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"INIA: Stress, Anxiety of Alcohol Abuse"

Abstracts

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Italy

CHRONIC ETHANOL INCREASE NEUROACTIVE STEROIDS AND CHANGE $GABA_A$ RECEPTOR PLASTICITY AND FUNCTION IN BRAIN OF SOCIALLY ISOLATED RATS

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ETHANOL INCREASES GABAergic TONIC CURRENTS IN RAT DENTATE GYRUS GRANULE CELLS IN A NEUROSTEROID-DEPENDENT MANNER

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Maine

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

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VOLTAMMETRIC EVALUATION OF STRIATAL DOPAMINE PARAMETERS IN C57/BL6 AND DBA/2 INBRED MOUSE STRAINS

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DOPAMINE HYPOFUNCTION LINKED TO ETHANOL INDUCED HYPERACTIVITY IN AN ENU-MUTATED MOUSE

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ACUTE ETHANOL CONSUMPTION DECREASES DHEAS LEVELS IN HEALTHY MEN AND WOMEN, WITHOUT CHANGING DHEA LEVELS

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GENE EXPRESSION CHANGES IN THE CEREBELLUM FOLLOWING CHRONIC ALCOHOL SELF-ADMINISTRATION

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PERIPHERAL BLOOD AS A BIOMARKER DISCOVERY TOOL FOLLOWING CHRONIC ALCOHOL SELF-ADMINISTRATION

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Oklahoma

ETHANOL EXPOSURE AND WITHDRAWAL ENHANCE LTP IN CENTRAL AMYGDALA

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Oregon

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) DISTINGUISH RHESUS MACAQUE ANCESTRIES.

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South Carolina

CHRONIC INTERMITTENT ETHANOL EXPOSURE THAT SUSTAINS HIGH BLOOD LEVELS IS CRITICAL FOR PRODUCING ENHANCED VOLUNTARY ETHANOL DRINKING IN MICE

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EARLY SOCIAL ISOLATION AND CHRONIC VARIABLE STRESS INDUCE LATER ELEVATED ETHANOL INTAKE IN ADULT C57BL/6J MICE

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SENSITIZATION TO LOCOMOTOR STIMULANT EFFECTS OF ETHANOL AND ETHANOL DRINKING IN ENU-INDUCED 7TNJ MUTANT MICE

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Tennessee

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NOREPINEPHRINE PRODUCES AN α1 ADRENERGIC RECEPTOR DEPENDENT LONG TERM DEPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Texas

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Virginia

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DEFINING ROBUST ACUTE ETHANOL-RESPONSIVE GENE NETWORKS BY EXPRESSION PROFILING ACROSS RECOMBINANT INBRED PANELS
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BRIEF REPEATED SOCIAL DEFEAT ALTERS ETHANOL DRINKING PATTERNS IN C57BL/6 MICE.
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CHRONIC ETHANOL INCREASE NEUROACTIVE STEROIDS AND CHANGE $GABA_A$ RECEPTOR PLASTICITY AND FUNCTION IN BRAIN OF SOCIALLY ISOLATED RATS

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Social isolation of rats elicits a marked reduction in the cerebrocortical and plasma concentrations of progesterone, 3α,5α-TH PROG and 3α,5α-THDOC. This effect is associated with an increase in the hippocampal level of α_2, α_4 and δ subunits immunoreactivity, while the amounts of α_1 and γ_2 were decreased. Ethanol increased the amplitude of $GABA_A$ receptor–mediated miniature inhibitory postsynaptic currents (mIPSC) recorded from CA1 pyramidal neurons with a greater potency in hippocampal slices prepared from socials isolated (SI) rats than in those from group-housed (GH), an effect inhibited by finasteride, an inhibitor of the 5α-reductase.

Voluntary consumption of ETH during social isolation abolished both the reduction of the brain and plasma concentrations of 3α,5α-TH PROG and the enhanced potency of ethanol on mIPSC recorded from CA1 pyramidal neurons. However, the amplitude of $GABA_A$ receptor–mediated tonic inhibitory currents in granule cells of the dentate gyrus, greater in hippocampal slices from SI rats than in those from GH animals, was further increased in SI animals. Since voluntary ETH consumption was associated with small, but significant, further enhancement of the amount of δ subunit immunoreactivity throughout the hippocampus, these data suggest that voluntary ETH consumption in SI animals results in the formation of an increased number of α_4 subunit–containing $GABA_A$ receptors that also contain the δ subunit. At variance, this treatment did not further modify the hippocampal level of α_1, α_2, α_4 and γ_2 subunits in SI rats.

Intermittent ETH vapour exposure for 8 days, a protocol involving multiple withdrawal episodes, induces a long-lasting increase of the brain concentrations of 3α,5α-TH PROG, both in SI and GH rats, an effect blunted in SI rats suggesting that housing condition is crucial in the development of tolerance to the steroidogenic effect of ethanol. The effect of intermittent ETH vapour exposure and withdrawal in SI rats on $GABA_A$ receptor hippocampal plasticity will be presented.

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ETHANOL INCREASES GABAergic TONIC CURRENTS IN RAT DENTATE GYRUS GRANULE CELLS IN A NEUROSTEROID-DEPENDENT MANNER

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Several lines of research have suggested that certain pharmacological effects of ethanol (EtOH) may result from an increased brain concentrations of neuroactive steroids such as 3α ,5α-THProg, a potent and efficacious positive modulator of GABAA receptors. We have also suggested that EtOH may stimulate hippocampal steroidogenesis independently from the activity of the HPA axis, an effect that results in an increased synaptic GABAA receptor function in CA1 pyramidal neurons. Given that extrasynaptic GABAA receptors containing the α4 and δ subunits, such as those expressed in dentate gyrus granule cells (DGGCs), are particularly sensitive to 3α,5α-THProg, in the present study we examined the effect of EtOH on tonic GABAergic currents in DGGCs and determined the role of hippocampal steroidogenesis. Whole-cell patch clamp recording of GABAergic tonic currents was performed in DGGCs (Vhold = -65 mV), present in coronal hippocampal slices prepared from Sprague-Dawley rats, before, during, and after perfusion of EtOH (50 -100 mM) for 30 min. EtOH increased tonic current noise variance in DGGCs in a time-dependent manner, with an onset of about 20-30 min. Perfusion for 30 min of 100 mM EtOH resulted in a 15 ± 2.5% increase ($p < 0.05$; $n = 19$) over the EtOH pre-exposure basal level. On the other hand, we found no significant change in tonic current noise variance during the initial 10 min of EtOH exposure (6 ± 2.1% change). In addition, EtOH did not alter tonic current shift produced by the exposure of 20 μM bicuculline at any time point. EtOH-induced increase in tonic current noise variance was completely prevented by the co-application of the 5α-reductase inhibitor finasteride (1 μM). These results demonstrate that exposure of hippocampal slices to EtOH for 30 min results in the stimulation of local neurosteroid concentration at relevant levels to modulate the function of DGGC extrasynaptic GABAA receptors.
Supported by INIA-Stress Consortium – NIAAA

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

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Alcohol withdrawal is associated with affective, chronobiological, neurophysiological and neuroendocrine disturbances, as well as increased alcohol craving, in both human alcoholics and in animal models of alcohol dependence. In general, these phenomena are potentiated by increased chronicity of prior alcohol exposure and by multiple prior episodes of withdrawal. In this study, we examined the effects of withdrawal from chronic intermittent ethanol vapor exposure (CIE) on circadian patterns of home-cage locomotor (running-wheel) activity in C57BL/6J mice. Following baseline activity measurements, mice were exposed to a 4-day CIE protocol in which 16 hours of ethanol vapor exposure alternated with 8 hours of withdrawal, while control animals were continuously exposed to plain air in an identical environment. During CIE, animals remained in their home cages with access to running wheels, but running-wheel activity was not recorded. Ethanol exposure began at the onset of the dark phase of the daily 12:12 light-dark cycle, and each exposure period was initiated by an injection of 1.6 g/kg ethanol and 1.0 mmol pyrazole, i.p., to rapidly stabilize blood ethanol concentrations, while air-exposed controls received pyrazole in saline only. Despite achieving lower than expected blood ethanol levels (approximately 60 to 100 mg%), CIE resulted in persisting reductions in locomotor activity, while activity levels were unchanged in air-exposed controls. Analysis of circadian waveforms indicated that reduced activity occurred throughout most of the dark phase of the LD cycle, but that daily activity patterns were otherwise unaltered. These results contrast with those of Kliethermes et al. (2005), who reported little or no effect of withdrawal from three days of (continuous) ethanol vapor exposure on circadian activity patterns. One important difference between the two studies is that Kliethermes et al. employed photobeam activity monitors rather than running wheels, which are thought to reflect a form of reward-seeking behavior. Subsequent experiments will examine the effects of ethanol dose, mouse strain, daytime vs. nighttime ethanol exposure, and repeated CIE cycles, on circadian activity patterns. Supported by NIAAA AA013893 (AMR) and AA13641 (INIA-Stress).

VOLTAMMETRIC EVALUATION OF STRIATAL DOPAMINE PARAMETERS IN C57/BL6 AND DBA/2 INBRED MOUSE STRAINS

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Inbred mouse strains are powerful tools in characterizing the genetic determinants of ethanol drinking behaviors. The C57/BL6 (C57) and DBA/2 (DBA) are some of the most studied inbred strains in alcohol research, because the C57 mice voluntarily drink large amounts of alcohol compared to the DBA mice. Ethanol elevates dopamine (DA) levels in brain areas such as the caudate-putamen (CPu) and nucleus accumbens (NAc), similar to other psychostimulants including cocaine and amphetamine. There has been extensive behavioral characterization of these two mouse strains with regard to ethanol effects; however, an in-depth evaluation of their DA-ergic systems and responsiveness to ethanol has not been performed. The primary focus of this study was to characterize the dopaminergic system using in vitro voltammetry to assess multiple DA parameters in the C57 and DBA mice and relate the findings to their ethanol responsiveness. Using in vitro voltammetry, the DBA mice exhibited a trend toward decreased DA release and lower uptake rates in the CPu compared to the C57 mice. However, in the NAc core, there was no difference in DA release or uptake between the C57 and DBA mice. Furthermore, we examined the DA D2 and D3 receptor function in the nucleus accumbens. Using the mixed D2/D3 agonist, quinpirole, the C57 appear to be more sensitive to the release-decreasing autoreceptor effects of quinpirole at low concentrations compared to the DBA mice. On the other hand, a preliminary study using the more selective DA D3 receptor agonist (±)PD 128907 revealed D3 supersensitivity in DBA compared to C57 mice. This is the first attempt to characterize the dopaminergic system of C57 and DBA strains using voltammetry. The data suggest there may be subtle differences between these strains which may in part explain their ethanol response differences.

DOPAMINE HYPOFUNCTION LINKED TO ETHANOL INDUCED HYPERACTIVITY IN AN ENU-MUTATED MOUSE

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Recently, the Integrative Neuroscience Initiative on Alcoholism generated a strain of mice, called 22 TNJ, by chemically-induced mutagenesis (N-ethyl nitrosourea, ENU). Initial characterization of these mice illustrated a 10-fold greater locomotor activity response to ethanol than controls. The rewarding, hyperlocomotive and addictive properties of drugs of abuse are often associated with an elevation in dopamine (DA) levels. Ethanol is known to activate DA cell bodies in the ventral tegmental area, leading to enhanced DA signaling in terminal fields such as the nucleus accumbens (NAc). Using in vivo microdialysis, we determined the 22 TNJ mice are supersensitive to the dopaminergic effects of cocaine and ethanol. To better understand this hyper-responsive phenotype, we examined extracellular DA levels in the NAc and caudate-putamen (CPu) of the 22 TNJ and control mice. Extracellular DA levels were lower in the CPu of the 22-TNJ mice (1.0 ± 0.4 nM) compared with their controls (3.7 ± 0.8 nM). Extracellular DA levels in the NAc showed a trend towards decreased extracellular levels (0.49 ± 0.09 nM and 0.76 ± 0.15 nM, 22 TNJ and controls, respectively). This decrease in extracellular DA levels is consistent with in vitro voltammetry results, which demonstrate faster DA uptake in the NAc of the 22 TNJ mice compared to controls. In addition, voltammetry and synthesis measurements were used to characterize D2/D3 autoreceptors using the nonselective agonist, quinpirole. Presynaptic D2-like autoreceptors controlling both release and synthesis were found to be supersensitive in the NAc of the 22 TNJ mice compared to their controls. Finally, the 22 TNJ mouse showed increased vertical activity in response to apomorphine (3.0 mg/kg), a non-selective DA receptor agonist, compared to controls. These results indicate that the 22-TNJ mice are hypo-dopaminergic, and consequently demonstrate supersensitive pre- and post-synaptic DA receptors. The drug-induced hyperactivity in the 22 TNJ strain may be the result of hypodopaminergic tone and supersensitive DA receptors. These mice exhibit hyperactivity in response to stimulating ethanol doses in spite of a reduction in DA levels. This could be an important example of the behavioral consequences of hypodopaminergia, revealed as a seemingly paradoxical supersensitivity to stimulant drugs.

ACUTE ETHANOL CONSUMPTION DECREASES DHEAS LEVELS IN HEALTHY MEN AND WOMEN, WITHOUT CHANGING DHEA LEVELS

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Acute ethanol administration to rodents (1.3 – 4.0 g/kg) induces a marked increase in the brain and plasma concentrations of the potent GABAergic neuroactive steroids 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP) and 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-THDOC), but does not alter the androgen steroids dehydroepiandrosterone (DHEA) and its sulfate derivative DHEAS (see Morrow et al., 2006 for review). In contrast, moderate doses of ethanol (0.8 g/kg) have no effect (Holdstock et al., 2006) or decrease plasma 3α,5α-THP concentrations (Pierucci-Lagha et al., 2006) in healthy human subjects, but effects on DHEA levels are controversial (Pierucci-Lagha et al., 2006; Sarkola et al., 2000). We hypothesized that androgen steroids might be more responsive to ethanol consumption in humans compared to rodents. To address this hypothesis we measured DHEA and DHEAS plasma levels in healthy volunteers after acute ethanol consumption. Men (n=8) and women (n=12) in the follicular phase of the menstrual cycle were included in the study. Each subject participated in two sessions on which they received ethanol (0.8 g/kg or 0.7 g/kg, respectively for men and women) or placebo. Blood was obtained 15 minutes before and 30, 60, 120 and 375 minutes after ethanol consumption and DHEA and DHEAS were measured in plasma samples by radioimmunoassay. Ethanol decreased DHEAS levels at all time points, compared to the basal sample taken 15 minutes before its consumption. In men, DHEAS levels were decreased by 8 and 11% at 30 and 60 minutes ($p<0.05$) and by 15 and 16% at 120 and 375 minutes ($p<0.01$). In women, DHEAS levels were decreased by 11% at 30 minutes ($p<0.05$) and by 16, 19 and 26% at 60, 120 and 375 minutes respectively ($p<0.001$). In contrast, ethanol consumption did not alter DHEA levels in men or women at any of the time points examined. These preliminary data suggest that acute ethanol consumption alters DHEAS, but not DHEA levels in human subjects. Thus, it is possible that DHEA is rapidly metabolized into its androgen derivatives; future studies will address this hypothesis.
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GENE EXPRESSION CHANGES IN THE CEREBELLUM FOLLOWING CHRONIC ALCOHOL SELF-ADMINISTRATION

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Patients with alcoholism present with cerebellar pathology including well-documented *in vivo* brain structural volume deficits and associated functional sequelae. There is a growing body of evidence that describes a role for the cerebellum in diverse higher cognitive functions, suggesting that cognitive deficits might also occur as a result of alcohol-induced cerebellar damage. The goal of this study, therefore, was to identify and characterize changes in gene expression in the cerebellum that have occurred as a result of chronic alcohol consumption. To identify changes in gene expression that may underlie cerebellar dysfunction, we employed a very robust nonhuman primate model of ethanol self-administration. Through the use of whole genome microarrays, cerebellum transcript levels were measured in twelve adult macaques (6 male; 6 female) that had self-administered ethanol chronically for 18 consecutive months, and eight adult animals (4 male; 4 female) that were alcohol-naïve. For each animal, gene expression was measured in each of three functionally and anatomically distinct regions of the cerebellar cortex: anterior lobe, posterior lobe and vermis. The discussion will focus on gene expression profile comparisons within individuals (for each of the three regions), between individuals (a measure of individual variability), and between groups (for drug effect: chronic alcohol versus alcohol-naïve).

PERIPHERAL BLOOD AS A BIOMARKER DISCOVERY TOOL FOLLOWING CHRONIC ALCOHOL SELF-ADMINISTRATION

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Biological markers that may be associated with risk for alcoholism, or used for assessing chronic ethanol consumption, are limited both by their nature (e.g., variable sensitivity and predictive value) and due to the heterogeneous nature of the human alcoholic population. In an effort to identify new potential alcohol biomarkers, this study examines peripheral gene expression in whole blood taken from macaque monkeys whose drinking behavior models human alcoholism. Eight adult male cynomolgus monkeys *(Macaca fascicularis*) were exposed to daily oral ethanol self-administration sessions for 18 consecutive months. Ethanol intake (g/kg/day) and blood ethanol content at 7 hours post session start (BEC; mg%) was recorded for each animal for the duration of the study. At 18 months a blood sample was taken from each of the alcohol self-administering animals, as well as from ten alcohol-naïve adult male cynomolgus monkeys. Total RNA isolated from these samples was used to query high density oligonucleotide arrays. Genes that were significantly over- or under-expressed in the drinking animals were identified. Cynomolgus monkeys in this study self-administered ethanol at average rates of between 1.2 and 3.9 g/kg/day. At intakes ≥ 2 g/kg the animals routinely had BECs ≥ 100 mg%. Gene expression analysis from whole blood revealed >7000 genes that were differentially regulated in the alcohol-consuming animals (@ fold change ≥ 1.5 and $p \leq 0.05$). Ontologic classes of upregulated genes that are overrepresented in this dataset include genes involved ribosome formation, cell communication and the complement and coagulation cascades. Overrepresented groups of downregulated transcripts include genes involved in long-term potentiation, antigen processing and presentation, and several biosynthetic pathways including oxidative phosphorylation, lipid metabolism, glycolysis/gluconeogenisis, and pentose phosphate pathway genes.

ETHANOL EXPOSURE AND WITHDRAWAL ENHANCE LTP IN CENTRAL AMYGDALA

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Recent reports suggest that the amygdaloid nuclear complex may play an important role in mediating emotional and stress-related responses associated with alcohol withdrawal, and alterations in amygdala function may contribute to the exacerbation of withdrawal severity that is sometimes observed after repeated withdrawal episodes. The current investigation examined the effects of chronic alcohol exposure in C57Bl/6J mice on the network properties of the amygdaloid nuclear complex. For these studies, animals received a priming dose of ethanol (1.6 g/kg, 8% w/v, i.p.) and the alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg) each day and were then exposed to ethanol in vapor-inhalation chambers for 16 hours. Following four days of ethanol treatment, acute in vitro amygdala slices were studied, 6-8 hrs after the last ethanol exposure. Control animals received similar housing, handling and daily pyrazole injections, but were not exposed to ethanol. Studies were conducted using a substrate-imbedded multi-electrode array (Panasonic Med64) that allowed the examination of electrical activity from 64 electrodes across each slice. Individual slices from each animal were examined for the development of long-term potentiation (LTP) in response to theta burst stimulation administered to neurons within the basolateral amygdaloid complex (lateral, basolateral, and basomedial nuclei of the amygdala) or the central nuclear group (central lateral and central medial nuclei). LTP was similar in control and in ethanol-exposed animals when stimulation was administered into the lateral or basolateral nuclei, but was significantly greater in ethanol-treated animals with stimulation in the central nuclei. These findings provide support for the conclusion that the function of the central nucleus of the amygdala is altered during acute withdrawal from chronic ethanol treatment and suggest that this effect may contribute to the neuronal hyperexcitability observed during the ethanol withdrawal syndrome.
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SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) DISTINGUISH RHESUS MACAQUE ANCESTRIES.

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Genetic variation contributes to phenotypic differences between both individuals and animal populations. For example, Chinese rhesus macaques are recognized as being more impulsive and aggressive than Indian rhesus macaques. The divergent genetic and phenotypic characteristics of these two rhesus populations make it important to consider animal ancestry in designing studies. In an effort to identify genetic markers that can 1) verify the geographic origin of rhesus (Chinese or Indian) and 2) allow us to explore the genetic basis of temperament and alcohol consumption, we undertook a SNP discovery effort focusing on 94 rhesus macaque genes. A region from the 3' UTR of each gene was sequenced in 20 unrelated Indian and Chinese rhesus monkeys. Among the 661 SNPs identified, 69% were unique to either the Chinese or Indian populations. We then used a subset of the SNPs to generate a low-cost, efficient SNP assay to genotype 90 additional Chinese and Indian rhesus. Our results identified a set of 50 SNP population markers that reliably distinguish Chinese from Indian rhesus macaques. This SNP assay is useful for both study design purposes and for breeding applications. In addition, this study identified the MAOA (monoamine oxidase A), NR3C1 (glucocorticoid receptor) and SNCA (synuclein) genes as having high frequency alleles (0.4-0.97) that are unique to the Chinese rhesus population. These alleles may be associated with different gene expression levels or protein functions that contribute to the more aggressive and impulsive Chinese rhesus temperament. Finally, a similar effort to identify SNPs that distinguish Cambodian, Indonesian and Philippine cynomolgus macaques was undertaken. We have developed a 35 SNP panel that is useful for verifying animal ancestry in this species as well.

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CHRONIC INTERMITTENT ETHANOL EXPOSURE THAT SUSTAINS HIGH BLOOD LEVELS IS CRITICAL FOR PRODUCING ENHANCED VOLUNTARY ETHANOL DRINKING IN MICE

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We previously demonstrated that chronic intermittent EtOH vapor exposure results in enhanced voluntary EtOH intake in C57BL/6J mice. This study examined whether other chronic EtOH procedures would yield similar increases in EtOH drinking. Adult male C57BL/6J mice were trained to drink 15% EtOH in a limited access (2 hr/d) 2-bottle choice paradigm. After stable baseline daily intake was established (~2.5 g/kg), mice were exposed to 3 cycles of chronic intermittent EtOH exposure for 1 week followed by 5 consecutive days of limited access drinking. In Experiment 1, mice were given chronic intermittent access to 15% EtOH (2-bottle choice) or only water in the home cage 16 hr/d (n= 12/group). Although mice consumed 12-14 g/kg/16 hr, limited access EtOH intake during intervening weeks remained unchanged, indicating that episodic, long-term (16 hr/d) access to EtOH in the home cage did not increase drinking. In Experiment 2, chronic intermittent EtOH exposure was delivered by gastric intubation. Mice were intubated with increasing doses of EtOH (4, 4.5 and 5 g/kg) or volume-matched sucrose (n= 8/group) once daily for 5 days during each cycle, respectively. Although EtOH intubation produced high, dose-dependent blood EtOH levels at 1 hr (4 g/kg: 375 mg%; 4.5 g/kg: 425 mg%; and 5 g/kg: 550 mg%), limited access EtOH intake did not change as a function of treatment, suggesting that high blood EtOH levels produced by once-daily intubation is not sufficient to increase subsequent drinking. In Experiment 3, mice were exposed to chronic intermittent EtOH vapor (maintaining BEC at 150-200 mg%) or air for 16hr/d (n= 23-29/group). Lickometer circuits tracked drinking at the EtOH and water tubes during limited access sessions. While baseline lick rates (~425 licks/2 hr) and intake (~2.8 g/kg) were similar, lick rates and intake progressively increased following each cycle of EtOH vapor exposure (~735 licks/2 hr; 3.8 g/kg) compared to controls (~460 licks/2 hr; 2.8 g/kg). Collectively, these results indicate that chronic intermittent EtOH exposure that enables sustained blood EtOH levels in the 150-200 mg% range (resulting in EtOH dependence) is necessary to subsequently increase voluntary EtOH drinking in C57BL/6J mice.
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EARLY SOCIAL ISOLATION AND CHRONIC VARIABLE STRESS INDUCE LATER ELEVATED ETHANOL INTAKE IN ADULT C57BL/6J MICE

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This study examined the effects of chronic social isolation and/or variable stress experiences during early development on later voluntary ethanol (EtOH) intake in adult male and female C57BL/6J mice. The experiment was defined by three factors; sex, housing (single- vs. group-housed), and chronic variable stress (CVS vs. control). All mice were born in our facilities and weaned at postnatal day 21 (PND21). At weaning, half of the mice were singly housed while the remaining mice were group housed (4 per cage) according to sex. Starting at PND32, the singly-housed and group-housed mice were either subjected to CVS or left undisturbed. CVS groups experienced random presentations of mild stressors twice a day for 14 days, including exposure to an unfamiliar open field, restraint, physical shaking, and forced swim, among others. At PND60 all mice were single-housed and three days later tested for voluntary EtOH intake for 3 weeks using a limited access procedure (2 hr/day), with 15% (v/v) EtOH presented in a 2-bottle choice with water. Mice were not food or water deprived at any time. Results indicated that for mice that did not experience CVS, early social isolation resulted in greater EtOH intake (males: 2.7 ± .2 g/kg; females: 3.5 ± .2 g/kg) compared to group-housed mice (males: 2.1 ± .2 g/kg; females: 2.2 ± .3 g/kg). CVS subsequently resulted in a significant increase in EtOH intake (Male CVS 2.7 ± .1, Females CVS 3.3 ± .2) in group-housed mice, but CVS failed to further increase EtOH intake in mice that experienced chronic social isolation early in life. Overall, females evidenced higher EtOH intake than males and water intake was not significantly affected by housing conditions, CVS or sex. These results confirm previous findings from our laboratory and others indicating that chronic social isolation experience early in development subsequently results in increased voluntary EtOH intake. In addition, experience with CVS early in life results in elevated EtOH intake later in adulthood. Taken together, these results emphasize the important role of early stress experiences that modulate later voluntary EtOH intake during adulthood. Supported by NIAAA grants AA014095, AA10761 and VA Medical Research.

SENSITIZATION TO LOCOMOTOR STIMULANT EFFECTS OF ETHANOL AND ETHANOL DRINKING IN ENU-INDUCED 7TNJ MUTANT MICE

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The 7TNJ ENU-induced mutant mouse pedigree has been reported to exhibit an exaggerated locomotor stimulant phenotype in response to ethanol (EtOH) challenge. This study was designed to examine whether repeated EtOH administration results in locomotor sensitization in these mutant mice, and whether such treatment influences EtOH drinking. 1TNH and C57BL/6J mice served as controls. Adult male and female 7TNJ, 1TNH and C57 mice were divided into 2 groups (n= 11-12/group). One group was allowed to drink EtOH (24 hr 2-bottle choice: 15% v/v vs. water) for 3 weeks before activity testing. Mice with or without a history of EtOH intake were then divided in two groups. One group received saline injections (ip) for nine consecutive days and then 2.0 g/kg EtOH on day 10. The remaining mice received 2.0 g/kg EtOH on day 1 followed by eight daily injections of 2.25 g/kg EtOH, and then 2.0 g/kg EtOH on day 10. All mice were tested for locomotor activity in an open field (15 min) 5 min after injection on day 1 and day 10. Following the final activity test, all mice were evaluated for EtOH intake for 3 weeks. Results indicated that 7TNJ mice failed to exhibit an exaggerated locomotor stimulant response to acute EtOH in comparison to saline, but developed locomotor sensitization following repeated EtOH injections (sensitization was greater in males than females). 1TNH mice displayed a depressant response to acute EtOH in comparison to saline, but an increased activity response following repeated EtOH injections (blunted in females compared to males). Male and female C57 mice showed no change in activity after acute EtOH, but demonstrated locomotor sensitization after repeated EtOH injections. Prior EtOH intake did not alter locomotor activity response to acute or repeated EtOH treatment. Overall, C57 mice consumed significantly more EtOH than 7TNJ and 1TNH mice, with the 7TNJ mutants showing a small trend toward greater drinking in comparison to the 1TNH genotype. Prior expression of sensitization to EtOH's effects on locomotor activity, or a history of EtOH drinking before locomotor sensitization did not influence subsequent EtOH intake in male and female mice of all genotypes. Supported by NIAAA grant AA014095 and NIMH grant MH061971.

ETHANOL MEDIATION OF STRESS RESPONSE IN LXS RI MICE: BEHAVIORAL AND QTL FINDINGS

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We have examined how ethanol affects behavioral response to restraint stress in mice from the LXS RI panel. Mice were either restrained or not and then given saline or 1.8 g/kg ethanol, i.p. Anxiety-related behavior in the elevated zero-maze was then evaluated. With the exception of a few RI strains, ethanol-treated animals displayed decreased anxiety-related behavior in the elevated zero-maze regardless of whether or not they were exposed to the stressor. Strains that showed lower baseline anxiety-related behavior appeared less sensitive to the effects of ethanol. While the behavioral results suggest general ethanol anxiolysis, a preliminary genome-wide scan suggests that the effects of ethanol on these behaviors are likely affected by a number of different quantitative trait loci. For example, suggestive QTL were identified on Chromosomes 8, 14, and 17 for the percentage of time spent in the open areas of the zero-maze in animals that were restrained and given saline whereas suggestive QTL were identified on Chromosomes 2,5,16, and 19 for the same variable in animals that were restrained and treated with ethanol. QTL were identified on Chrs. 13, 14, and 19 for animals not restrained, but ethanol treated and on Chrs. 13 and 15 for animals not restrained, but saline treated. These preliminary results suggest anxiolytic effects of alcohol and behavioral responses that likely involve complex genetic mechanisms. These studies are ongoing.

MICROARRAY ANALYSIS OF THE BED NUCLEUS OF THE STRIA TERMINALIS FOLLOWING CHRONIC EXPOSURE TO ETHANOL

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Neuroadaptations produced by chronic exposure to alcohol are hypothesized to play important roles in the concomitant alterations in behavior, in particular increased alcohol drinking. One region that has been suggested to be critical in the development of this behavioral adaptation is the bed nucleus of the stria terminalis (BNST). In order to begin to understand the changes that occur in this region following ethanol exposure, we have performed microarray analysis on punches obtained from the dorsal anterolateral BNST following chronic ethanol exposure in mice. The results obtained indicate robust alterations in expression of genes following chronic intermittent ethanol exposure. In particular, we observed significant up-regulation of several transcripts related to inhibitory synaptic transmission; specifically GAD67 and the GABAA receptor alpha 2 subunit. In order to examine the impact of withdrawal on the alterations in gene expression in the BNST, we have performed similar experiments in animals that have been exposed to chronic continuous ethanol. Further, in order to examine the temporal regulation of gene expression following ethanol exposure we have obtained samples from animals 4 days following removal from ethanol exposure. Future analysis will focus on comparing these treatment conditions in order to identify molecules and pathways that are differentially involved in withdrawal.

CHRONIC INTERMITTENT ETHANOL EXPOSURE LEADS TO DIVERGENT CONTROL OF CATECHOLAMINE SYNAPSES IN DISTINCT TARGET REGIONS

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Neuroadaptations produced by chronic exposure to alcohol are hypothesized to play important roles in the concomitant alterations in behavior, in particular increased alcohol drinking. Dopaminergic signaling plays a key role in reward-related behavior, with evidence suggesting it undergoes modification following exposure to drugs of abuse. Using a model of chronic intermittent ethanol (CIE) exposure in mice, we have begun to investigate the effects of alcohol intake on dopaminergic signaling by examining levels of catecholamine and dopamine synapse proteins in target fields of heavy dopamine innervation, the anterolateral bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAc) and dorsal striatum. Following alcohol exposure, animals were killed, and tissue punches collected from the BNST, NAc and striatum for Western and neurochemical analysis. Strikingly, we found divergent regulation of tyrosine hydroxylase (TH) protein levels across these three regions. CIE exposure produced a clear decrease in TH levels in the dorsal anterolateral BNST, while having no significant effect on TH levels in NAc or striatum. Because TH is present in multiple classes of catecholamine terminals, we also performed Western blot analysis for the dopamine transporter (DAT) in these regions. Consistent with the decrease in TH levels in the BNST, we also observed a clear trend for a decrease in DAT levels in this region. DAT levels were dramatically elevated in the NAc, and unaffected in the striatum, reinforcing the idea of divergent dysregulation of DA transmission following ethanol exposure. In total, these data suggest that CIE divergently regulated dopamine transmission in different target structures.

NOREPINEPHRINE PRODUCES AN α1 ADRENERGIC RECEPTOR DEPENDENT LONG TERM DEPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Anxiety and alcoholism have a complex relationship. Although often reported as comorbid pathologies, anxiety is often a predictor for relapse in abstinent alcoholics. The bed nucleus of the stria terminalis (BNST) is a component of the extended amygdala implicated in models of anxiety and addition. The BNST is anatomically situated to integrate stress and reward pathways and in addition receives an extremely dense adrenergic projection. Disrupting adrenergic transmission in the BNST, using α2 adrenergic receptor agonists and β adrenergic receptor antagonists, can attenuate relapse in animal models of stress-induced relapse to drug seeking. Furthermore, α1 adrenergic receptors (α1-AR) in the BNST have been identified to play a role in stress induced anxiety and the regulation of stress hormones. Very recent evidence has demonstrated in an animal model of ethanol dependence that administration of α1-AR antagonists during the withdrawal phase attenuates relapse to ethanol self-administration (Walker et al., Abstract Viewer, The Society for Neuroscience, Atlanta, GA, 2006).

A previous study in our lab characterized the modulatory effect of α2 and β adrenergic receptors on glutamatergic synaptic transmission in the BNST using a 10 minute bath application of norepinephrine (NE). Here we show that a 20 minute application of NE results in a long term depression (LTD) of excitatory transmission in the BNST. This LTD is dependent on α1 adrenergic receptors, and can be mimicked by the α1 specific agonist methoxamine. α1-AR dependent LTD has been previously reported in other brain regions, however, unlike previous studies the α1-AR LTD in the dlBNST expresses independently of NMDA receptor activation and concurrent glutamatergic input. Further experiments demonstrated that expression of the α1-AR LTD in the BNST occludes metabatropic glutamate receptor type 5 (mGluR5) LTD in the same region. Our lab is utilizing a chronic intermittent ethanol exposure paradigm that produces enhanced ethanol drinking and heightened anxiety-like responses in behavioral tasks to test whether this α1-AR LTD is engaged and/or modified by repeated ethanol exposure.

ONTOLOGICAL DISCOVERY ENVIRONMENT: A WEB-BASED TOOL FOR INTEGRATING GENE-CENTERED ALCOHOL RESEARCH ACROSS MULTIPLE SPECIES

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High-throughput genomic technologies have enabled researchers to rapidly ascribe large sets of genes to alcohol related phenomena in a variety of sequenced organisms. Now commonly used techniques including mutant analysis, microarray analysis, and even gene-centric literature analysis have all been used to identify sets, networks and pathways of genes and gene products associated with the various behavioral and neurobiological constructs of alcohol effects and alcohol addiction. Integration of these diverse data is performed both within species and across species via orthologous genes to identify generalizable and robust gene-phenotype associations. The Ontological Discovery Environment is a new, Web-based tool for community submission and integrative analysis of phenotype-centered gene sets, developed specifically to address questions pertinent to alcohol research. Submitted gene sets can be compared to existing gene sets for similarity, intersection and overlap. Combinatorial algorithms are applied to the entire database of submitted gene sets to construct a map of the phenome space, an inferred ontology of the alcohol phenome. This approach allows the comparison of animal models for aspects of human alcoholism, examination of the overlap of stress, alcoholism, anxiety, and depression, and identification of the molecular substrates of these relationships. The integration of gene-phenotype associations can be used to address a variety of research questions around the biological substrates that are common or unique to sets of alcohol related phenotypes.

STANDARDIZED PARADIGM FOR INDUCTION OF ANTICIPATORY ANXIETY DURING FMRI

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Alcohol-dependent patients exhibit a muted hypothalamic-pituitary-adrenal (HPA) axis response to pharmacological and environmental stressors. Two key brain regions involved in the stress response, the hippocampus and amygdala, have a high concentration of glucocorticoid (GC) receptors; accordingly, a blunted GC response may impair the central nervous systems' response to stressors, thereby increasing relapse risk. This study sought to establish a reliable paradigm for the exploration of anticipatory anxiety and the limbic / HPA axis system interaction in alcohol-dependent patients using functional magnetic resonance imaging (fMRI). A modified version of the paradigm described by Ploghaus et al. (1999) was used.

During fMRI scanning, a visual conditioned stimulus (CS) signaled the impending application of either a low heat (LH) or high heat (HH) by a thermal stressor (unconditional stimulus, US). A triangle CS was followed by LH; a square CS was followed by either LH or HH. Subjects rated their anxiety from 1 to 4 during initial CS presentation (4 s). The CS then continued (6 to 14 s) prior to application of the US, followed by concurrent CS and US application (8 s). Each CS-US sequence took 18 to 26 s, and was separated from the next sequence by a baseline circle (9 - 11 s). Each session consisted of 42 trials over 22 minutes. Plasma ACTH, cortisol, and neurosteroids were obtained prior to and following the session. Prior to scanning, maximal tolerable heat thresholds were determined for each subject and a mock scan was performed to train subjects in the paradigm, condition behavioral responses, and acclimate subjects to the fMRI environment.

Thus far, 6 subjects have been studied (4 controls, 2 abstinent alcohol-dependent). Subjects rated their anxiety as 3.12 ± 0.24 (mean±SD) following presentation of the square compared to 1.51 ± 0.81 (mean±SD) following presentation of the triangle (square vs. triangle $p<0.005$). This paradigm demonstrates the feasibility of reliably inducing anticipatory anxiety in an fMRI setting, allowing concurrent assessment of neural processes.

Reference: Ploghaus et al. *Science*. 1999;284:1979-81.

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HYPOCRETIN EFFECTS ON LOSS OF RIGHTING DUE TO ETHANOL (LORE)

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Several quantitative trait loci (QTL) for Loss of Righting due to Ethanol (LORE) have been identified and confirmed. One QTL, *Lore4*, maps to a region on mouse chromosome 11. We report here evidence for and against hypocretin (*Hcrt*) as a candidate gene for the *Lore4* QTL. Hypocretin (orexin) is a peptide produced almost exclusively in the lateral hypothalamus. Orexinergic neurons project profusely throughout the brain, including regions known to play a role in drug addiction. While orexin is best characterized for its' role in both feeding and sleep/arousal, more recently it has been implicated in the actions of several drugs of abuse, including barbiturates, morphine, amphetamine and cocaine. Using Affymetrix microarrays, we found that *Hcrt* has differential baseline expression in the ventral tegmental area (VTA) in Inbred Long-Sleep (ILS) and Inbred Short-Sleep (ISS) mice (ILS > ISS), selectively bred for susceptibility and resistance to the hypnotic effect of ethanol. In addition, congenic mice that have a *Lore4* QTL region from ILS introgressed onto an ISS background had greater expression of *Hcrt* compared to ISS controls. These expression differences were verified in VTA and hypothalamus by q-PCR and northern blot analysis. Immunohistochemistry showed ILS had a greater number of orexin-positive neurons than ISS in hypothalamus. However, behavioral data do not support *Hcrt* as the *Lore4* QTL. *Hcrt* knockout mice showed a LORE duration similar to that observed in wildtype controls. Intracerebroventricular (ICV) administration of either Orexin A or Orexin B into third ventricle decreased LORE duration in both ILS and ISS; Orexin administration by itself had little effect on activity in either strain. Systemic injection of SB334867, an orexin receptor antagonist, increased LORE duration in both ILS and ISS. Thus, while manipulation of the orexin system has effects on LORE, these effects are similar in ILS and ISS and do not support *Hcrt* mediating differential sensitivity in ILS and ISS. Preliminary DNA sequencing shows no ILS/ISS polymorphisms in either of the two *Hcrt* exons or a known upstream regulatory element; additional upstream sequencing is ongoing.

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ANALYSIS OF GENE EXPRESSION PROFILE UNDERLYING ETHANOL DEPRIVATION EFFECT IN C57BL/6 MICE.

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The ethanol deprivation effect (EDE) is a widely used animal model of ethanol craving. Renewed access to ethanol solutions after a period of deprivation for several days/weeks leads to a pronounced, although temporary, increase in voluntary ethanol intake. While the EDE is widely studied in rats, the molecular mechanisms underlying EDE are not clearly understood. Recently, we characterized a simple behavioral protocol that rapidly and reliably induced EDE in C57BL/6 mice (Khisti et al., Alcohol 2007, *In Press*). In the present study, we studied genomic changes in medial prefrontal cortex (mPFC), ventral tegmental area (VTA) and nucleus accumbens (NAC), brain areas involved in the mesolimbic reward pathway, during EDE in mice. Briefly, adult male C57BL/6NCrl mice (n = 36) were presented at the beginning of dark phase with 2-tubes containing either 10 % w/v ethanol or tap water for 18hrs/day, as well as food *ad libitum*. Following establishment of stable baseline drinking for 13 days, mice were deprived of ethanol for 4 days. To study genomic changes following EDE, mice (n = 18) were sacrificed at 96-hrs after ethanol deprivation and brain regions dissected. Ethanol deprivation-induced change in ethanol drinking was studied in the remaining mice (n = 18) by reinstating availability of ethanol (10 % w/v). A robust EDE was observed after reinstatement of ethanol, as evidenced by 1.48 fold increases in ethanol consumption ($p<0.001$ vs. prior to EDE) and 1.73 fold increases in % ethanol preference ($p<0.01$). Oligonucleotide microarrays (MG430_A2, Affymetrix) were used to characterize patterns of gene expression in three brain regions of the mesolimbic reward pathway. Using a stepwise method for microarray analysis, we identified genes differentially expressed in control versus ethanol deprived mice in the PFC, VTA and NAC. Pathway analysis of differentially expressed genes in NAC and VTA identified over-representation of G-protein coupled receptor signaling, IGF-1 signaling, insulin receptor signaling and ERK/MAPK signaling. Analysis of significant genes from mPFC revealed important over-represented signaling pathways. In conclusion, this study identifies important molecular pathways involved in the ethanol deprivation effect. Simultaneous genomic and behavioral analysis during EDE provides potential therapeutic targets for intervention in ethanol craving. *Supported by NIAAA Grant RO1 AA014717 to MFM.*

DEFINING ROBUST ACUTE ETHANOL-RESPONSIVE GENE NETWORKS BY EXPRESSION PROFILING ACROSS RECOMBINANT INBRED PANELS

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We previously used expression profiling to identify genes differentially regulated by acute ethanol in C57BL/6 (B6) and DBA/2 (D2) mice, two strains with divergent responses for numerous ethanol behaviors. However, correlating gene networks to behaviors by using only two strains is prone to significant false positives. Genetic influences on acute ethanol behavioral responses have been studied using recombinant inbred panels (RI) of mice, particularly the BXD RI lines derived from B6 and D2 progenitor strains. To define gene networks having causal relationships with ethanol behaviors, we initiated microarray studies on brain regions of the mesolimbocortical dopamine pathway across 27 BXD strains following treatment with saline or ethanol. Initial work was focused on prefrontal cortex (PFC). To increase the stringency of our analysis, we compared these BXD results with our recent work on PFC across 42 lines of the LXS RI panel. We expected to identify robust ethanol-responsive gene networks, map common chromosomal regions (expression QTLs, eQTLs) controlling such networks and correlate expression networks with acute behavioral responses to ethanol. Brain tissue was harvested 4 hours after injection of saline or ethanol (1.8 g/kg) and processed for microarray analysis using Affymetirx 430 type 2 oligonucleotide arrays. A limited number of genes showed consistent responses to ethanol across all 69 mouse lines, suggesting strong genetic influences on most ethanol-responsive gene expression. Several large clusters of ethanol-responsive genes showed eQTLs in common chromosomal regions and some of these overlapped between the BXD and LXS panels (e.g. distal Chr 7). Strikingly, many genes showing linkage to the same chromosomal region differed between the two RI panels but there was strong conservation of gene network function linked to the eQTL site. This suggests conservation of the functional outcome of ethanol-responsive gene expression, despite divergence in individual gene responses across the two RI panels. These studies may provide novel insight into mechanisms and functions of gene expression responses to acute ethanol across the mesolimbocortical dopamine pathway. *Supported by NIH Grants RO1 AA13678 and RO1 AA014717 to MFM.*

GENETICAL GENOMICS ANALYSIS OF ANXIOLYTIC RESPONSES TO ETHANOL IN BXD RECOMBINANT INBRED MICE.

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Individual differences in acute behavioral responses to ethanol are hypothesized to contribute to the susceptibilty of ethanol abuse and alcoholism. Although there has been a long-standing interest in understanding the relationship between anxiety and ethanol abuse due to high comorbidity and human reports of consuming ethanol to relieve anxiety, little attention has been focused on the anxiolytic response to ethanol in rodent models. The identification of ethanol-induced anxiolysis-like behavioral quantitative trait loci (QTL) will offer an opportunity to elucidate the underlying molecular mechanisms involved in the variance of this trait. Therefore, anxiety-related responses to ethanol were measured across the BXD recombinant inbred panel using the light-dark transition model of anxiety. Animals were restrained for 15 minutes, immediately injected (I.P.) with either 0.9% saline or 1.8 g/kg ethanol and tested in the light-dark box for 10 minutes. QTL analysis has identified potential genetic loci associated with various anxiety-related behaviors. Saline-control anxiety QTL identified include suggestive QTL on chromosome 1, 11, 13, 17 while a highly significant QTL on chromosome 12 appears to control the susceptibility to ethanol-induced anxiolysis. These QTL are consistent across multiple anxiety-related behaviors including percent time spent in the light and percent distance traveled in the light. In addition, identification of the ethanol-induced anxiolysis QTL on chromosome 12 was replicated using independently derived BXD lines. Further analyses of these QTL included the identification of gene expression patterns in the prefrontal cortex across BXD recombinant inbred lines using Affymetrix oligonucleotide microarrays. The combination of expression profiling and genetic marker mapping allowed us to correlate ethanol-responsive expression patterns with behavioral QTL data. This genetical genomic approach has yielded a number of candidate genes that may control select ethanol expression networks and the ethanol-induced anxiolysis phenotype. *Supported by NIAAA Grants RO1 AA014717 to MFM and F31 AA016052 to AHP.*

BRIEF REPEATED SOCIAL DEFEAT ALTERS ETHANOL DRINKING PATTERNS IN C57BL/6 MICE

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Humans have long reported increased drinking following stressful periods. Stress has been shown to increase ethanol drinking in a number of behavioral models, although not in all studies. Repeated social defeat (RSD) is an ethologically relevant model of stress for rodents representing situations the animal would normally encounter in its natural environment and for which it may have developed evolutionary defenses. RSD has been shown to alter neurochemical measures of anxiety, increase cardiac and adrenocortical responsiveness as well as increase ethanol drinking in low preference mice. This model, however, has also been shown to decrease or have no effect on ethanol drinking in rodents. Despite a number of reports looking at the effects of social stress on ethanol drinking, direct study of the molecular networks involved in this behavior has not been investigated. We are interested in determining the molecular pathways activated by social stress which contribute to drinking behaviors. The current experiments were designed to test the effects of RSD on ethanol drinking. Male C57BL/6 mice were given voluntary access to 10% (w/v) ethanol and tap water in a 2 bottle choice paradigm. Following stable drinking patterns, mice were exposed to brief social defeat by an aggressive male conspecific or briefly exposed to a clean cage as control for 5 consecutive days. In one study, mice had no access to ethanol during RSD but access was resumed for two weeks following defeat. In this study, ethanol drinking levels decreased in the defeated mice at 2 days up to 2 weeks upon ethanol reinstatement. However, mice with initial low drinking levels increased their ethanol intake following social defeat. These results suggest that the level of ethanol drinking prior to social stress may be a key determinant for drinking responses following stress. Additional experiments investigating the effect of RSD with concurrent ethanol access will be presented. Brain regions from individual mice subjected to social defeat and voluntary ethanol drinking will then be analyzed using Affymetrix microarrays to identify gene networks contributing to social stress modulation of ethanol drinking. *Supported by NIAAA Grant R01 AA14717 to MFM.*