTL and compared to the values for the EtOH group. Withdrawal from CIE exposure (8 hr) produced region-specific effects on immunohistochemical detection of allopregnanolone levels across limbic brain regions. We observed significant increases in cellular allopregnanolone-like immunoreactivity in the prefrontal cortex, ventral tegmental area and the lateral amygdala. There was a trend for increased immunoreactivity in the nucleus accumbens and the medial division of the central nucleus of the amygdala. Ethanol-exposed mice showed ~2.5-fold increase in corticosterone levels. These data suggest that specific adaptations in GABAergic neurosteroids may be present in regions of brain that mediate anxiety, stress and drinking responses related to ethanol dependence. The present data are consistent with previous studies, which showed the peak of ethanol withdrawal at the 8 hr time point is characterized by elevated plasma corticosterone levels. Alterations in neurosteroid levels may have functional consequences that mediate behavioral adaptations to ethanol.

P0715

PKA MODULATES PHYSIOLOGICAL CHANGES IN GABAA RECEPTORS ASSOCIATED WITH ETHANOL EXPOSURE IN CULTURED CEREBRAL CORTICAL NEURONS

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Ethanol elicits bidirectional changes in GABAA receptor surface expression, causing a decrease in GABAA alpha 1 subunits and an increase in GABAA alpha 4 subunits. These changes are likely mediated largely by activation of various second messenger pathways, including protein kinase C (PKC) and protein kinase A (PKA). While PKC activation decreases alpha 1 and increases alpha 4 subunit surface expression, biochemical data suggest that PKA

contradicts these changes. This study sought to investigate the functional consequences of PKA activation by ethanol with electrophysiological recording in primary cultures of cerebral cortical neurons prepared from rat pups on postnatal 0-1 and maintained in culture for at least 17 days. Cells were exposed to ethanol (50 mM) for one hour  $\pm$  PKA activators and inhibitors. Ethanol decreased zolpidem responses in the presence of 1  $\mu$ M GABA (20%,  $p < 0.05$ ), which was exacerbated by the addition of Rp-cAMP (45%,  $P < 0.01$ ). In addition, the PKA activator Sp-cAMP increased (+41%,  $p < 0.05$ ) zolpidem responses with 1  $\mu$ M GABA, suggesting adaptations in GABA $\alpha$ 1 subunit receptors. Next, we examined the effects of ethanol and PKA modulators on mIPSC kinetics. No effects of ethanol or PKA modulators alone were observed. However, in the presence of zolpidem, an increase in mIPSC decay  $\tau_2$  and decay 90-37 was observed after Sp-cAMP exposure, consistent with an increase in synaptic  $\alpha 1$  subunits. Interestingly, Ro15-4513 (1  $\mu$ M) also increased the decay  $\tau_2$  of mIPSCs in ethanol-exposed cells, and this effect was also prevented by Rp-cAMP, suggesting adaptations of multiple  $\alpha$  subunit receptors in the cells. The results support the hypothesis that PKA modulates ethanol-induced changes in GABA $\alpha$  receptor expression. PKA activation may ameliorate some of the changes in receptor expression induced by ethanol, and may represent an important target for development of drugs involved in the treatment of alcoholism.

P0988

#### VIRAL VECTOR MEDIATED OVEREXPRESSION OF CYTOCHROME P450 SIDE CHAIN CLEAVAGE IN THE VTA PRODUCES LONG-TERM REDUCTIONS ON ETHANOL SELF-ADMINISTRATION

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Neuroactive steroids are endogenous neuromodulators synthesized in the brain, adrenal glands, and gonads. Systemic administration of endogenous and synthetic neuroactive steroids can alter ethanol self-administration in rodents. We have hypothesized that neuroactive steroids alter ethanol self-administration by modulating mesolimbic circuitry. In the current study, a previously characterized adeno-associated serotype 2 viral vector (AAV2) expressing the mitochondrial cytochrome P450 side chain cleavage (P450 $\text{scc}$ ) enzyme was used to produce long-term increases in P450 $\text{scc}$  expression. P450 $\text{scc}$  converts cholesterol to pregnenolone, which is the rate-limiting enzymatic reaction in neurosteroidogenesis. Overexpression of P450 $\text{scc}$  should allow us to investigate how sustained increases in steroidogenesis, isolated to specific brain regions, affect ethanol self-administration. The P450 $\text{scc}$  expressing vector (AAV2-P450 $\text{scc}$ ) or control green fluorescent protein (GFP) expressing vector (AAV2-GFP) were injected bilaterally into the ventral tegmental area (VTA, 2  $\mu$ L/hemisphere) or nucleus accumbens (NAc, 3  $\mu$ L/hemisphere) of alcohol preferring (P) rats ( $n = 7-8$ /group) previously trained to self-administer ethanol. Following viral vector injection the animals were given 1 week to recover from surgery before operant self-administration sessions resumed. P450 $\text{scc}$  overexpression in the VTA reduced ethanol responding by 25% compared to controls (2-way ANOVA,  $p < 0.01$ ) over the 3 week test period. The reduction in responding following injection was due in part to a persistent reduction in responses (28%, 2-way ANOVA w/Bonferroni post-test,  $p < 0.001$ ) during the first 5 minutes of operant sessions over 3 weeks of testing. In contrast, P450 $\text{scc}$  overexpression in NAc did not significantly alter long-term ethanol self-administration. General locomotor activity was not altered by vector

administration in VTA or NAc. P450scc overexpression did not increase allopregnanolone-like immunoreactivity in NAc; however, vector delivery in the VTA produced a 34% increase in allopregnanolone positive cells in the VTA, which did not reach statistical significance. These results provide evidence that P450scc overexpression, and presumably increased steroidogenesis, in the VTA reduces ethanol reinforcement. Investigating how neuroactive steroids modulate ethanol reinforcement may lead to the development of new therapeutic strategies for treating alcoholism.

S209

STRESS, TRAUMA AND ALCOHOL EFFECTS UPON BIOLOGIC STRESS RESPONSE SYSTEMS AND PROSPECTIVE RELAPSE IN ALCOHOL DEPENDENT SUBJECTS

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Both alcoholism and trauma produce similar disruptions in HPA axis responsivity, and environmental stressors can further alter this response. In order to parcel out the relative contributions of trauma/adversity, alcohol use, and stress in the development of HPA axis dysregulation, we assessed the interaction of previous childhood and lifetime trauma/adversity, alcohol use, and recent chronic and episodic stress upon multiple measures of HPA axis functioning (oCRH and cosyntropin pharmacological challenges and a public speaking task) in 25 controls and 69 4-6 week abstinent, treatment-seeking alcohol-dependent subjects as well as the interaction of baseline measures of HPA reactivity with post-treatment episodic stress upon alcohol relapse. Stress measures included ACTH, cortisol, BDNF, NPY, and neurosteroids. The neural response to an anxiety-inducing conditioned stressor in a subset of subjects was assessed with fMRI (n=15/group). Significant findings include: childhood trauma/adversity predicted recent alcohol use; the ACTH, but not cortisol, response to oCRH was blunted in alcohol-dependent subjects relative to controls; and self-reported childhood trauma/adversity moderated the cortisol response to the public-speaking stressor. Whereas control participants showed marked increases in the BOLD response of striatal-limbic regions during high relative to low anticipatory anxiety, alcohol-dependent participants did not demonstrate activation of these areas. Striatal-limbic activation in the alcohol-dependent group was negatively correlated with childhood adversity, particularly in the caudate. These findings provide evidence that early trauma effects both future drinking and HPA axis/striatal-limbic reactivity in alcohol-dependent subjects. Effects upon prospective drinking and stress will also be presented. An integrative approach of the contributions of trauma, stress, HPA axis regulation, and neural responsivity upon alcohol use will be discussed.



## **Oregon – Grant Lab**

P0377

### **SEX AND AGE OF CHRONIC ETHANOL DRINKING AFFECTS ETHANOL INTAKE AND SYNAPTIC TRANSMISSION ONTO MEDIUM SPINY NEURONS IN THE STRIATUM OF THE RHESUS MONKEY**

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As many as 1 in 12 adults abuse alcohol or are alcohol dependent. Studies suggest that alcohol-related problems and prevalence of binge or excessive drinking are highest among young adults (18–29 years). Similarly, the risk of alcohol dependence increases the younger the onset of drinking to intoxication. There are also differences in alcohol abuse and its effects due to gender. In general more men than women are alcohol dependent. However due to differences in drinking patterns as well as the effects of alcohol due to gender, women are at a greater risk to develop alcohol related problems.

Here we examine the effect of age of onset and gender on daily EtOH intake, pattern of drinking and the accompanying changes in synaptic transmission in MSNs of putamen, a brain region that we have previously discovered shows decreased GABAergic transmission that correlates with BEC/intake in chronic EtOH drinking monkeys. To examine age-of-onset effects on EtOH consumption and changes in synaptic transmission, young adult (age at drinking onset 5–6 years) and adult (age of onset drinking onset 7–11 years) male rhesus monkeys were trained to orally self-administer EtOH under “open access” for approximately one year. To examine potential sex differences, these young adult males were compared to age-matched females. Younger male monkeys had average daily intakes between 2.7 – 4.1 g/kg (yielding BECs 45.8–142.3 mg/dl) whereas older males averaged from 0.3 – 2.6 g/kg (yielding BECs 0–96.1 mg/dl). Young females, on the other hand, averaged between 4.1 – 5.6 g/kg (yielding BECs 47.0–99.4 mg/dl). This suggests that younger individuals drank more than older yielding higher BECs. What is interesting is that females drank more than their male counterparts yet had similar BECs. Our data suggests that this may be in part due to the pattern of drinking that differed between the sexes of rhesus monkeys. After necropsy, acute slices containing the dorsal striatum were obtained. Whole-cell patch clamp electrophysiology examined GABAergic miniature inhibitory postsynaptic currents (mIPSC) in putamen MSNs. Chronic EtOH drinking was associated with decreased mIPSC frequency in the putamen that correlated with EtOH intake. We are currently examining the interaction between gender and pattern of drinking with alterations in GABAergic transmission. The observed changes in striatal physiology may shed light on the increased risk for habitual EtOH drinking in younger onset and women drinkers.

P0422

### **EXAMINING THE RELATIONSHIP BETWEEN ANXIOUS AND AGGRESSIVE BEHAVIOR AND HEAVY DRINKING IN FEMALE RHESUS MONKEYS**

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Anxiety is associated with heavy drinking in humans. However, it is unclear whether anxiety is a risk or a consequence of heavy drinking. The current study assessed anxiety using a human intruder test (HIT) and a novel objects test (NOT) in a group of female rhesus monkeys (n=6)

prior to introducing them to ethanol. These tests have reliably been shown to assess individual differences in anxiety and the stress response in primates. The HIT assesses three different conditions: acclimation, profile (stranger stands on side of the cage without facing monkey, with profile towards monkey), and stare (stranger stares at monkey while maintaining eye contact). The NOT sequentially measures responses during three conditions: a novel food offer, a novel object test, and a novel object with familiar food test. Ethanol self-administration was induced via a schedule induced polydipsia procedure, which consisted of daily induction of specified volumes of water or 4% ethanol. Every thirty days, the consumed dose was increased by 0.5 g/kg/d up to a final dose of 1.5 g/kg/day. Following this induction procedure, ethanol (4% w/v) and water were available concurrently 22 h/d.

The HIT consisted of two anxiety-inducing conditions; the stare and profile phases. During these phases, 100% of the monkeys displayed anxious and/or aggressive behavior. Significant correlations were found between several measures of anxiety and drinking, such as the positive correlation between teethgrinding during the stare phase and 22 hour ethanol intake (g/kg) over 6 and 12 month periods ( $r_s = 0.94$ ,  $p = 0.005$ ). During the NOT, latencies to touch the novel objects ranged from 1 second to 300 seconds, with latencies of more than 90 seconds indicating anxiety in response to the object. The same monkeys tended to exhibit anxious behavior across the NOT phases, with monkeys showing increased latency to inspect one novel object also showing increased latency to inspect the other novel objects. Monkeys were characterized as having high or low levels of anxiety based on their responses during the NOT, and non-significant differences in their ethanol consumption were found. When instead characterized based on their aggressive-anxious behaviors during the HIT, significant differences in their blood ethanol content were found ( $t = 3.2$ ,  $p = 0.03$ ), indicating higher ethanol consumption in the aggressive-anxious group. An aggressive-anxious temperament may predict heavier ethanol consumption.

P0733

#### ETHANOL SELF-ADMINISTRATION IN RELATION TO GO/NO-GO PERFORMANCE WITH VARYING STIMULUS CONDITIONS

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Impulsivity has been linked to alcohol abuse. Further, it has been suggested that cognitive and physiological challenges may affect individuals' regulation of behavior. The current study tested the hypothesis that a cognitive challenge would alter the regulation of drinking. Female cynomolgus monkeys (*Macaca fascicularis*,  $n = 3$ ) were trained to self-administer ethanol and to perform a go/no-go task to measure impulsivity. In the go/no-go task, monkeys were reinforced with banana pellets (which comprise their meals) during two types of trials: 1) responding during go trials, and 2) omitting a response during no-go trials. The monkeys performed this task for their meals three times per day every 2 hours. The time that the monkeys were required to inhibit responding to receive a banana pellet was manipulated. Specifically, the total duration of no-go stimuli presented during each session was systematically varied (0 s, 30 s and 150 s) by manipulating their number and duration. Two monkeys drank doses of ethanol resulting in measurable blood-ethanol concentration prior to the third meal each day (mean  $\pm$  SD: 77,  $0.78 \pm 0.30$  g/kg; 82,  $0.83 \pm 0.32$  g/kg). On days in which the monkeys had to inhibit the longest for their banana pellets, they drank a similar dose of ethanol prior to the third meal (77,  $1.0 \pm 0.3$  g/kg; 82,  $1.0 \pm 0.1$  g/kg) compared to when the no-go time was brief (77,  $0.8 \pm 0.3$  g/kg; 82,  $0.8 \pm 0.2$  g/kg;  $F(1, 2) = 4.4$ ,  $p = 0.17$ ),

the opposite of water intake, which was slightly but not significantly lower during sessions with long (77, 169 ± 58 ml; 82, 236 ± 51 ml) compared to brief (77, 247 ± 94 ml; 82, 302 ± 57 ml) waits,  $F(1, 2) = 5.7$ ,  $p = 0.14$ . Response accuracy did not vary significantly across conditions. Thus, the data suggest that the cognitive challenges in this study did not influence ethanol consumption. Additional studies are needed to evaluate the role of psychogenic (cognitive or psychosocial) stress in the precipitation of binge drinking in heavier drinking subjects

P0736

DISCRIMINATION OF ETHANOL AND NICOTINE MIXTURES: PARADOXICAL MECHANISMS OF OVERSHADOWING AND SYNERGY

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Training subjects to discriminate ethanol and nicotine as a compound interoceptive cue offers the advantage of studying a situational context in which individuals experience a history of conditioning with both drugs. This approach departs from the traditional one of training one drug to acquisition criteria, and then testing generalization, facilitation, or antagonism by the other drug. Earlier work from our laboratory suggested that a moderate ethanol dose (1.5 g/kg) overshadowed the discriminative stimulus (SD) effects of nicotine, irrespective of nicotine dose magnitude (0.4–1.2 mg/kg) within the training drug mixture. The current work further explored interactions between ethanol and nicotine at the level of SD effects by using a dose ratio strategy whereby incremental increases in ethanol dose (0.5, 1.0 and 2.0 g/kg = E) were trained in combination with a fixed nicotine dose (0.8 mg/kg base = 0.8N). Four groups of male C57BL/6 mice ( $n = 10$ /group) were trained to discriminate 0.8N, 0.5E+0.8N, 1.0E+0.8N, or 2.0E+0.8N from saline in standard 2-lever conditioning chambers. The 1.0E+0.8N group met acquisition criteria in significantly fewer training sessions than the 0.8N group (46±3 versus 70±7 sessions). Nicotine fully substituted (± 80% drug appropriate responding) in the 0.8N and 0.5E+0.8N groups at test doses of 0.8 and 1.2 mg/kg, respectively, but only partially substituted in the 1.0E+0.8N and 2.0E+0.8N groups. Conversely, ethanol exhibited no substitution in the 0.8N group, partial substitution in the 0.5E+0.8N group, and full substitution in the 1.0E+0.8N and 2.0E+0.8N groups. Pretreatment blockade with mecamylamine revealed that the direct activation of nicotinic acetylcholine (nACh) receptors was essential for producing the SD effects in the 0.8N group, played a minor role in the 0.5E+0.8N group, and was unnecessary for the 1.0E+0.8N and 2.0E+0.8N groups. Assessment of ethanol generalization gradients in combination with 0.8 mg/kg nicotine resulted in significant leftward shifts in the ethanol dose-response curves when compared to ethanol only tests. Therefore, although the SD effects of nicotine are overshadowed by discriminable doses of ethanol, nicotine does augment the potency of the ethanol cue. Identification of the receptor mechanisms underlying these interactive effects will aid in the development of pharmacological interventions for ethanol and nicotine co-abuse. Supported by NIH grant AA16849 (to MMF).

P0759

MENSTRUAL CYCLES DURING ETHANOL SELF-ADMINISTRATION IN FEMALE RHESUS MONKEYS (MACACA MULATTA)

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Alcoholism in women and chronic ethanol self-administration in animals has been reported to disrupt reproductive function, although with wide species and individual differences. The current longitudinal study assessed menstrual cycle quality during ethanol self-administration using 3.3–4 year-old female Indian rhesus monkeys (*Macaca mulatta*,  $n = 13$ ). Most rhesus monkeys have begun ovulating by 3.5 years of age (Zehr et al. 2005 *Biol Reprod* 72). Menstrual data were obtained before (9 months) and during (9 months) 22 h/d self-administration of ethanol (4% w/v) and water ( $n = 6$ ) or isocaloric maltose-dextrose and water ( $n = 3$ ), or during consumption of a laboratory diet for 18 months ( $n = 4$ ). Under the laboratory diet 33.5  $\pm$  10.1% of cycles were of normal duration (25–31 days; Quadri and Spies 1976 *Biol Reprod* 14) and ovulatory (defined as peak progesterone  $\neq$  4 ng/ml; peak progesterone, 10.9  $\pm$  0.8 ng/ml). Prior to the introduction of maltose-dextrose or ethanol (4 months), normal ovulatory cycles occurred in 1/1 maltose-dextrose and 1/3 ethanol subjects for which plasma progesterone was measured at this early stage of the experiment when the monkeys were being trained for awake venipuncture, with peak serum progesterone 6.1 ng/ml and 5.5  $\pm$  1.3 ng/ml, respectively. During the 5 months of introduction of maltose-dextrose or ethanol induction, respectively, 39  $\pm$  6% (peak progesterone, 8.7  $\pm$  1.1 ng/ml) and 56  $\pm$  13% (10.1  $\pm$  1.7 ng/ml) of cycles were normal and ovulatory. All monkeys that had 22 h/d access to ethanol and water were heavy drinkers ( $> 3.5$  g/kg/d). For three (of 6) monkeys, a majority (77–100%) of menstrual cycles were of irregular duration and/or anovulatory. For the other three monkeys, a majority (57–88%) of menstrual cycles were of normal duration and ovulatory (peak progesterone 9.8  $\pm$  1.0 ng/ml), despite drinking  $> 4.0$  g/kg ethanol per day on average (equivalent of 16 drinks). The data highlight individual differences in sensitivity to reproductive dysfunction due to ethanol. The monkeys with resistance to ethanol-induced disruption of menstrual cycles had the greatest peak progesterone and percentage of normal ovulatory cycles prior to ethanol access. Thus, heavy ethanol drinking during peri-pubescence, prior to the onset of regular ovulatory cycles, could impair reproductive capacity and circulating progesterone, with implications for neuroactive steroid interactions with ethanol.

P0995

ETHANOL-INDUCED DIFFERENTIAL GENE EXPRESSION OF 5-HT1A AND YIF1B IN HIPPOCAMPAL PYRAMIDAL AND GRANULE CELLS IN CYNOMOLGUS MACAQUES

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Heavy drinking is known to produce neuroadaptive changes that can facilitate the transition to compulsive consumption of alcohol, one of the hallmarks of addiction. The hippocampus is particularly vulnerable to ethanol-induced neuroplasticity and the 5-HT1A receptor plays an important modulatory role in this region. We have shown previously that chronic ethanol self-administration

is associated with 43% greater hippocampal 5-HT1A receptor density. We

hypothesized that increased 5-HT1A receptor density may be paralleled by concomitant alterations in 5-HT1A gene expression as well as changes in Yif1B, a trafficking protein crucial for 5-HT1A dendritic targeting. To this end, RNA was isolated from hippocampal CA1 pyramidal neurons and dentate gyrus granule cells from male cynomolgus macaques.

Drinkers voluntarily self-administered ethanol during daily 22 hour sessions in their home cage

for at least 12 months (n=9) while their control counterparts remained ethanol naïve (n=8). At necropsy, brains were blocked, flash-frozen and processed for laser capture microdissection (LCM). Eight hundred to 1,200 CA1 pyramidal neurons and the entire dentate gyrus granule cell layer from four sections per brain were microdissected from each subject. Isolated RNA from each sample was reverse transcribed and processed for qPCR. qPCR values for 5-HT1A and Yif1B were normalized to three endogenous control genes that were stable across all subjects. No significant between group differences in 5-HT1A receptor gene expression were observed in either the dentate gyrus or CA1 of the hippocampus ( $p \neq 0.3$ ). In contrast, a preliminary analysis uncovered a trend for greater gene expression of the 5-HT1A receptor trafficking protein, Yif1B, in both dentate gyrus granule cells ( $p=0.06$ ) and CA1 pyramidal neurons ( $p=0.07$ ) in ethanol drinkers. These data suggest that the greater hippocampal 5-HT1A receptor density observed in chronic drinkers is not due to an increase in receptor gene expression but perhaps an increase in the trafficking of the receptor to specific dendritic sites.

S111

#### EFFECT OF CHALLENGING REINFORCEMENT CONTINGENCIES FOR MEAL DELIVERY ON ETHANOL SELF-ADMINISTRATION IN CYNOMOLOGUS MONKEYS

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Subjects used in animal models of ethanol self-administration typically have all of their physiological requirements met without exerting any effort. For example, they are maintained in a shelter with constant temperature and food and water are predictably delivered by experimenters. This contrasts with the contingencies of daily life for humans, who must work to earn money for maintaining a shelter and for purchasing food. Contingencies of reinforcement mediating the acquisition of essential commodities in humans may limit ethanol drinking, and conversely, the absence of these contingencies in animal models may contribute to excessive drinking. Studies of ethanol self-administration in non-human primates in this laboratory previously delivered three daily meals every two hours, with each banana pellet being delivered according to a fixed-ratio (FR) 1 schedule (one finger poke, one pellet). In place of the FR-1 schedule for meals, the current study substituted two-choice visual discrimination (go/no-go) approximately once per week, and then every day, for four female cynomolgus monkeys (*Macaca fascicularis*). Inaccurate responding resulted in postponement of pellet delivery for at least two hours. For two monkeys, drinking to intoxication was controlled by the contingencies of meal delivery. When required to perform the go/no-go task for their meals, these two monkeys consumed significantly less ethanol (about two fewer drinks) compared to during the FR-1 contingencies. The two monkeys showing lower ethanol intake during go/no-go sessions also drank significantly more water, indicating that the cognitive task specifically suppressed intake of ethanol. Regulation of ethanol drinking by the contingencies of meal delivery occurred as a greater time between bouts, fewer bouts of ethanol drinking, lower drink and bout volumes. Monkeys with low ethanol intake did not drink less when required for perform the go/no-go task. Thus, the control of ethanol drinking by meal contingencies in the other two monkeys was likely related to experience with ethanol intoxication. Overall, the data suggest that feedback regarding the effect of ethanol intoxication on maximization of reinforcement may have regulated intake among moderate drinkers. Future studies could determine whether such feedback could be lost after prolonged, excessive ethanol intake.

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### **South Carolina – Becker, Lopez and Mulholland Labs**

P0349

#### TOLERANCE TO ETHANOL'S AVERSIVE EFFECTS IN ETHANOL DEPENDENCE: THE ROLE OF NMDA RECEPTOR SIGNALING IN THE BASOLATERAL AMYGDALA

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Repeated cycles of chronic intermittent ethanol exposure (CIE) results in tolerance to ethanol's aversive effects using a Conditioned Taste Aversion (CTA) procedure. This study examined the role of NMDA receptor signaling in the basolateral amygdala (BLA) in mediating ethanol tolerance in ethanol dependent mice. Adult male C57BL/6J mice were implanted with bilateral guide cannulae positioned above the BLA. After 7 days of recovery, mice were exposed to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers (16 hr/d for 4d), followed by a week of home cage rest. This pattern was repeated for 3 more cycles. At 72hr after CIE, EtOH and CTL mice were tested in the CTA paradigm. Thirty minutes of access to a saccharin solution after the 3rd cycle (1% w/v) or kool-aid (1% w/v) after the 4th cycle served as the conditioned stimulus (CS). Mice were microinjected with test drugs at the end of the CS access period followed immediately by an IP injection of ethanol (1 or 2 g/kg), which served as the unconditioned stimulus (US). Intake of the CS was evaluated 24 hr after conditioning, with data expressed as a percent of CS intake from the conditioning session. Microinjection of vehicle (PBS) coupled with 2 g/kg ethanol revealed significant ethanol-induced CTA in CTL mice (67±8% reduced CS intake) while only moderate aversion in EtOH mice (31±15% reduced CS intake), consistent with tolerance. Separate mice microinjected with AP-5 (5 ng/side) followed by 2 g/kg ethanol showed this NMDA receptor antagonist partially blocked the ethanol-induced CTA in CTL mice (39±10% reduced CS intake) but did not alter aversion in EtOH mice (32±13% reduced CS intake). Finally, another group of mice were microinjected with NMDA (0.3 nmol/side) to enhance CTA with 1 g/kg ethanol, a dose that does not produce aversion. NMDA enhanced ethanol-induced CTA in CTL mice (27±7% reduced CS intake) as expected, but this was not found in the EtOH mice. Taken together, these data clearly implicate NMDA receptor signaling in the BLA as critical for the development of aversion to ethanol. Further, our data suggest that there are adaptations in NMDA receptor signaling in ethanol dependent mice that may underlie tolerance to the aversive effects of ethanol. Ongoing studies are continuing to examine NMDA related mechanisms of tolerance to ethanol's aversive effects. Supported by NIAAA grant AA018036 and VA Medical Research.

P0350

#### ROLE OF GLUTAMATERGIC NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS IN ETHANOL DRINKING IN ETHANOL DEPENDENT AND NON-DEPENDENT MICE

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We have shown that chronic intermittent ethanol (CIE) exposure significantly increases ethanol consumption in C57BL/6J mice and, using in vivo microdialysis procedures, that CIE exposure also increases extracellular glutamate (GLUEX) levels in the nucleus accumbens (NAc). In the present study, we pharmacologically manipulated glutamatergic tone in the NAc to test the hypothesis that increased GLUEX in the NAc may drive escalation of drinking in this

CIE model. After implanting bilateral guide cannula positioned above the NAc, mice were trained to drink ethanol (15% v/v) in a 2-bottle choice, limited access paradigm (water as the alternate fluid). After establishing stable baseline ethanol intake, mice received 4 weekly cycles of chronic intermittent exposure (16 hr/d for 4d) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers, with each exposure cycle alternating with a week of limited access drinking test sessions. As expected, ethanol drinking increased in EtOH compared to CTL mice ( $3 \pm 0.1$  vs  $2.5 \pm 0.1$  g/kg). During the 4th drinking test period, mice were microinjected with either vehicle (PBS), the non-selective GLU reuptake inhibitor DLthreo-beta-Benzyloxyaspartic acid (TBOA; 0, 250 or 500 IM/side), or the mGluR2/3 agonist LY379268 (0.5, 1 and 5 nmol/side) into the NAc 30 min prior to their usual access to ethanol. Therefore, TBOA increased GLUEx while activating mGluR2/3 receptors with LY379268 reduced presynaptic glutamate release. After vehicle injection, EtOH mice continued to drink more ethanol than CTL mice ( $p < 0.05$ ) while TBOA dose-dependently increased ethanol drinking in both groups ( $p < 0.05$ ). Conversely, LY379268 dose-dependently reduced drinking ( $p < 0.05$ ) and EtOH mice were more sensitive to this effect at lower doses than CTL mice. Taken together, these data are consistent with the idea that a hyperglutamatergic state in the NAc that results from CIE exposure underlies increased ethanol consumption in dependent (EtOH) mice. Additionally, the apparent greater sensitivity of EtOH mice to the intake reducing effects of LY379268 suggests that the increased GLUEx is derived from neuronal rather than glial sources. Supported by NIAAA grant P50 AA10761 and VA Medical Research.

P0353

#### GLUTAMATE TRANSPORTER EXPRESSION IN NUCLEUS ACCUMBENS AND DORSAL STRIATUM AFTER CHRONIC INTERMITTENT ETHANOL EXPOSURE

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Using an established model of ethanol dependence and relapse drinking, previous studies have demonstrated increased glutamate levels in the nucleus accumbens (NAc) and dorsolateral striatum (DLS) of mice that have experienced repeated cycles of chronic intermittent ethanol (CIE) exposure. This increase in basal striatal glutamate tone has been demonstrated at least 1 week following final CIE exposure, and may mediate increased ethanol drinking in dependent mice. However, the source of this elevation in extracellular glutamate levels is as yet unclear. One potential cause is altered excitatory amino acid transporter (EAAT) expression in the dorsal and ventral striatum. Removal of glutamate from the extracellular space in these brain regions occurs predominately through EAAT2 and EAAT3. This study tested the hypothesis that reduced EAAT2 and/or EAAT3 expression contributes to elevated glutamate levels in the NAc and DLS in ethanol dependent mice. Adult male C57BL/6J mice received repeated cycles of CIE exposure in inhalation chambers (16 hr/day, 4 days/week to achieve BECs at 175 – 225 mg/dL) alternated with weekly limited access two-bottle choice ethanol (15%) consumption (2 hr/day, 5 days/week, water as the other available fluid). CIE exposed mice showed significant escalation of voluntary ethanol consumption compared to air-exposed controls over 4 weekly test cycles ( $3.7 \pm 0.7$  vs  $2.2 \pm 0.3$  g/kg for CIE and control groups, respectively). Following the 4th test drinking period, Western blot analysis was used to measure total, surface, and intracellular EAAT2 and EAAT3 expression in the NAc and DLS. Mice were sacrificed 7 days post-chamber, 18–22

hours following their last 2-hr drinking session. Results showed that when comparing ethanol dependent and non-dependent mice, no difference was found in total, surface and intracellular EAAT2 and EAAT3 expression in the NAc or DLS brain regions. These data suggest that differences in glutamate release mechanisms (e.g., via system Xc) rather than altered uptake mechanisms may be responsible for the elevated extracellular glutamate levels observed in CIE mice. Ongoing studies will address the role of system Xc in the CIE model as well as the possibility that neuronal hyperexcitability may contribute to increased extracellular glutamate levels in the NAc and DLS which, in turn, may mediate increased ethanol consumption associated with ethanol dependence. Supported by grants T32 AA007474 and P50 AA010761.

P0419

EFFECT OF SOCIAL DEFEAT STRESS ON VOLUNTARY ETHANOL INTAKE IN ETHANOL DEPENDENT AND NON-DEPENDENT C57BL/6J MICE

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Repeated cycles of chronic intermittent ethanol (CIE) exposure produces escalation of voluntary ethanol drinking in C57BL/6J mice. Previous studies have also indicated that social defeat (SD) stress can increase intake in mice. This study evaluated the effect of repeated SD on voluntary ethanol intake in ethanol-dependent and control non-dependent mice. C57BL/6J mice were trained to drink 15% (v/v) ethanol using a limited access (2 hr/day) 2-bottle choice paradigm. Once stable intake was established ( $\approx 2.6$  g/kg/day), mice received 4 weekly cycles of chronic intermittent exposure (16 hr/day x 4 days) to ethanol vapor (EtOH group) or air (CTL group), with each exposure cycle alternating with a week of limited access drinking test sessions. Separate groups of EtOH and CTL mice experienced SD stress either during every ethanol intake test, only during the last (fourth) test cycle of ethanol intake, or were not exposed to stress. During SD stress sessions, each C57BL/6J mouse was placed in the home cage of a resident CD1 mouse for 30 min (5 min of interaction, 25 min separated by a wire mesh) at 4 hr before each ethanol intake session. As expected, EtOH mice that did not experience SD showed a significant increase in ethanol intake (up to  $\approx 3.4$  g/kg/day) compared to CTL no stress mice that evidenced relatively stable intake across all test cycles. EtOH mice that experienced SD stress during every test cycle did not show an increase in ethanol intake above baseline levels while CTL mice showed a significant decrease in intake during the first two test cycles compared to baseline (down to 1.9 g/kg/day). EtOH mice that experienced SD stress only during the last test cycle showed a reduction in intake to baseline levels while CTL mice that experienced SD stress during the last test cycle did not show a change in ethanol intake. In summary, these results indicate that SD stress does not increase ethanol consumption in C57BL/6J mice. However, SD stress did differentially impact ethanol intake in ethanol-dependent and control (non-dependent) mice. SD stress reduced ethanol intake in control non-dependent mice and prevented the escalation of drinking in ethanol-dependent mice.

Supported by NIAAA grant U01 AA014095 and VA Medical Research.

P0698

ALLOPREGNANOLONE IMMUNOHISTOCHEMICAL STAINING IS REGIONALLY ALTERED



DURING WITHDRAWAL FROM CHRONIC INTERMITTENT ETHANOL EXPOSURE IN  
C57BL/6J MICE

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The GABAergic neuroactive steroid allopregnanolone ((3 $\alpha$ ,5 $\alpha$ )-3-hydroxypregnan-20-one) has been studied during ethanol withdrawal in humans, rats and mice. Previous work has shown that acute ethanol (2g/kg) did not alter serum levels of GABAergic neuroactive steroids in male C57BL/6J mice. Immunohistochemical detection of allopregnanolone allows brain region specific analysis of the effects of chronic intermittent ethanol (CIE) exposure and withdrawal. Given CIE exposure increases subsequent voluntary ethanol drinking, we examined brain regions known to influence this behavior. Adult male C57BL/6J mice were exposed to the CIE model of ethanol dependence. Briefly, after establishing stable baseline drinking using a limited access (2 hr/day) 2-bottle choice (15% ethanol vs. water) paradigm, mice received four cycles of chronic intermittent exposure (16 hr/day x 4 days) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers. Exposure cycles 1–3 were followed by a week of daily limited access drinking. All mice were sacrificed and perfused at 8 hr following the final exposure cycle. Free floating brain sections (40 microns; 3–4 sections/region) were immunostained and analyzed for each animal. Data were transformed and expressed as a percent of CTL and compared to the values for the EtOH group. Withdrawal from CIE exposure (8 hr) produced region-specific effects on immunohistochemical detection of allopregnanolone levels across limbic brain regions. We observed significant increases in cellular allopregnanolone-like immunoreactivity in the prefrontal cortex, ventral tegmental area and the lateral amygdala. There was a trend for increased immunoreactivity in the nucleus accumbens and the medial division of the central nucleus of the amygdala. Ethanol-exposed mice showed ~2.5-fold increase in corticosterone levels. These data suggest that specific adaptations in GABAergic neurosteroids may be present in regions of brain that mediate anxiety, stress and drinking responses related to ethanol dependence. The present data are consistent with previous studies, which showed the peak of ethanol withdrawal at the 8 hr time point is characterized by elevated plasma corticosterone levels. Alterations in neurosteroid levels may have functional consequences that mediate behavioral adaptations to ethanol.

P0016

ACTIVITY-DEPENDENT PROCESSES UNDERLIE CHRONIC ETHANOL-INDUCED  
ENHANCEMENT OF SYNAPTOPODIN EXPRESSION IN HIPPOCAMPAL NEURONS

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Synaptopodin (SP) is a proline-rich actin-associated protein that regulates the activity of actinin, an actin crosslinking microfilament protein. SP is found in a subset of mature dendritic spines in hippocampal neurons where it is tightly associated with and regulates expression of the spine apparatus. Spines that contain SP have prolonged decay kinetics of intracellular Ca<sup>2+</sup> transients and persistent activity-dependent increases in volume. Expression of SP is increased following induction of long-term potentiation (LTP) in hippocampus, and mice lacking SP exhibit decreased hippocampal LTP and more working memory errors on the radial arm maze task in comparison to wild-type mice. Chronic ethanol exposure is associated with enlargement of dendritic spines and altered synaptic plasticity in hippocampus. However, it is unknown if ethanol affects SP expression, and if so could this contribute to altered synaptic and morphological plasticity. To begin to address this, we examined changes in SP clustering in primary hippocampal neurons treated with chronic ethanol. Hippocampal neurons were costained with SP, phalloidin to label F-actin-rich dendritic spines, and PSD-95, a postsynaptic density scaffolding protein. Confocal images of SP staining were acquired, and image analysis was performed using the Imaris 3D imaging program. As expected, we observed punctate SP clustering in the proximal and distal dendrites and the soma of hippocampal neurons. SP clusters were also observed in the head, neck and base of dendritic spines in ethanol-naïve neurons. Treatment of neurons with 50 mM ethanol for 4 d significantly increased SP cluster volume and density (n = 7–8 dishes/group; 640 total SP clusters). Because ethanol inhibits NMDA receptors and regulation of SP is thought to be activity-dependent, we treated neurons chronically with D-APV, an NMDA receptor antagonist. Similar to our findings with ethanol, prolonged inhibition of NMDA receptors with D-APV (50 μM) markedly enhanced SP cluster volume and density in comparison with untreated neurons (n = 7 dishes/group). These data suggest that the effects of chronic ethanol on SP expression are dependent upon ethanol inhibition of NMDA receptor activity. Thus, ethanol-induced changes in SP may be part of the homeostatic adaptive plasticity of dendritic spines and may contribute to aberrant synaptic and behavioral plasticity associated with chronic ethanol exposure.

Supported by NIH grant AA010983.

P0017

CHRONIC ETHANOL EXPOSURE DECREASES KV4.2 CHANNEL AND KCHIP3  
EXPRESSION IN THE HIPPOCAMPUS

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It is well documented that chronic ethanol exposure results in significant, long-term adaptations in neuronal homeostasis of glutamatergic synapses that alter cellular responses and signal transduction. However, it is unknown how chronic ethanol affects overall dendritic plasticity and ion channel surface expression at the synapse. Particularly, A-type K<sup>+</sup> channels (Kv4.2) have an important role in determining neuronal firing properties and regulating neuronal excitability. Previous findings in our lab in ethanol-treated organotypic hippocampal

slice cultures indicate an increase in backpropagating action potential amplitude, due to a decrease in transient A-type K<sup>+</sup> current. Backpropagating action potentials may provide information on distance-dependent modulation of dendritic and synaptic plasticity. Additionally, an increase in phosphorylated Kv4.2 channels along with a decrease in Kv4.2 channel surface expression and Kv channel interacting proteins (KChIPs) were also observed. Kv channel interacting proteins are important for the formation and functional properties of Kv4.2 channels. Loss of KChIP3 in particular results in a decrease in A-type current densities. KChIP3 also serves as a negative regulator of NMDA surface expression. Chronic ethanol exposure increases NMDA surface expression with in vitro and in vivo models. In an in vivo model of chronic ethanol exposure, preliminary data using western blot also suggest a decrease in KChIP3 and Kv4.2 channel expression in the rat hippocampus. These decreases in Kv4.2 channel and KChIP3 expression, as well as increases in NMDA surface expression, may have important implications in the homeostatic adaptations at glutamatergic synapses during chronic ethanol exposure.

P0064

#### BIOCHEMICAL ADAPTATIONS IN THE DORSOLATERAL STRIATUM ASSOCIATED WITH ESCALATION OF VOLUNTARY ALCOHOL CONSUMPTION IN RODENTS

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It is thought that a fundamental switch from goal-directed behavior, governed by the medial prefrontal cortex (mPFC) to habitual behavior, mediated by the dorsolateral striatum (DLS), contributes to high rates of consumption in individuals with alcohol use disorders. Repeated episodes of ethanol exposure and withdrawal are associated with enhanced relapse vulnerability, possibly contributing to this switch. Similarly, rats will develop a preference for ethanol and escalate their drinking if given ethanol in an intermittent alcohol access (IAA) paradigm. Studies have shown that IAA reduces the ability of small conductance potassium (SK) channels to regulate firing in the nucleus accumbens (NAc), another key brain region associated with the alcohol circuitry. Here, we examined the underlying biochemical adaptations associated with the escalation of voluntary drinking, specifically in regions relating to goal-directed and addictive behavior. Long-Evans rats were given intermittent access to ethanol under a two-bottle choice (20% ethanol or water) model. We measured ethanol consumption and drinking patterns with a lickometer system for 9 weeks. After one week of withdrawal from IAA consumption, we assessed changes in glutamate receptor and SK channel expression levels in PSD-enriched fractions. As expected, rats demonstrated an escalation in drinking from 2.2 g/kg to 4.1 g/kg after 9 sessions of IAA exposure. This level of drinking remained stable for an additional 17 sessions, with rats showing a slight preference for ethanol over water. We found a strong, positive correlation between g/kg ethanol consumed and number of licks in the first four drinking sessions ( $R^2 = 0.716$ ) and after the rats reached their baseline drinking level ( $R^2 = 0.931$ ). The rats consumed ethanol in discrete episodes every 2–3 hours during the dark cycle, with some rats also showing an isolated episode of drinking 1–2 hours before the onset of the light cycle. Western blot analysis indicated a significant decrease of SK3 expression in the DLS of IAA rats compared with control rats. No significant differences in the expression of glutamate receptor subunits or SK channels were observed in either the mPFC or NAc. The reduction in SK3 channels by IAA may contribute to enhanced glutamatergic activity reported in the DLS. Thus, these data suggests a possible role for SK3 channels in the DLS in regulating escalation of habitual

alcohol consumption. This work was supported by NIH grants AA019722 and AA007474.

P0342

#### ADOLESCENT ETHANOL EXPOSURE REDUCES ADULTHOOD COMT LEVELS IN THE MEDIAL PREFRONTAL CORTEX

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During adolescence, the prefrontal cortex (PFC) undergoes a critical period of cortical development and refinement of neuronal circuits that occurs in conjunction with the maturation of complex cognitive behaviors. Although accumulating evidence suggests that binge-like alcohol consumption during adolescence adversely affects the development of the PFC, little is known about the long-term consequences of this on PFC-dependent behaviors in adulthood. Efficient information processing of glutamatergic networks in the mPFC depends upon an optimal extracellular concentration of dopamine (DA), and enzymatic breakdown of DA by catechol-O-methyltransferase (COMT) plays a primary role in determining the extracellular concentration of DA in the PFC where the expression of DA transporters is very low. The aim of this study was to determine the effects of adolescent binge-like alcohol exposure on the expression of proteins associated with DAergic, glutamatergic and GABAergic neurotransmission in the mPFC of adult rats. Long-Evans rats were exposed to repeated cycles of binge-like intermittent ethanol exposure by vapor inhalation during postnatal days (PD) 28 to 42 (AIE). Following AIE exposure, rats were grown to adulthood (PD90) prior to preparation of mPFC tissue for Western blot analysis. We isolated PSD (synaptic) and soluble (or extrasynaptic) fractions, and examined AIE-induced prolonged neuroadaptations in glutamatergic and DAergic systems. We did not observe significant changes in synaptic expression levels of subunits of the AMPA (GluR1) or NMDA (GluN1, GluN2A, and GluN2B) receptors. Also, AIE did not alter extrasynaptic GluR1 subunit expression levels. However, AIE exposure significantly down-regulated COMT levels in adulthood (control,  $96.9 \pm 13.3$ ; AIE,  $60.4 \pm 13.4$ ;  $p < .05$ ). These data suggest that while AIE exposure does not appear to produce persistent neuroadaptations in synaptic glutamate receptors in the mPFC, AIE may affect adulthood extracellular DA concentrations through a reduction in COMT. These data are consistent with the suggestion that AIE exposure may disrupt DA modulation of optimal PFC performance in adulthood, leading to degradation of PFC-dependent control of complex cognitive behaviors.

P0353

#### GLUTAMATE TRANSPORTER EXPRESSION IN NUCLEUS ACCUMBENS AND DORSAL STRIATUM AFTER CHRONIC INTERMITTENT ETHANOL EXPOSURE

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Using an established model of ethanol dependence and relapse drinking, previous studies have demonstrated increased glutamate levels in the nucleus accumbens (NAc) and dorsolateral striatum (DLS) of mice that have experienced repeated cycles of chronic intermittent ethanol (CIE) exposure. This increase in basal striatal glutamate tone has been demonstrated at least 1 week following final CIE exposure, and may mediate increased

ethanol drinking in dependent mice. However, the source of this elevation in extracellular glutamate levels is as yet unclear. One potential cause is altered excitatory amino acid transporter (EAAT) expression in the dorsal and ventral striatum. Removal of glutamate from the extracellular space in these brain regions occurs predominately through EAAT2 and EAAT3. This study tested the hypothesis that reduced EAAT2 and/or EAAT3 expression contributes to elevated glutamate levels in the NAc and DLS in ethanol dependent mice. Adult male C57BL/6J mice received repeated cycles of CIE exposure in inhalation chambers (16 hr/day, 4 days/week to achieve BECs at 175 – 225 mg/dL) alternated with weekly limited access two-bottle choice ethanol (15%) consumption (2 hr/day, 5 days/week, water as the other available fluid). CIE exposed mice showed significant escalation of voluntary ethanol consumption compared to air-exposed controls over 4 weekly test cycles ( $3.7 \pm 0.7$  vs.  $2.2 \pm 0.3$  g/kg for CIE and control groups, respectively). Following the 4th test drinking period, Western blot analysis was used to measure total, surface, and intracellular EAAT2 and EAAT3 expression in the NAc and DLS. Mice were sacrificed 7 days post-chamber, 18–22 hours following their last 2-hr drinking session. Results showed that when comparing ethanol dependent and non-dependent mice, no difference was found in total, surface and intracellular EAAT2 and EAAT3 expression in the NAc or DLS brain regions. These data suggest that differences in glutamate release mechanisms (e.g., via system Xc) rather than altered uptake mechanisms may be responsible for the elevated extracellular glutamate levels observed in CIE mice. Ongoing studies will address the role of system Xc in the CIE model as well as the possibility that neuronal hyperexcitability may contribute to increased extracellular glutamate levels in the NAc and DLS which, in turn, may mediate increased ethanol consumption associated with ethanol dependence. Supported by grants T32 AA007474 and P50 AA010761.

P0440

SHORT TERM ETHANOL EXPOSURE INDUCES CALPAIN DEPENDENT A-SPECTRIN  
PROTEOLYSIS AND NEURODEGENERATION IN THE HIPPOCAMPUS

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The calpain family of cysteine proteases is crucial to the initiation, regulation and execution of cell death and is implicated in neurodegenerative states such as stroke and traumatic brain injury. The present studies examined accumulation of the calpain-dependent 145kDa spectrin breakdown product (SBDP) and changes in the neuronal markers NeuN, MAP-2, and propidium iodide (PI) following 1, 3 or 5 days of binge-like exposure to EtOH (50 mM) in organotypic hippocampal slice cultures. Western blot analysis demonstrates time-dependent accumulation of the 145kDa SBDP in homogenized hippocampal tissue with significance observed after 5 days of EtOH exposure. Uptake of the non-vital marker PI was increased in the pyramidal cell layers of the CA1 and CA3 and the granule cell layer of the DG of the hippocampus following 1 day of exposure to EtOH, with increases evident in the granule cell layer on day 3 as well. Immunoreactivity (IR) of MAP-2, a known calpain substrate, showed time-dependent decreases in each subregion, while NeuN IR was significantly reduced in all subregions after 5 days of EtOH exposure. These data suggest the activation of calpains with prolonged exposure to binge-like EtOH concentrations and the associated development of

neurodegeneration within the hippocampus. In sum, the calpain family of cysteine proteases may represent a novel therapeutic target for the treatment of ethanol abuse disorders. Supported by NIAAA.

S195

THE PHOSPHODIESTERASE-TYPE 4 INHIBITORS ENHANCE CLUSTERING BUT NOT SURFACE EXPRESSION OF NMDA AND AMPA RECEPTORS

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Previous studies have shown that prolonged inhibition of synaptic activity during chronic ethanol exposure promotes homeostatic increases in NMDA receptor expression at the synapse through a process that requires cAMP/PKA. Additional studies have shown that increases in cAMP associated with activation of NMDA receptors is specifically regulated by phosphodiesterase-type 4 (PDE4). Additional studies have implicated PDE4 and cAMP in the adaptive responses to alcohol. Therefore, we examined whether prolonged inhibition of PDE4 activity alters the synaptic trafficking of NMDA receptors. Exposure of rat hippocampal neuronal cultures to the PDE4 selective inhibitor rolipram (50-100 IM, 2-4 days) resulted in a significant increase in punctate clustering of membrane NMDA receptors. Similar increases were observed following prolonged exposure to another selective PDE4 inhibitor, RO-20-1724 (50 IM), the non-selective PDE inhibitor IBMX (25 IM), or a combination of rolipram (10 IM) and forskolin (4 IM) that alone were ineffective. Inhibitors of PDEs that degrade cGMP did not alter NMDA receptor clustering. The increase in NMDA receptor clustering induced by chronic inhibition of PDE4 activity occurred in both the synaptic and extrasynaptic membrane compartments, and was not altered by co-incubation with a protein synthesis inhibitor or by co-incubation with a low concentration of NMDA. Chronic rolipram treatment also increased clustering of the AMPA receptor subunit GluR1, but this occurred only in the synaptic pool of receptors. Surprisingly, although rolipram enhanced NMDA receptor clustering, no changes were observed in NMDA receptor-mediated whole-cell currents or surface expression. The increase in synaptic GluR1 was associated with an increase in kainate-induced AMPA wholecell currents but no change in surface expression of GluR1. Overall, the results of this study suggest that while chronic increases in the PDE4-regulated pool of cAMP can dramatically increase NMDA and AMPA receptor membrane clustering, this is not associated with an overall increase in their surface expression.

## **Tennessee – Delpire Lab**

P0989

### **MIDBRAIN AND FOREBRAIN CRF-R1 INVOLVEMENT IN EXCESSIVE ALCOHOL DRINKING: PHARMACOLOGICAL AND MOLECULAR GENETIC APPROACHES**

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Extrahypothalamic CRF1 receptors (CRF-R1) are thought to mediate anxiety-related aspects in the transition to alcoholism (i.e. binge drinking alternating with withdrawal). Escalated consumption is largely modulated by brain sites important to reward and affect such as the ventral tegmental area (VTA) and the dorsal raphe nucleus (DRN). Efferent projections from different sources in the midbrain to the prefrontal cortex may be differentially inhibited by action of CRF-R1. However, it is also unknown what role forebrain CRF-R1 may wield on alcohol consumption. The current studies investigated the roles of CRF-R1 within this network using both pharmacological and molecular genetic approaches. Adult, male C57BL/6J mice were given 24-hour access to 20% ethanol or water on an intermittent schedule (3 days per week). Mice were surgically implanted with cannulae targeting the VTA or DRN. On test days, 2-, 4-, and 24-hour fluid consumption were measured after a microinfusion of a CRF-R1 antagonist (aCSF vehicle, 0.3 ug CP-154,526, and 0.6 ug CP-154,526). Microinfusion of the CRF-R1 antagonist into the VTA dose-dependently suppressed ethanol drinking, and had a mild effect on water drinking. Intra-DRN microinfusion suppressed ethanol drinking at the highest dose. To investigate blockade of CRF-R1 through an inducible genetic deletion, mice were generated to have a functional knock-out (KO) of forebrain-specific CRF-R1. Deleted *crh1* transcript is controlled by a tetracycline-controlled transactivator protein through removal of doxycycline from the diet, and the deletion is specific to the forebrain due to a cre-lox system. Male and female littermates are tested since 75% expected progeny are functionally wild-type (WT) and 25% expected progeny are CRF-R1 KO. Ongoing studies explore the CRF-R1 KO phenotype in several alcohol drinking protocols, including drinking-in-the-dark, continuous access, and intermittent access. Males and females are tested with doxycycline in the diet and without. After testing baseline alcohol drinking and alcohol preference, mice will be challenged both systemically and intra-VTA and intra-DRN with a CRF-R1 antagonist. These experiments may reveal differential roles of CRF-R1 in midbrain sites and forebrain sites, or an important communication between the two, as possible systems that mediate excessive drinking.

## **Virginia – Miles Lab**

P0004

### ETHANOL REGULATION OF GSK3B IN PFC

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Alcohol dependence is a chronic neuropsychiatric disorder in which symptoms persist despite negative physical, mental and psychological consequences. We have previously shown using microarray analysis that glycogen synthase kinase 3b (GSK3b) is an ethanol responsive gene in mouse prefrontal cortex (PFC). Mice overexpressing GSK3b in the PFC exhibit a potentiation of ethanol drinking behavior (40% increase in 2-bottle choice drinking paradigm), an increase in alcohol withdrawal-induced anxiety and alterations in genes involved in synaptic transmission. Here, we have examined the effects of ethanol on GSK3b S9 phosphorylation levels in PFC. We found that acute ethanol treatment (2 g/Kg i.p.) of DBA/2J mice results in a significant induction in GSK3b expression in the PFC compared to saline treated mice using RT-PCR. We are currently assessing the effects of chronic intermittent ethanol exposure on GSK3b S9 phosphorylation levels in the PFC using western blot. We hypothesize that chronic ethanol exposure may result in a downregulation of GSK3b S9 phosphorylation levels and therefore an increase in GSK3b signaling, contributing to the potentiation of ethanol drinking behavior observed in mice overexpressing GSK3b. Unraveling the molecular and neuronal processes responsible for the development and persistence of these pathological behaviors might lead to the development of new strategies to treat alcohol dependence. This work was supported by NIAAA grant AA0106667 to MFM.

P0005

### ALTERATIONS TO THE SYNAPTIC TRANSCRIPTOME IN RESPONSE TO ETHANOL-INDUCED SENSITIZATION

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Characteristic behaviors associated with chronic drug use, such as sensitization, tolerance, craving and relapse, are thought to be caused by long-term molecular adaptations precipitated by the drug's continual administration. Ethanol-induced behavioral sensitization can provide a valid, testable model to study the neural plasticity that mediates chronic drug-related behavior. The lasting activity-dependent changes that underlie behavioral adaptation to chronic ethanol are thought to, in part, depend on new protein synthesis and remodeling at the synapses. It is well established that mRNA can be transported to neuronal distal processes, where it can undergo localized translation regulated in a spatially restricted manner in response to stimulation. These concepts have led to our hypothesis that behavioral sensitization to repeated ethanol exposure results, at least in part, from alterations in the trafficking of mRNAs to distal processes, contributing to synaptic remodeling and plasticity. To identify molecular targets involved in synaptic plasticity underlying ethanol behavioral sensitization, DBA/2J mice (n=16 per treatment) were subjected to a standard 14-day sensitization paradigm using 2.5g/kg i.p. ethanol. Four hours following the final treatment on day 14, frontal pole tissue was dissected and utilized for a synaptoneurosome preparation which concentrates synaptic mRNA encapsulated within vesicularized elements. Profiling of RNA obtained from these samples (Affymetrix\_ GeneChip\_ Mouse 1.0 ST Arrays) allowed for discrimination of expression changes in synaptically localized genes which may otherwise go undetected when



studying the entire transcriptome. RMA expression data was analyzed to identify synaptically targeted genes differentially expressed as a result of ethanol treatment. Examination of gene expression patterns revealed a number of genes whose levels were altered in response to acute ethanol, but habituated in response to repeated, sensitizing treatment. Further investigation revealed enrichment for genes involved in the processes of synaptic transmission, kinase activity, and endoplasmic reticulum function. Further analysis of these data will focus on novel networks of genes whose alterations in synaptic transcript levels contribute to ethanol-induced behavioral sensitization. Supported by NIAAA grants F31AA021035 to MAO and AA016667 to MFM.

P0057

ROLE OF ADRENAL GLUCOCORTICOID SIGNALING IN PREFRONTAL CORTEX GENE EXPRESSION AND ACUTE BEHAVIORAL RESPONSES TO ETHANOL

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Adrenal glucocorticoid hormones, the final step in activation of the hypothalamic-pituitary-adrenal (HPA) axis, modulate acute and chronic behavioral and molecular responses to drugs of abuse including psychostimulants and opioids. There is growing evidence that glucocorticoids might also modulate behavioral responses to ethanol. Acute ethanol activates the HPA axis, causing glucocorticoid hormone release. Our prior genomic studies suggest glucocorticoids play a role in regulating gene expression in the prefrontal cortex (PFC) of DBA2/J (D2) mice following acute ethanol administration. However, little work has been done regarding HPA axis regulation of gene expression and glucocorticoid regulation of behavioral responses to ethanol. Such work could be significant, given the predictive value for level of response to acute ethanol in the risk for alcoholism. We studied whether the glucocorticoid receptor (GR) antagonist, RU-486, or adrenalectomy (ADX) altered male D2 behavioral responses to acute (locomotor activation, anxiolysis or loss-of-righting reflex (LORR)) or repeated (sensitization) ethanol treatment. Whole genome microarray analysis and bioinformatics approaches were used to identify candidate genes that may be responsible for altered behavioral responses to ethanol following ADX. We show that ADX and RU-486 both impair acute ethanol (2 g/kg) induced locomotor activation in D2 mice without affecting basal locomotor activity. However, neither ADX nor RU-486 alter initiation of ethanol sensitization, ethanol-induced anxiolysis or LORR. We also show differing expression of previously identified ethanol-responsive genes in the PFC of SHAM vs. ADX mice. *Fkbp5* was significantly decreased and *Gpr6* was significantly increased basally in ADX mice. Our studies suggest that ethanol's activation of adrenal glucocorticoid release and subsequent GR activation may partially mediate ethanol's acute locomotor activating properties in male D2 mice. In addition, adrenal glucocorticoid basal tone can regulate PFC gene expression. This suggests that glucocorticoid regulated PFC gene expression may be an important factor modulating acute behavioral responses to ethanol. This work was supported in part by NIAAA grants F31 AA20141-0 to BNC and AA016667 to MFM.

P0326

CHLORIDE INTRACELLULAR CHANNELS (CLICS) MODULATE ACUTE ETHANOL BEHAVIORS IN DROSOPHILA, C. ELEGANS AND MICE

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Identifying genes that influence behavioral responses to alcohol is critical for understanding the molecular basis of alcoholism and ultimately developing therapeutic interventions for the disease. Using an integrated approach that combined the power of the *Drosophila*, *C. elegans* and mouse model systems with bioinformatics analyses, we established a novel, conserved role for Chloride Intracellular Channels (CLICs) in alcohol-related behavior. CLIC proteins might have several biochemical functions including intracellular chloride channel activity, modulation of TGF- $\beta$  signaling, and regulation of ryanodine receptors and A-kinase anchoring proteins. We initially identified vertebrate *Clic4* as a candidate ethanol-responsive gene via bioinformatic analysis of data from published microarray studies of mouse and human ethanol-related genes. We confirmed that *Clic4* expression was increased by ethanol treatment in mouse prefrontal cortex and also uncovered a correlation between basal expression of *Clic4* in prefrontal cortex and the locomotor activating and sedating properties of ethanol across the BXD mouse genetic reference panel. Furthermore, we found that disruption of the sole *Clic* *Drosophila* orthologue significantly blunted sensitivity to alcohol sedation in flies, that mutations in two *C. elegans* *Clic* orthologues, *exc-4* and *exl-1*, altered behavioral responses to acute ethanol in worms, and that viral-mediated overexpression of *Clic4* in mouse brain decreased the sedating properties of ethanol. Together, our studies demonstrate key roles for *Clic* genes in behavioral responses to acute alcohol in *Drosophila*, *C. elegans* and mice. Ongoing studies are exploring the possibility that *Clic4/Clic* influences ethanol sensitivity by functioning within the TGF- $\beta$  and other signaling pathways.

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NOVEL MU-OPIOID RECEPTOR SELECTIVE ANTAGONIST NAQ SELECTIVELY REDUCES CONSUMPTION OF HIGH-CONCENTRATION ETHANOL IN MICE

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The non-selective opioid antagonist naltrexone (NTX) is one of few drug therapies approved by the FDA for alcoholism, all of which have shown limited long-term efficacy. NTX acts at mu (MOR) and kappa, and to a lesser degree delta, opioid receptors, and the role of each of these subtypes in its effects on alcohol consumption is unknown. Until recently the only mu-selective ligands available were conformation-constrained peptides with limited experimental utility. However, the recent development of a pair of small molecule MOR antagonists, NAQ and NAP, has allowed for the examination its role in alcohol consumption, with the goal of hypothesis-driven development of new pharmacological therapies for alcoholism. Toward this end C57BL/6J mice were employed in a 3-bottle-choice alcohol self-administration model, in which the effects of NAQ, NTX, and saline vehicle on ethanol consumption (g/kg/day) and preference (over water) were examined. Mice were allowed 24 hour access to 15% and 30% (v/v) ethanol and water, in 3 separate tubes. Two experiments were performed on the same mice, with all drugs administered IP 1x/day in saline. First, following 14 days of baseline ethanol access, the effects of NTX and NAQ (both at 1.00 mg/kg) were examined. Next, following 14 days of abstinence, mice were exposed to intermittent alcohol access (IAA) on a 3 day per week (M/W/F) schedule to examine the effects of prior NAQ, NTX, or saline pretreatment

(in experiment 1) on the development of elevated alcohol intake after abstinence. In the first experiment, NAQ and NTX significantly reduced total ethanol consumption compared to baseline on the first test day. On test day 2 the NTX mice had recovered to baseline consumption levels, while NAQ mice continued to show significantly decreased intake. NAQ significantly reduced consumption and preference for 30% ethanol compared to baseline on both test days, while NTX produced non-significant reductions. In the IAA model, the saline group significantly increased total ethanol preference on the first reinstatement day, while NAQ and NTX groups showed little change until week 3, and never reached the high levels of the saline group. These results show that selective MOR antagonism is sufficient to reduce alcohol consumption and preference in mice, with selectivity for high concentrations, and that NAQ may be a treatment for alcoholism with some potential benefits over NTX. This work was supported in part by NIAAA grant AA016667.