INTEGRATIVE NEUROSCIENCE INITIATIVE ON ALCOHOLISM
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"INIA: STRESS, ANXIETY AND ALCOHOL ABUSE"

Abstracts

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Maryland

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PRENATAL ETHANOL EXPOSURE DISRUPTS SUBSEQUENT HABIT FORMATION IN ADULT MICE
C.M. Gremel, V.C. Carlson, D.M. Lovinger
Section on In Vivo Neural Function, Laboratory for Integrative Neuroscience, NIAAA, NIH, Bethesda, MD 20852

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Section on Behavioral Science and Genetics, Laboratory for Integrative Neuroscience, National Institute on Alcoholism and Alcohol Abuse, NIH, Rockville, MD

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Oregon

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Charleston Alcohol Research Center, Medical University of South Carolina and Department of Veterans Affairs Medical Center, Charleston, SC 29425

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W.C. Griffin III, C.L. Hazelbaker, H. Haun, V. Ramachandra, H.C. Becker
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2University of Tennessee, Memphis, TN 3Virginia Commonwealth University, Richmond, VA

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Virginia

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Canada

0745

COMPARISON OF THE RELATIONSHIP BETWEEN ETHANOL-RELATED, SLEEP AND ANXIETY PHENOTYPES USING A BIOINFORMATIC APPROACH IN BXD MICE

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Alcohol addiction, sleep disturbances, and anxiety disorders are complex interrelated phenotypes. However, the degrees to which these phenotypes share common genetic underpinnings are currently unknown. As part of a large phenotyping project (Philip et al., Genes Brain Behav., 2010) we tested an expanded panel of BXD recombinant inbred lines of mice that were generated from C57BL/6J and DBA/2J parental strains. As many as 55–60 strains were tested for a range of alcohol-related, sleep and anxiety-related phenotypes with separate groups of mice tested for the different phenotypes. Both males and females were tested allowing for the analysis of sex-specific differences. A number of alcohol-related phenotypes were examined including alcohol-induced locomotor activation in an activity chamber, alcohol-induced anxiolysis in an elevated plus maze, and alcohol-induced motor incoordination on an accelerating rotarod. Anxiety measures included evaluation in an elevated plus maze following saline and analysis of morphological parameters in the adrenal gland. Sleep phenotypes were assessed in a noninvasive manner using the Piezo system and included percent sleep time, length of sleep bouts and time of peak activity. Identification of the QTLs (quantitative trait loci) that controlled each phenotype were assessed using GeneNetwork.org. QTLs were compared to determine if similar regions were identified among these phenotypes. For the sleep and alcohol-related phenotypes, there was little overlap in the QTLs. Comparisons between the anxiety-related and alcohol-related QTLs showed more overlap providing evidence of some genetic relatedness. Correlations between phenotypes provided another means of assessing genetic relatedness among traits and was also conducted using GeneNetwork.org. Several significant correlations were found among different types of traits such as those between ethanol-induced locomotor activity and several anxiety measures, and a positive correlation between sleep time and time in the closed arms of the elevated plus maze. Interestingly, sex-specific relationships were also found including a correlation between adrenal weight and percent sleep time in females. These phenotypic correlations provide evidence of the interrelatedness among these phenotypes.

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Maryland

004 - Symposium

PRENATAL ETHANOL EXPOSURE DISRUPTS SUBSEQUENT HABIT FORMATION IN ADULT MICE

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Persistent drinking during pregnancy often results in prenatal ethanol exposure-induced changes in fetal brain development processes that can contribute to learning deficits in adulthood. Our preliminary work suggests that prenatal ethanol exposure alters cannabinoidergic transmission and GABAergic synaptic transmission in the dorsal striatum. Interestingly, the dorsal medial striatum (DMS) is necessary for goal-directed actions, while the dorsal lateral striatum (DLS) and CB1 receptors are necessary for habit formation (Yin et al., 2004; 2005; Hilario et al., 2009). In the current experiments, we examined whether prenatal ethanol exposure would alter goal-directed or habitual behaviors. C57Bl/6J mice were either exposed to air or ethanol vapor throughout gestation and through P10 yielding blood ethanol concentrations of ~80 mg/dl. In adulthood, we trained the same mouse to make the same self-initiated lever press for the same reward using both goal-directed and habitual learning strategies differentiated by context. Importantly, we probed why the mouse pressed the lever during devaluation testing. We found that control mice would be goal-directed or habitual depending on the training schedule/context, while prenatal ethanol-exposed mice were goal-directed in both training contexts. Since different dorsal striatal regions have been implicated in learning and executing actions, we used multisite single unit recordings to simultaneously record neuronal activity in DMS and DLS in the same animal as it performed a similar action on an identical manipulandum in a goal-directed versus habitual manner. We observed that prenatal ethanol exposure differentially altered action-related activity in striatal circuits during learning, and prevented a shift in action-related activity that normally occurred during performance of goal-directed actions and habits. During devaluation testing, we found dramatic shifts in the activity of the same neurons when control mice executed goal-directed versus habitual actions, with the magnitude of shift correlating with degree of goal-directed behavior. This shift was not observed in prenatal ethanol-exposed mice. These results suggest that prenatal ethanol exposure disrupts subsequent habit formation by disrupting functional pathways in the dorsal striatum that are necessary for shifting between goal directed and habitual actions that underlie flexible and efficient decision making.

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CHRONIC ALCOHOL VAPOR EXPOSURE ALTERS EXECUTIVE FUNCTIONS AND CORTICO-STRIATAL NEURONAL MORPHOLOGY IN MICE.

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Abuse of alcohol is thought to impact cognitive and executive functions and produce functional shifts in the neural systems mediating these processes. The objective of the current study was to assess the consequences of chronic ethanol exposure for various corticostriatal mediated forms of learning and executive function, and for corticostriatal dendritic morphology. Male C57BL/6J mice were exposed to either eight or sixteen rounds of ethanol vapor (one round = sixteen continuous hours of exposure, producing BECs of 175 ±25 mg/dL). Three days after the final exposure, mice were tested for either (i) pairwise visual discrimination learning, (ii) visual reversal learning, (iii) Pavlovian-instrumental transfer, or (iv) cued reinstatement of a visual stimulus-response. In additional cohorts, protein expression of NMDAR subunits in dorsolateral striatum and ventromedial prefrontal cortex was measured via Western blot, and dendritic morphology and spine density was quantified via Golgi-Cox impregnation in neurons in dorsolateral striatum, orbitofrontal cortex and prelimbic cortex. Results show that eight rounds of ethanol vapor exposure facilitated reversal (but not discrimination) learning and enhanced Pavlovian-instrumental transfer. These behavioral changes were paralleled by a significant increase in dendritic material in dorsolateral striatal, but not prelimbic cortical, neurons. These data demonstrate that this form of ethanol exposure produces profound and selective changes in cognitive/executive behaviors, possibly via altering the function of cortico-striatal circuits. Research supported by the NIAAA-intramural research program.

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GESTATIONAL ETHANOL EXPOSURE CAUSES DYNAMIC CHANGES IN GABAERGIC NEUROTRANSMISSION OF THE DORSAL STRIATUM IN THE ADULT MOUSE

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Ethanol (EtOH) abuse during pregnancy can lead to birth defects and behavioral disabilities, such as motor dysfunction, hyperactivity, and learning and memory deficits, collectively known as fetal alcohol spectrum disorder (FASD). Since the dorsal striatum (DS) plays a role in executing movements and associative learning, DS insults may contribute to FASD-associated behavioral abnormalities. We hypothesized that gestational EtOH exposure alters synaptic transmission in the DS, specifically the dorsolateral striatum (DLS), an area important for habit learning. To test this, mice were exposed to EtOH from embryonic day 0.5 to postnatal day 10 yielding blood EtOH concentrations of ~80mg/dl. In the adult offspring, EtOH-induced changes were examined in the GABAergic neurotransmission onto MSNs. Gestational EtOH exposure decreased the frequency and amplitude of mIPSCs, and produced a loss of the inhibitory effects of acute EtOH exposure and CB1-receptor agonist on mIPSC frequency. Immunohistochemistry with an antibody raised against the CB1-receptor and CB1-receptor binding studies suggest slightly higher protein expression and similar number of binding sites, respectively. However, exposure to a CB1-receptor antagonist increased mIPSC frequency, suggesting a gestataional EtOH-induced increase in ambient endocannabinoid levels. Habit formation was impaired in EtOH-exposed mice, without a change in goal-directed task performance, similar to CB1 KO mice. Thus, altered cannabinoidergic transmission or a floo reffect of depressed GABAergic neurotransmission in the DLS could contribute to the observed behavioral changes. These mechanisms and DS subregional differences in synaptic transmission are addressed in ongoing studies. Overall, our findings suggest that gestational EtOH produces impairments in corticostriatal circuits governing habit formation.

0188

CHRONIC ADOLESCENT METYHLPHENIDATE EXPOSURE ALTERS ETHANOL EFFECTS ON ADULT STRIATAL SYNAPTIC PLASTICITY AND DOPAMINE IN MICE

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Ritalin (MPH) is a psychostimulant prescribed during adolescence for the treatment of ADHD. The aim of this study was to elucidate how chronic adolescent administration of MPH affects synaptic transmission and future susceptibility to addiction. C57BL/6J mice were administered 6–9mg/kg MPH in drinking water from PN30–60. Twenty four hours following MPH treatment, mice were used for microdialysis, sacrificed for electrophysiological experiments and confocal imaging, or tested behaviorally. Changes in the dorsolateral striatum (DLS) were assessed, as this subregion has important roles in addiction. For microdialysis experiments, mice were implanted with guide cannulae into the DLS. On testing days, probes were inserted and allowed to equilibrate for one hr, and were continuously superfused with artificial cerebrospinal fluid. Baseline levels of dopamine (DA) were measured every 20 min for two hr, with MPHtreated and control mice showing similar levels. Mice received 0.8 or 1.6 g/kg EtOH (i.p.) and DA levels were measured every 20 min. While control mice showed moderate transient changes in DA levels in response to EtOH, MPH-treated mice showed increased DA levels, persisting for over one hr. Field potential recordings in slices estimate synaptic activation of DLS neurons using the amplitude of the population spike (PS). The DA D2R-dependent high frequency stimulation-induced long-term depression (HFS-LTD) was enhanced in MPH mice as compared to controls. However, when 50mM or 80mM EtOH was applied in combination with HFS, MPH mice showed long-term potentiation while controls showed LTD. These results indicate that chronic adolescent methylphenidate administration causes synaptic changes in the DLS, which alters EtOH actions in this brain region. It appears that DA levels may be elevated in MPH treated mice in response to EtOH challenge. This alteration in DA appears to alter DA-dependent plasticity and EtOH effects on plasticity. These results are consistent with voltammetry data indicating MPH mice have greater striatal DA release. These synaptic changes have implications for addiction. Previously presented behavioral data indicate that MPH treated mice show decreased locomotor effects of various EtOH doses, as compared to controls. Interestingly, they do not show any clear differences in EtOH self-administration. Ongoing experiments are addressing alterations in medium spiny neuron dendritic spine density and operant behavioral measures of impulsivity.

0206

PRETREATMENT WITH D9-TETRAHYDROCANNABINOID INCREASES THE SENSITIVITY OF a1 GLYRS TO ETHANOL-INDUCED POTENTIATION

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Alcohol and marijuana are the most widely used and abused substances in the western world. There is strong clinical evidence showing that concurrent use of both drugs can synergistically suppress brain function. Accumulating evidence has suggested that glycine receptors (GlyRs) are one of the primary targets of alcohol and D9-tetrahydrocannabinol (THC) actions in the brain. Both drugs can potentiate glycine-activated current (IGly). However, the interaction of THC with ethanol (EtOH) to affect GlyR function at cellular level has not been reported. We observed that THC at 1 lM alone increased the amplitude of IGly by 812 % in HEK 293 cells expressing a1GlyRs. IGly reached the peak amplitude after 3–5 min during continuous incubation with THC and returned to the baseline immediately after discontinuation of THC application, indicating that THC potentiation was fully reversible. The magnitude of potentiation induced by EtOH was significantly less than that of THC; EtOH at 100 mM alone enhanced IGly by 48% in cells expressing the a1 GlyRs. However, the magnitude of EtOH-induced potentiation was significantly enhanced by 4-fold (from 48% to 213 %) following THC incubation. Such enhancement lasted for nearly 30 min after discontinuation of THC application. Cytoplasmic application of THC (10 lM) via the patch pipette did not significantly alter the sensitivity of GlyRs to EtOH. This suggests that the interaction between THC and EtOH may not be mediated through an intracellular signaling mechanism. The interaction of THC with EtOH on GlyRs could contribute to some of the THC and alcohol-induced brain and behavioral effects. Supported by the NIAAA intramural research program.

0247

ALTERED ETHANOL RESPONSES IN KNOCK-IN MICE WITH GLYCINE RECEPTORS THAT ARE INSENSTIVE TO GBC MODULATION

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Glycine receptors (GlyRs) are potentiated by low, clinically relevant ethanol concentrations and they play a critical role in neuronal excitability in the mammalian brain stem and spinal cord. GlyRs are also present in higher brain structures. Recently, we reported that GlyRs are modulated by G protein bc subunits via the TM3-4 intracellular loop of a1-containing GlyRs. Importantly, mutants that were resistant to Gbc dimer modulation were insensitive to potentiation by ethanol. To test the physiological relevance of Gbc modulation of GlyRs, we created and characterized gene knock-in mice in which two amino acids in the TM3-4 loop of the a1 subunit were mutated to render the GlyRs insensitive to Gbc modulation. Knock-in mice were viable and overtly normal. Electrophysiological experiments are examining glycinergic synaptic transmission, GlyR function and acute ethanol effects using isolated brainstem neurons from control and knock-in mice. Preliminary patch clamp recordings of glycinemediated IPSCs in 13 to 20-day old hypoglossal neurons revealed no differences between genotypes in basic current properties. Knock-in mice exhibited a mild impairment in ability to perform an accelerating rotarod task. In a novel open field task, knock-in mice showed normal basal ambulatory activity but were significantly more sensitive to the locomotor stimulatory effects of 1.0 g/kg ethanol compared to control littermates. The motor ataxic effects of 2.0 g/kg ethanol did not differ between genotypes on a fixed speed rotarod task. Duration of loss of the righting reflex following 3.5 g/kg ethanol was increased in knock-ins compared to controls. In conclusion, Gbc modulation of GlyRs function is required for normal behavioral responses to ethanol.

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0489

ASSOCIATION OF VARIANTS IN GABA(A) RECEPTOR SUBUNIT GENE CLUSTER ON CHROMOSOME 5 WITH SUBJECTIVE EFFECTS OF ALCOHOL

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Low response to alcohol, a risk factor for alcohol dependence (AD), has a genetic component. Because GABAA receptors mediate behavioral effects of alcohol, genes encoding GABArelated proteins are candidates to influence risk of AD. Markers in the cluster of GABAA receptor genes on chromosome 5q, which includes GABAA a-1 (GABRA1), GABAA a-6 (GABRA6), GABAA b2 (GABRB2), and GABAA c2 (GABRG2) receptor subunit genes, have shown mixed results in association with AD, highlighting the importance of examining the association of these genes with endophenotypes related to the development of AD.
Methods: 69 healthy social drinkers [59% males, 78% Caucasian, 24.2 yrs old (SD=2.6)] participated in an alcohol sensitivity challenge session. Subjects received a placebo drink at time 0, followed by three equal alcohol doses at 45 min intervals, achieving a mean peak alcohol concentration of 99 mg/dL (SD=18). The Biphasic Alcohol Effects Scale (BAES), Subjective High Assessment Scale (SHAS) and Drug Effect Visual Analog Scale (VAS) were completed at baseline, following each alcohol drink and at 45-minute intervals over the 5-hr session. Genotype effects were analyzed using longitudinal analysis by GEE methods. Ancestry was estimated by the Structure 2.3.3 Software and was adjusted for in the association analyses.
Results: Subjects homozygous for the minor allele for SNPs in the GABRG2 and GABRA6 region reported lower alcohol stimulant and sedative effects (BAES) (rs 211029 p = 0.009 and p = 0.01, respectively; rs 169793 p = 0.01 and p = 0.01, respectively; rs 7704209 p = 0.01, sedative) compared to those heterozygous or homozygous for the common allele. In addition, subjects homozygous and heterozygous for the minor allele reported lower SHAS total effects (rs 211029 p = 0.03; rs 169793 p = 0.03) and lower ‘‘bad, dislike and worst’’ VAS effects (rs 211029 p = 0.02; rs 169793 p = 0.01) compared to those homozygous for the common allele.
Conclusion: Our results suggest that variations in the GABAA receptor genes under study modulate subjective responses to alcohol and thus may increase susceptibility to AD. Greater understanding of the role of genetic variation mediating alcohol effects may contribute to the development of genetic approaches in the screening and pharmacological management of alcohol use disorders. [Supported by NIH grants AA10158(GSW), AA12303(GSW), K23AA017466(MU), AA12837(MEM), AA017704 (EMW)].

0490

SEROTONIN TRANSPORTER GENE PROMOTER POLYMORPHISM IS ASSOCIATED WITH CORTISOL RELEASE FOLLOWING NALOXONE CHALLENGE

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BACKGROUND: Hypothalamic-pituitary-adrenal axis dysregulation may increase risk of alcoholism and relapse following alcohol withdrawal. Both the serotonergic and opioid neurotransmitter systems are involved in regulating the HPA axis through excitatory and inhibitory input, respectively, and serotoninergic agents and opioid receptor antagonists are employed to treat alcohol dependence. Our laboratory and others have found that individuals at high risk for problematic drinking show increased cortisol response to naloxone-induced opioid blockade compared to low risk individuals, suggesting that high risk individuals have altered inhibitory opioid tone on the HPA axis. With respect to serotonin, both high and low gene expression variants (L and S bialleles) of the functional serotonin transporter (5HTT) gene promoter polymorphism (5HTTLPR) have been associated with risk of alcohol use disorders. Further study has indicated that a SNP contained on the L allele (a vs. g triallele) is associated with greater 5HTT expression. This study is the first to our knowledge to consider the interactive effect of 5HTT triallele genotype and hypothalamic opioid tone on HPA function.
METHODS: Healthy social drinkers (n = 79; ages 18 to 32; 52 males) received IV placebo followed by sequential doses of IV naloxone (50, 100, 200, and 400 ug/kg) every 30 minutes. Following 4 baseline blood samples, blood was collected every 15 minutes for 2.5 hrs for cortisol measurements. Participants were grouped by triallelic genotype (LaLa vs. LaLg/LaS vs. LgLg/LgS/SS). Cortisol levels were compared using general linear mixed models, with gender and population genetic substructure as covariates.
RESULTS: A significant group by time interaction ($p < 0.05$) demonstrated that the LaLa group had the greatest and the LgLg/LgS/SS group had the lowest cortisol response to naloxone challenge.
CONCLUSIONS: Cortisol response to opioid receptor blockade varied with respect to 5HTT genotype. Expression of La alleles leads to higher cortisol levels when endogenous inhibitory tone is removed. This observation raises the question of whether the polymorphism influences the course of alcoholism and/or influences treatment responses to modifiers of serotonergic activity and/or the opioid receptor antagonist, naltrexone.

North Carolina

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A HISTORY OF BINGE-LIKE ETHANOL DRINKING IN NONDEPENDENT MICE ALTERS THE EFFECTS OF NPY ON GABAERGIC TRANSMISSION

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The central extended amygdala, composed of the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CeA), has been suggested to be a critical substrate for dependence induced alterations in ethanol drinking behavior. However, the impact of binge-like ethanol drinking on synaptic transmission and modulation in these regions has not been studied. Using whole-cell voltage clamp recordings in an ex vivo slice preparation, we investigated the effect of binge-like ethanol drinking on inhibitory transmission in both the CeA and the BNST. We found that exposure to multiple binge episodes caused a significant increase in basal GABAergic transmission, specifically an increased in spontaneous inhibitory postsynaptic currents, in the BNST but not the CeA. Next, given the ability of manipulations of NPY to alter binge-like drinking, we examined the ability of NPY to modulate inhibitory transmission in mice with a history of binge-like ethanol drinking. Interestingly, we found that the ability of NPY to inhibit GABAergic transmission in the CeA was significantly enhanced following a history of binge-like ethanol drinking. Future studies will examine the impact of binge-like ethanol drinking on NPY function in the BNST, as well as the persistence of these adaptations. Our current results support the possibility that excessive binge-like drinking may engage neurochemical systems similar to those seen during dependence and that these systems may be suitable targets for reducing alcohol binge drinking.

0192

ETHANOL AND OPIOID EFFECTS ON GABAERGIC TRANSMISSION IN DOPAMINERGIC CELLS OF THE VENTRAL PERIAQUEDUCTAL GRAY (VPAG)

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Numerous studies have pointed to the important role that endogenous opioid peptides play in regulation of alcohol related behaviors, potentially through modulation of dopamine neuron activity. Most of this effort has focused on the modulation of dopamine neurons in the VTA. However, several recent studies have demonstrated the presence of a population of dopamine (DA) neurons in the ventral periaqueductal gray (vPAG). These vPAG DA neurons are of particular interest for alcohol abuse, as they project to both the bed nucleus of the stria terminalis and central nucleus of the amygdala, regions that have been implicated in behavioral deficits and increased drinking following chronic alcohol exposure. Moreover, chronic exposure to alcohol leads to altered expression of enkephalin and dynorphin, endogenous agonists for mu and kappa opioid receptors respectively. To date, there have been no studies examining the impact of either alcohol or opioids on dopamine neuron function in this brain region. The goal of this study is to characterize the influence of alcohol and opioids on GABAergic activity of vPAG dopamine neurons using a transgenic mouse line that expresses eGFP driven by the tyrosine hydroxylase (TH) promoter. Briefly, we performed whole-cell recordings in eGFP positive neurons from acutely prepared brain slices containing the vPAG, examining the impact of ethanol and opioid receptor agonists on GABAergic transmission. We found that bath application of 50mM ethanol had no effects on either the frequency or amplitude of spontaneous inhibitory postsynaptic currents, nor did it alter evoked inhibitory postsynaptic currents. In contrast, we found that both kappa and mu receptor opioid agonists reduced GABAergic transmission in vPAG dopamine neurons in a dose-dependent manner. Current studies are focused on (i) determining if there is any acute functional interaction between ethanol and opioid receptor signaling on these neurons and (ii) determining the mechanism that underlies both mu and kappa opioid receptor inhibition of GABA transmission. In conclusion, we found that kappa and mu opioid receptor agonists, but not ethanol, altered GABAergic transmission in vPAG DA neurons. These findings support the possibility that PAG DA neurons are differentially regulated than DA neurons located in the VTA.

0193

CHRONIC ETHANOL-INDUCED D1R-MEDIATED POTENTIATION OF LOCAL, BUT NOT LPC GABAERGIC TRANSMISSION IN THE BASOLATERAL AMYGDALA

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Withdrawal-induced anxiety has been shown to be mediating by the basolateral amygdala (BLA). It has been suggested that this anxiety results from a disruption in the balance between BLA glutamate and GABA neurotransmission. Dopaminergic innervation of the BLA can also alter this balance by differentially modulating GABAergic transmission from local feed-forward interneurons and feed-back lateral paracapsular cells (LPCs) within the BLA. Therefore, we hypothesize that chronic ethanol exposure and withdrawal might disrupt the balance between glutamate and GABA by interfering with DA modulation of GABAergic transmission. To test this, animals were exposed to intermittent ethanol vapor inhalation (12hrs on/off) for 10 consecutive days (CIE) or the CIE treatment followed by 24hrs room air alone (WD). Electrophysiological recordings were then performed on BLA principal neurons within brain slices prepared from CIE and WD animals. Preliminary data shows that in control (CON) slices dopamine inhibits GABAergic IPSCs from both local and LPC synapses. However, dopamine potentiates local GABAergic transmission in CIE slices, and this effect is reversed by a D1R antagonist. This facilitatory effect appears to diminish at 24 hours of withdrawal. In contrast, dopamine modulation of GABAergic transmission at LPC synapses was not affected by CIE or WD. These findings suggest a possible role for the BLA dopamine system in WD-induced anxiety. Furthermore, because studies have shown that dopamine facilitates local GABAergic transmission in naive animals younger than our CON group, we are the first to demonstrate a potential functional developmental shift in dopamine modulation of the BLA GABA system.

0194

INPUT SPECIFIC ALTERATIONS OF LATERAL/BASOLATERAL AMYGDALA GLUTAMATE SYNAPTIC TRANSMISSION FOLLOWING CHRONIC INTERMITTENT ETHANOL AND WITHDRAWAL

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The lateral and basolateral amygdala (BLA) are major amygdala subdivisions that process environmental stimuli in an associative process that ultimately results in anxiety-like or fearful behavioral responses. These nuclei receive glutamatergic input via two major pathways differentiated by their anatomical arrangement and the upstream brain areas giving rise to the pathways. Cortical information flows into the BLA via the external capsule (EC) while the stria terminalis/longitudinal association bundle ('medial' input) brings information from more mid-line structures like medial prefrontal cortex and thalamus. Recent work from our lab suggests that the long-term anxiogenic effects of alcohol withdrawal may be related to adaptations in BLA glutamatergic neurotransmission. These studies have indicated that the Lateral and Medial inputs into the BLA can be functionally isolated in a coronal brain slice. This allows us to investigate input specific alterations of glutamate function following both chronic intermittent exposure (CIE) to ethanol vapor and 24 hr withdrawal (WD). Our electrophysiological recordings indicate that the lateral EC glutamatergic input undergoes predominantly postsynaptic alterations in response to CIE and WD treatments. Conversely, these treatments produce only pre-synaptic alterations at the medial input with no change at post-synaptic sites. We also utilized biochemical approaches to more precisely characterize the post-synaptic changes that occur in the BLA following our treatments. Our preliminary data indicate that changes in AMPA receptor phosphorylation/surface expression and changes in total protein and phosphorylation state of several protein kinases may be associated with these postsynaptic alterations. Together, these data detail CIE and WD induced changes in the BLA that likely contribute to long-term increases in anxiety-like behavior following chronic ethanol exposure and highlight potential targets for therapies to reverse these long term effects. This work was supported by NIH grant #AA014445.

0196

CHRONIC STRESS AND BINGE-LIKE ETHANOL DRINKING DIFFERENTIALLY IMPACT NEUROPEPTIDE Y SIGNALING IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Exposure to stress is a major risk factor for various neuropsychiatric conditions, including posttraumatic stress disorder and alcoholism. Chronic alcohol and stress exposure are associated with lasting changes in emotional behaviors, including anxiety, due in part to alterations in function of specific brain regions. In this study, we used slice electrophysiology in male C57BL/6J mice to examine whether long-term binge-like ethanol drinking and repeated restraint stress produce similar alterations in the brain's antistress system, specifically signaling of neuropeptide Y (NPY). We found that three cycles of binge-like ethanol drinking significantly reduced NPY's ability to decrease GABAergic transmission in the bed nucleus of the stria terminalis (BNST), a structure in the extended amygdala that is critical for the production of anxiety-type behaviors. However, ten days of restraint stress did not affect this measure. These data suggest that long-term alcohol use, but not chronic stress, may contribute to anxiogenesis by causing prolonged dysregulation of NPY signaling in pathways involved in anxiety-related behaviors. Histological examination is currently underway to determine whether binge-like ethanol drinking and repeated restraint stress alter overall levels of NPY and expression of its receptors in the BNST. Results may reveal potential targets for the treatment of alcohol and/or stress-induced anxiety.

0213

SYSTEMIC ETHANOL ADMINISTRATION DECREASES GABAERGIC NEUROACTIVE STEROID IMMUNOREACTIVITY IN RAT NUCLEUS ACCUMBENS AND CENTRAL AMYGDALA

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The 5α-reduced pregnane neuroactive steroids (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP or allopregnanolone) and (3α,5α)-3,21-dihydroxypregnan-20-one (3α,5α-THDOC) are potent positive modulators of GABAA receptors capable of modulating neuronal activity. Systemic administration of moderate dose ethanol increases plasma and cerebral cortical levels of these steroids, but the effects of ethanol in limbic brain regions are unknown. 5α-reduced neuroactive steroids enhance ethanol sensitivity and produce some of the behavioral and subjective effects of ethanol. Endogenous and synthetic GABAergic neuroactive steroids also modulate drinking behavior in both operant and free choice drinking paradigms. In the present study, we used immunohistochemistry (IHC) to quantify ethanol-induced changes of cellular 5α-reduced steroids in the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), CA1 hippocampus, central nucleus of the amygdala (CeA), basolateral amygdala (BLA), and dentate gyrus (DG). Male Sprague-Dawley rats (n = 7–8/group) were habituated to handling and injections for 7 days prior to the experiment. Animals were administered ethanol (2g/kg) or saline intraperitoneally and after 60 minutes sacrificed/transcardially perfused. IHC was performed on free floating sections (3–4 sections/animal/brain region) using a purified antibody that labels 5α-reduced pregnane steroids and a biotinylated secondary antibody. Tissue was processed using the avidin biotin complex kit and immunoreactivity was visualized with 3,3'-diaminobenzidine. Ethanol increased 5α-reduced steroid immunoreactivity by 24±6% ($p < 0.01$) in mPFC, 32±12% ($p < 0.03$) in the hippocampal CA1 pyramidal cell layer and 31±6% ($p < 0.001$) in the polymorph cell layer of the DG, similar to changes measured by radioimmunoassay in the cerebral cortex and hippocampus. In contrast, ethanol administration reduced 5α-reduced steroid immunoreactivity by 22±5% ($p < 0.01$) in the NAc and 21±3% ($p < 0.03$) in the CeA. No changes in 5α-reduced steroids were observed in the BLA. Studies are currently in progress to investigate ethanol-induced changes in 5α-reduced steroids in other limbic brain regions. Visualization of brain region specific ethanol-induced changes in 5αreduced steroids may lead to the development of new therapeutic targets for treating alcoholism.

0215

ETHANOL EXPOSURE INCREASES STRIATAL TYROSINE PHOSPHATASE (STEP) ACTIVITY IN CULTURED CEREBRAL CORTICAL NEURONS: EFFECTS ON GABAA RECEPTOR EXPRESSION

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Ethanol exposure is known to increase GABAA a1 receptor endocytosis and a4 subunit receptor surface expression both in vivo and in vitro, but the mechanisms of this regulation remain unclear. Previous studies have shown that the functional activity of GABAA receptors is regulated by protein kinases such as PKC, PKA and Fyn. Further, the actions of ethanol are dependent upon both PKC and PKA activation. The actions of these kinases are countered by phosphatases, but there is no current evidence for ethanol effects on phosphatase expression. Recent evidence shows that STriatal-Enriched protein tyrosine Phosphatase-61 (STEP61) mediates effects of ethanol on NMDA receptor function and surface expression. Therefore, we investigated the possible involvement of STEP61 in ethanol actions on GABAA receptor expression. Cultured cerebral cortical neurons were prepared from rat pups on postnatal day 0–1 and maintained for at least 18 days. Cells were exposed to 50 mM ethanol for 1 or 4 hours and its effects on STEP61 as well as GABAA a1 and a4 subunit expression were assessed following subcellular fractionation. Ethanol increased STEP61 levels in the P2 fraction of cortical neurons after 1 hour by 19 ± 6.9% ($p < 0.03$, $n = 7$) and after 4 hours by 24.5 ± 8.5% ($p < 0.01$, $n = 7$). In contrast, there was no effect of ethanol on the expression level of the serine phosphatase PP1A. To determine if STEP61 mediates the effects of ethanol on GABAA receptor a1 or a4 subunits, we investigated the effect of the STEP construct TAT-STEP46 [C/S] that lacks catalytic activity, but can still bind and trap substrates. Exposure of cultured neurons to TAT-STEP46 [C/S] (2 lM) did not alter basal expression of GABAA a1 or a4 subunits in the P2 fraction. However, preliminary results suggest that TAT-STEP46 [C/S] prevented the effect of ethanol on GABAA a1 subunit expression ($p < 0.03$, $n = 3$). In contrast, ethanol-induced increases in GABAA a4 subunit expression remain unchanged in the presence of TAT-STEP46 [C/S]. These findings suggest that STEP may be involved in ethanol regulation of a1, but not a4 subunit expression in cultured cortical neurons. These studies suggest an important role of tyrosine phosphatase in ethanol actions involving both excitatory and inhibitory synapses. Supported by AA11605 (ALM).

0216

CHRONIC ETHANOL ALTERS GABAA RECEPTOR AND ALPHA SUBUNIT DENSITY IN THE MONKEY SUPERIOR TEMPORAL SULCUS, INFERIOR TEMPORAL GYRUS, AND INSULA

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Chronic ethanol consumption alters GABAergic transmission and has been shown to alter the subunit composition and functional properties of GABAA receptors in some regions of the cerebral cortex. The present study examines the changes in total GABAA and a1 and 4 subunit containing receptors in the superior temporal sulcus (STS), inferior temporal gyrus (ITG), and the insula using a nonhuman primate model of chronic ethanol self-administration. Adult male cynomolgus macaques (n = 4) self-administered ethanol or concurrently available water during 22 hour sessions in their home cage over 18 months. Control monkeys (n = 3) were exposed to the same environment and diet for eight months without an operant panel or ethanol. At necropsy, brains were processed for in vitro receptor autoradiography. The GABAA receptor inverse agonist [3H]RO15-4513 was used to measure total GABAA receptor density; coincubation in the presence of 75nM Zolpidem or 100nM Diazepam allowed us to quantify the densities of the a1 or 4 subunits, respectively. Binding was measured in areas TPO and TEa in the STS, TE in the ITG, and at two levels of the dysgranular insula. Two-way ANOVA revealed no differences in TPO, but a main effect of decreased a4 subunit-containing receptors was seen in TEa in ethanol drinkers. There was a main effect of decreased total GABAA binding as well as a4 subunit-containing receptors in ethanol drinkers in TE. Changes in the insula depended on the rostrocaudal level of the section. In the insula, a1 subunit containing receptor binding density was lower in ethanol drinkers. The insula was also analyzed at two separate cortical levels. At the more rostral level, a1 subunit-containing receptor binding density was lower in ethanol drinkers. At the caudal level, a4 receptor binding density was higher in ethanol drinkers. The upper bank of the STS is involved in multimodal processing. TE is involved with higher level visual object recognition. The insula plays a role in processing interoceptive information and has been implicated in addiction. Changes in both phasic and tonic GABAergic tone in the insula would alter information processing that allows physiological states to be related to drug-associated reward. Overall, this study indicates regionally specific differences in GABAA receptor binding density.

0218

EFFECTS OF D2 TYPE AUTORECEPTOR ANTAGONISTS ON ETHANOL-INDUCED MODULATION OF DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS

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In general, microdialysis studies have provided compelling evidence that acute ethanol enhances dopamine (DA) neurotransmission in the mesolimbic DA system originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAcc) and other limbic structures. However, using fast scan cyclic voltammetry (FSCV), another story has emerged, demonstrating that acute ethanol decreases DA signals in the NAcc (Jones et al., 2006). There are profound temporal and spatial differences between microdialysis and FSCV, with FSCV providing real-time measurements from more focal sources. Moreover, FSCV yields separable kinetic data regarding the temporal aspects of evoked DA release and subsequent uptake. It has been suggested that presynaptic DA autoreceptors may be activated by the accumulation of DA in the NAcc, and inhibit electrically stimulated DA release (Jones et al., 2006), which may partially explain why ethanol decreases evoked DA amplitudes. We evaluated the effects of D2-type autoreceptor antagonists on ethanol-induced modulation of DA release and uptake in the NAcc. Using FSCV in isoflurane-anesthetized mice and horizontal brain slices in vitro, we evoked DA signals in the shell of the NAcc by electrical stimulation of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (60 Hz, 24 pulses) or by local stimulation in the slice (1–10 pulses). Intraperitoneal (IP) administration of ethanol (1.0–3.0 g/kg) dose-dependently decreased the amplitude of the MFB-evoked NAcc DA signal. IP administration of the D2 antagonist eticlopride (1 mg/kg) markedly increased (250%) the amplitude of the evoked DA signal. When ethanol was administered after eticlopride it increased the amplitude of the DA signal an additional 42%. Application of ethanol to the slice preparation (20–60 mM) decreased DA release in the shell without affecting uptake kinetics, while D2 antagonists had no effect on DA release. As proposed by Jones et al, these findings suggest that ethanol induced decreases in evoked DA release may be due to autoreceptor feedback. However, the site of action is not in the shell, and may be in the VTA. Work is in progress to further characterize these effects and to explore the role of midbrain GABA neurons and D2 receptors in regulating DA release in the NAcc, as we have recently demonstrated that D2 antagonists block ethanol suppression of VTA GABA firing rate. This work is supported by PHS grant AA13666 to SCS and AA014091 to SRJ.

0220

SOCIAL ISOLATION REARING CHANGES DOPAMINE KINETICS

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Alcohol addiction is a chronic relapsing disease which can lead to devastating physical, psychological, and social consequences. Although the mechanisms underlying the reinforcing effects of ethanol are not well-understood, there is extensive evidence suggesting that these effects may involve the mesolimbic dopamine (DA) system. For example, acute ethanol increases DA levels throughout the brain, an effect that is common to psychostimulants and other drugs of abuse. Additionally, pharmacological manipulations that alter DA neurotransmission produce robust alterations in ethanol seeking behaviors. Rats reared under socially isolated (SI) conditions, a model of adverse early life events, show a variety of ethanol-related behavioral and neurochemical alterations including increased ethanol consumption and ethanol seeking during extinction. Additionally, SI animals show elevated DA neurotransmission in a variety of neural structures, including the nucleus accumbens (NAc). However, it remains to be elucidated as to whether presynaptic DA function is altered in these animals. In the present studies, we examined differences in presynaptic DA transmission between SI and group housed (GH) animals, under baseline conditions and in response to ethanol administration. Animals were GH 4 rats per cage or SI at postnatal day 21. After six weeks, rats were tested in an elevated plus-maze and response to novelty assay and, immediately following these behavioral procedures, all subjects were single housed and ethanol drinking was assessed. SI rats exhibited a significant increase in open arm exploration time on the elevated-plus maze as well as a greater response to a novel object. In addition, SI rats drank significantly more ethanol in the intermittent two-bottle choice procedure (20% ethanol/water available 3 days/week). At the end of the drinking period, NAc DA release and uptake were monitored in SI and GH rats using fast scan cyclic voltammetry (voltammetry). Brain slices containing the NAc were electrically stimulated (1 pulse) while performing local voltammetric recordings. SI animals showed increased electrically evoked DA release, as well as increased maximal rate of uptake (Vmax) for baseline measurements. When examined for changes in autoreceptor activity, SI and GH animals showed no significant difference in D2 sensitivity. SI rearing appears to alter DA transmission, which could be important for the increased consumption observed in these animals.

0223

CHRONIC INTERMITTENT ETHANOL INDUCES KAPPA OPIOID RECEPTOR SUPERSENSITIVITY AND REVERSES DOPAMINE RESPONSE TO ACUTE CHALLENGE

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Despite a large body of work examining the effects of alcohol on the central nervous system, the mechanism(s) underlying alcohol addiction remain unknown. Evidence from human studies suggests that ethanol consumption is often increased after stressful events, and the increase may be due to a combination of the anxiolytic and rewarding effects of ethanol. Becker and colleagues have shown increased drinking in mice following a chronic intermittent ethanol vapor exposure with repeated withdrawal periods, a paradigm which allows the examination of the neurobiological consequences of chronic ethanol as well as the stress of withdrawal. Ethanol, along with all other abused substances, activates the mesolimbic dopamine system, and systemic administration of ethanol increases extracellular dopamine levels in the nucleus accumbens of rats and mice following acute administration. Currently, it remains unclear how the dopamine system responds to acute ethanol challenge following chronic ethanol exposure. The current studies were designed to explicitly examine whether acute ethanol challenge elicits differential dopamine responses in control versus ethanol exposed mice. Studies were conducted using either 1 or 3 cycles of vapor exposure along with microdialysis in freely moving mice and voltammetry in NAc brain slices. A cycle of ethanol vapor exposure consists of 4 days of 16 hour exposure to volatilized ethanol in a chamber followed by 3 days of withdrawal. Following 1 cycle of exposure, the controls showed normal DA-elevating responses to acute ethanol (2 g/kg i.p.), but ethanol vapor-exposed mice had blunted responses, with very little increase from baseline. In contrast, following 3 cycles of exposure, control animals also showed a normal elevation of DA response to acute ethanol (2 g/kg i.p.), whereas ethanol vapor-exposed mice showed a reduction in response to below baseline levels. Voltammetry studies revealed no differences in release or uptake under baseline conditions between the ethanol and air exposed controls at either one or three cycles. Interestingly, three cycles of ethanol exposure resulted in a unexpected supersensitivity of kappa opioid receptors in the NAc core in ethanol exposed mice. Together results suggest the possibility that the reversal in DA responses to acute ethanol in ethanol exposed mice is regulated by kappa opioid mechanisms. Additional studies are aimed at exploring kappa opioid receptor function in ethanol exposed mice.

0232

BINGE-LIKE ETHANOL CONSUMPTION RECRUITS CRF SIGNALING IN THE CENTRAL NUCLEUS OF THE AMYGDALA

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Binge drinking is a common pattern of alcohol intake that increases the risk of developing alcohol dependence. Mounting preclinical evidence indicates that binge-like (BL) ethanol consumption (i.e., drinking associated with BECs ‡80 mg/dl) recruits the corticotropinreleasing factor (CRF) system. To date, the effects of BL ethanol consumption on the expression and functional activity of CRF are unknown and the specific brain regions in which CRF modulates BL ethanol consumption have not been identified. All experiments used a 4-day drinking-in-the-dark procedure (DID) to study BL ethanol intake in male C57BL/6J mice. In Experiment 1, the effects of BL ethanol consumption on central CRF-immunoreactivity (CRFIR) were assessed. In Experiment 2, the effects of BL ethanol consumption on the modulation of GABA transmission (assessed by in vitro electrophysiological recordings from slice preparations) produced by CRF application were assessed in tissue collected from the the CeA. In Experiment 3, bilateral injections of antalarmin into the CeA were used to assess the role of CRF-1 receptor (CRF1R) signaling in the CeA in BL ethanol consumption. Results showed that BL ethanol consumption significantly increased CRF-IR in the CeA and ventral tegmental area. CRF augmented the peak amplitude of eIPSCs in the CeA of water drinking controls, an effect which was abolished in animals with a history of BL ethanol consumption. Basal sIPSC frequency and amplitude in the CeA did not differ by ethanol history. Preliminary results suggest that site-directed injection of the CRF1R antagonist, antalarmin, into the CeA protected against BL ethanol consumption. Together, these converging novel results strongly suggest that BL ethanol consumption recruits CRF signaling in the CeA. Importantly, these results provide strong evidence suggesting that pharmacological treatments targeting the CRF system may be effectively applied in clinical settings to reduce binge drinking prior to the onset of alcohol dependence. (Supported by NIH grants AA013573, AA015148, and AA017803 and Department of Defense grants W81XWH-06-1-0158 and W81XWH-09-1-0293 to TET and NIH Grants AA019454, AA017668, INIA-Stress, ABMRF, NARSAD to TLK).

0459

IMPACT OF CHRONIC ETHANOL SELF-ADMINISTRATION ON WHITE MATTER IN VERVET MONKEY BRAIN: A DIFFUSION TENSOR IMAGING STUDY

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Long-term alcohol abuse has many deleterious effects on the brain but it is unclear when morphological and functional deficits may begin over the course of drinking in part because human studies are usually conducted in older treatment-seeking alcoholics with long histories of drinking. They likely differ from drinkers who are earlier in their drinking careers, and who may meet the criteria for alcohol use disorders, but not dependence. A nonhuman primate (NHP) model of ethanol (EtOH) self-administration avoids many confounds inherent in clinical studies and allows the study of progression of EtOH's CNS effects from the naive state through various stages of drinking. Our study used a NHP model of chronic EtOH self administration (Vivian et al., 2001; Grant et al., 2008) that elicits heaving drinking in monkeys.
Daily EtOH consumption levels range from < 1 g/kg to > 3–4 g/kg and early drinking patterns are predictive of subsequent heavy drinking (Grant et al., 2008).
It is known that microstructural changes in white matter (WM) precede volumetric changes in the alcoholic brain as shown by decreased fractional anisotropy (FA) in major WM regions such as the corpus callosum (Pfefferbaum et al.,2006; Liu et al., 2010). Because diffusion tensor imaging (DTI) methods are more sensitive to microstructural changes than conventional MRI methods, we applied DTI to assess whether changes in WM occur early in the drinking process. A between group comparison was conducted in a group of vervet monkeys that selfadministered EtOH or a control solution for 22 hr/day over 12 months. Using an NHP 8 channel phased array coil (Dr. Cecil Hayes, University of Washington, Seattle, WA) on a GE 3T MRI scanner, DTI data was acquired following chronic EtOH self-administration in a group of vervet monkeys to assess the impact of chronic EtOH exposure on WM integrity. WM integrity was assessed using MedInria (http://www-sop.inria.fr/asclepios/software/MedINRIA) in white matter tracts including multiple areas of the corpus callosum. A between group comparison between the EtOH drinking animals and control animals demonstrated significant differences in FA measures in the genu ($p < 0.01$), splenium ($p < 0.02$) and body ($p < 0.0001$) of the corpus callosum. Additional WM tracts are currently being assessed, but these data suggest that some of the changes in FA reported in the human literature may begin early in the drinking history.
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0461

THE EFFECTS OF ACUTE AND CHRONIC ETHANOL EXPOSURE IN NONHUMAN PRIMATE BRAIN NETWORKS

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Neuropathological and neuroimaging studies have demonstrated morphological and functional consequences of alcoholism in humans. However, these data do not provide a clear sense of how whole-brain connectivity is affected by ethanol exposure. Network science is a valuable tool for understanding the brain as a system by showing how different regions of the brain interact with each other. Using a nonhuman primate (NHP) model of ethanol self administration (Vivian et al., 2001; Grant et al., 2008), we investigated the effects of acute and chronic exposure to ethanol in NHP brain networks.

Resting state fMRI data was collected in an anesthetized rhesus macaque in the naive state and again following an intravenous infusion of 1g/kg EtOH (equivalent to 4 drinks) over 5–8 minutes. After administration of the ethanol bolus, a decrease in the clustering coefficient ($C = 0.13$ to 0.09) and path length ($L = 5.6$ to 4.8) was observed. Comparing these metrics to an equivalent random network ($C = 0.06$, $L = 3.35$), it appears that acute ethanol causes the network to become more random. In hub maps generated for both states, well-defined hubs, such as one in the anterior cingulate cortex, were no longer apparent following administration of the ethanol bolus. In a follow-up study, two EtOH naive vervet monkeys were compared to a vervet that chronically self-administered EtOH under free access conditions for 10 months. The blood ethanol concentration level in this animal was 0 mg% at the time of data acquisition. Network statistics for clustering coefficient and path length of the EtOH animal ($C = 0.22$, $L = 3.63$) were significantly lower than the animals in the naive group ($C = 0.27 \pm 0.02$, $L = 4.13 \pm 0.10$).

These results suggest that chronic EtOH exposure causes cortical networks to devolve into a more random topology, and this persists even when there is no EtOH in the system. Disrupted networks may decrease the ability of the brain to appropriately process information. Of equal import, however, these results show that network metrics can be used to detect and track over time changes in the brain due to exposure to ethanol.

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0748

GENETIC DIFFERENCES IN THE EFFECTS OF ETHANOL ON BRAIN 3alpha, 5alpha-THP LEVELS ACROSS SELECTED BXD RECOMBINANT INBRED MOUSE STRAINS

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Acute ethanol administration increases brain and plasma levels of (3a,5a)-3-hydroxypregnan-20-one (3a,5a-THP or allopregnanolone) and its precursors progesterone and pregnenolone in rats. The increase in neurosteroid levels contributes to the anxiolytic, anticonvulsant, sedative and proaggressive actions of ethanol. In contrast, acute ethanol administration increases brain but not plasma levels of 3a,5a-THP in DBA/2J (D2) mice but not in C57BL/6J (B6) mice, suggesting that genetic background may explain the effects of ethanol on neurosteroid levels. To further explore this hypothesis, we examined ethanol-induced changes in 3a,5a-THP levels in the cerebral cortex and the olfactory bulb and tubercle of selected D2xB6 (BXD) recombinant inbred strains. Mice were injected with ethanol (2 g/kg, i.p.) or saline at 9:00 am and were sacrificed 1 hour later. 3a,5a-THP levels were measured by radioimmunoassay in the cerebral cortex and by gas chromatography/mass spectrometry in the olfactory bulb and tubercle. Basal cerebral cortical 3a,5a-THP levels across 8 BXD strains plus the parental strains range between 1.81 and 3.72 ng/g, equivalent to a 2.0-fold genetic variation [$F(9,79) = 6.27$, $p < 0.0001$] and heritability (h2) of 0.40. Basal 3a,5a-THP levels in the olfactory bulb range between 0.60 and 1.80 ng/g equivalent to a 3.0-fold genetic variation [$F(9,59) = 2.46$, $p = 0.021$] and h2 of 0.20. The ethanol-induced changes in 3a,5a-THP levels range between +4% and +63% in the cerebral cortex and between)28% and +65% in the olfactory bulb. We also examined genetic correlations with behavioral phenotypes previously determined in the BXD strains and available in GeneNetwork (www.genenetwork.org). Both basal and ethanol-induced 3a,5a-THP levels are correlated with some anxiety and ethanol phenotypes. Interestingly, the ethanol-induced changes in cerebral cortical 3a,5a-THP levels are negatively correlated with consumption of 3% ethanol in the two bottle choice paradigm (Spearman)0.82, $p = 0.02$, $n = 7$, Phillips et al., 1994) and consumption of 10% ethanol over 24 hours (Spearman)0.82, $p = 0.02$, $n = 7$, Rodriguez et al 1994). Those strains with increased 3a,5a-THP levels in response to acute ethanol consume less alcohol. This finding, albeit observed in a small number of strains, supports the hypothesis that neurosteroid responses to ethanol may be putative biomarkers of excessive alcohol consumption. Future studies are required to expand and validate these preliminary results.

0939

CaMKII EXPRESSION IS INCREASED IN THE AMYGDALA FOLLOWING ALCOHOL CONSUMPTION AND FUNCTIONALLY REGULATES ALCOHOL SELF-ADMINISTRATION

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Repeated alcohol exposure causes neuroadaptive changes in brain regions that regulate addictive behaviors, including the neurocircuitry of the amygdala, a major component of the brain's reward system uniquely positioned to process alcohol reward, alcohol-related cues, and influence alcohol-seeking behavior. We hypothesize that maladaptive alterations in the activity and expression of proteins that regulate synaptic plasticity play a central role in the development and expression of addictive behaviors. Using an unbiased proteomics approach, the alpha subunit of calcium/calmodulin-dependent protein kinase II (CaMKIIa) was identified as a neuroplasticity-related protein increased in the amygdala of C57BL/6J mice following 28 days of alcohol consumption. Immunohistochemistry analysis revealed that CaMKIIa and phosphorylated GluR1 (pGluR1ser831), a CaMKII phosphorylation site, is increased in the central amygdala (CeA) of alcohol-drinking mice. Due to its role in modulating AMPA receptor activity, increased expression of CaMKIIa was predicted to alter the electrophysiological properties of CeA neurons. Using whole cell patch clamp electrophysiology, we found that the AMPA/NMDA ratio and sEPSC frequency are increased and paired-pulse ratio and sEPSC amplitude are unaffected in alcohol-drinking mice indicating that there are an increased number of postsynaptic, functional AMPARs at previously silent synapses in the CeA following chronic alcohol consumption. Alcohol consumption increased CaMKIIa in the amygdala; however, the role of CaMKII in alcohol self-administration is unknown. In a separate set of experiments, we used operant self-administration procedures to elucidate the role of CaMKII in the reinforcing properties of alcohol. We determined that phosphorylated CaMKII (pCaMKIIthr286) and pGluR1ser831 are increased in the CeA following operant selfadministration of sweetened alcohol (9% alcohol (v/v)/2% sucrose (w/v)) compared to sucrose (2% sucrose (w/v)). Targeted inhibition of CaMKII phosphorylation with microinjections of the synthetic peptide m-AIP (1 tg/per side) into the amygdala decreased alcohol but not sucrose self-administration without affecting spontaneous locomotor activity. Our results demonstrate that increased CaMKII expression and synaptic activity in the amygdala are a consequence of voluntary alcohol consumption and the phosphorylation of CaMKII functionally and selectively regulates the reinforcing effects of alcohol.

Oregon

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REGULATION OF CHRONIC, HEAVY, ETHANOL SELF-ADMINISTRATION IN MONKEYS

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Self-administration of ethanol by laboratory animals is a novel instrumental behavior that is believed to transition from a highly integrative response involving executive (cortical) monitoring and control to an autonomous behavior (habit). In humans, a symptom of alcoholism is a narrowing of the behavioral repertoire towards drinking alcohol and the term ‘‘habitual user’’ is a common descriptor. In our studies of over 50 cynomolgus monkeys trained to self-administer ethanol with fixed-time schedules of food delivery (schedule-induced polydipisa), monkeys learn to drink large volumes of ethanol rapidly (i.e., gulping in bouts) and subsequently experience ethanol intoxication. The drinking typography of gulping in bouts established early in training appears to become a ‘‘unit’’ of alcohol drinking behavior that is insensitive to alcohol devaluation, even up to circulating BECs of 200 mg/dl. In chronic, open access to ethanol (22 hr/day to concurrent 4% (w/v) ethanol and water for over 1 year), very heavy drinking days (>4.0 g/kg or 16 to 18 drink equivalent) have a characteristic increase in bout volume, indicating an increase in the stereotypical or habitual gulping typography of drinking. When the monkeys are subjected to repeated abstinence periods of 28 days interspersed with at least 4 months of open access to ethanol, intakes become highly regulated and patterns of intake are shifted even further to gulping in bouts and attaining daily BECs between 100 and 400 mg/dl. Associated with the highly regulated intake of chronic heavy drinkers is a selective change in the excitatory output of the putamen compared to the caudate of these monkeys. Thus, both behavioral and neurobiological adaptations suggest that alcoholic patterns of drinking are highly regulated with the apparent outcome of being able to sustain high circulating concentrations of ethanol.

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REGULATION OF ETHANOL INTAKE AND ETHANOL SEEKING BY NEUROSTEROIDS AND ACTIVATION OF EXTRASYNAPTIC GABAA RECEPTORS

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Neurosteroids have rapid membrane actions as positive or negative modulators of caminobutyric acidA (GABAA) receptors, with endogenous levels fluctuating in response to various stressors (including ethanol injection and consumption), during the menstrual/estrous cycle, and during pregnancy. While ethanol influences many neurotransmitter systems, the interaction at GABAA receptors appears to be integral for ethanol's reinforcing and discriminative stimulus effects. Thus, parallel interactions of neurosteroids and ethanol at GABAA receptors suggest that fluctuations in neurosteroid levels (and the resultant change in GABAergic inhibitory tone) may alter sensitivity to ethanol, leading to changes in the positive motivational effects of ethanol. Ongoing studies in the laboratory indicate that manipulation of neurosteroid levels, or other modulators of GABAA receptors, can influence voluntary ethanol intake. Using both lickometer chambers and operant self-administration procedures, we found that intracerebral and systemic injections of the GABAergic neurosteroid allopregnanolone (ALLO) produced a biphasic effect on ethanol intake by promoting the onset and blunting the maintenance of consumption during the limited access session. A priming dose of ALLO also promoted reinstatement of ethanol seeking. Recent data with ganaxolone (GAN), a synthetic neurosteroid analog of ALLO that is in Phase II clinical trials as an anticonvulsant, and with gaboxadol (THIP), a GABAA receptor agonist with selectivity for extrasynaptic receptors that is in Phase III clinical trials for the treatment of insomnia, also will be described. Lickometer studies indicated that both GAN and THIP produced biphasic effects on continuous access ethanol intake, with differences in time course and persistence of the effects. The similarities in the biphasic effects of GAN and THIP on ethanol intake to previous results with ALLO are consistent with the hypothesis that neurosteroid levels and activation of extrasynaptic GABAA receptors are an important determinant of ethanol intake and reinforcement. Evidence stemming from this work will likely generate a new target site for therapeutics aimed at alcohol abuse.

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PREFRONTAL CORTICAL GLUTAMATE AND GABA GENE EXPRESSION IN CHRONIC ETHANOL SELF-ADMINISTRATION IN MACAQUE MONKEYS

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The prefrontal cortex is a target for understanding alcohol-mediated deficits in cognitive effects. The cortical fields involved in cognitive actions include the anterior cingulated cortex (ACC), the dorsolateral prefrontal cortex (DLPFC) and the orbitofrontal cortex (OFC). The macaque primate cortex has extensive anatomical and biochemical similarities with human cortex in these higher-order associative cortical regions. Ethanol is known to alter glutamate and GABA neurotransmission. One mechanism may be through the reconfiguration of the subunits constituting ionotropic glutamate and GABA receptors. GABAergic and glutamatergic transmission have integral roles in cortical processing, influencing cortico-cortical and corticosubcortical communication. Thus, we have focused studies on understanding alcohol-induced alterations in these ionotropic receptors in prefrontal fields. For these studies we use tissue from a nonhuman primate model of excessive alcohol self-administration that recapitulates important aspects of human alcohol consumption including: alcoholic patterns of intake; 2–4 day ‘‘sprees’’ with blood ethanol concentrations over 200 mg/dl; pathological changes in hepatic, cardiovascular, and reproductive systems; clear signs of metabolic stress proportional to alcohol intakes. To characterize the effects of chronic ethanol self-administration on the prefrontal cortical fields, GABAA, NMDA, AMPA (GRIA) and kainate (GRIK) subunit mRNA expressions were studied in the OFC, DLPFC and ACC of male monkeys. In DLPFC, AMPA splice variant expression and kainate receptor subunit expression were significantly decreased in alcohol treated monkeys. Expression levels of over 20 GABA and glutamate receptor subunits were measure with rtPCR from control and alcohol drinkers. Overall, the pattern of GABAA expression was complex and decreased primarily in the OFC (alpha 2 and 4; gamma 1, 2 and 3) although beta 1 and gamma 1 were decreased in OFC and DLPFC. NMDA receptor expression changes were discrete (GRIN 1-1 in both OFC and DLPFC), with a notable increase in GRIN1-2 only in the OFC. In both OFC and DLPFC, the expression of AMPA subunit GRIA-4 was decreased. Notably, there were no changes in GABA, NMDA, AMPA or kainate receptor subunit expression in the ACC. Results from these studies provide strong evidence of selective transcriptional regulation of GABA and glutamate ionotropic receptor subunits in the primate brain following alcohol self-administration.

0450

MONKEY ALCOHOL TISSUE RESEARCH RESOURCE

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Excessive alcohol ingestion is comorbid both with brain and behavioral disorders and peripheral organ damage. Much of what is known about risk for and the results of heavy alcohol consumption derives from rodent studies or retrospective human studies. We are now funded by NIAAA to supply a unique resource for alcohol research, a Monkey Alcohol Tissue Research Resource (MATRR).

Both tissue and associated bioinformatics tools will be made available to the wider alcohol research community from this resource. The basis of the MATRR is tissue derived from our experimental model of ethanol self-administration in monkeys (Vivian et al., 2001; Grant et al., 2008). In this model, monkeys show a range of drinking from low (<1.0 g/kg/day) to excessive amounts of alcohol(>3.0 g/kg) over long periods of time (12–30 months) with concomitant pathological changes in endocrine, hepatic, cardiovascular, immunological and central nervous system (CNS) processes. These longitudinal designs span ‘‘stages of drinking’’ from ethanol?]naive to early exposure to chronic excessive drinking in cynomolgus and rhesus macaques and vervet monkeys. This resource will allow investigators to generate novel data for hypothesis testing relating the risk for and consequences of ethanol self-administration. These CNS and peripheral tissues comprise a unique translational resource that will serve to bi-directionally bridge the gap between rodent and human subject studies. The demand for, and the quality of, the tissues are high as reflected by over 50 peer-reviewed publications, 60 abstracts, 19 GenBank sequences and numerous data presentations using tissue supplied by the resource. These tissues also offer the opportunity to collect preliminary data for grant submissions and the animal model that is the basis for this tissue resource has resulted in at least 32 extra- and intramurally funded projects.

Our primary goal is to continue to build the resources of this tissue bank and distribute these tissues and/or associated bioinformatics, to the broader alcohol research community.

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0460

HIPPOCAMPAL 5-HT1A RECEPTOR DENSITY FOLLOWING CHRONIC ETHANOL SELF-ADMINISTRATION IN CYNOMOLGUS MACAQUES

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Treatment with pharmacotherapeutics targeting the serotonin system, and in particular, the 5-HT1A receptor, may reduce alcohol intake and craving in some populations, it is still unclear how these drugs exert their effects. The hippocampus is one of several limbic structures that modulate the mesolimbic dopamine system, or reward pathway. It is heavily innervated by the serotonergic system and contains a particularly high density of 5-HT1A receptors relative to other brain regions. Previous studies investigating the effects of alcohol on 5-HT1A receptor density in humans and rodents have produced equivocal results. By utilizing a well-established nonhuman primate model of chronic, voluntary ethanol self-administration, we hope to contribute to the current understanding of the effects of ethanol on this receptor system while avoiding confounds that often exist in studies using other species. Male cynomolgus macaques were induced to self-administer ethanol using a schedule-induced polydipsia procedure. After induction, the monkeys 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 naive (n = 8). At necropsy, brains were blocked, flash-frozen, and processed for in vitro receptor autoradiography using the 5-HT1A receptor antagonist [3H]MPPF. Nonspecific binding was determined using WAY-100635. Images were analyzed using AIS software and regions of interest were defined using overlaid nissl stained images of adjacent sections. In agreement with previous literature, the most densely bound regions of the hippocampus included the molecular layer of the dentate gyrus, the pyramidal cell layer of CA1 and CA2, and the stratum lacunosum-moleculare of CA1 and CA2. The molecular layer of the dentate gyrus was significantly more densely bound in ethanol drinkers compared to controls ($p = 0.03$). Furthermore, 5-HT1A receptor density in this region positively correlated with average daily ethanol intake (g/kg; $p = 0.02$). No significant between group differences were observed in CA1 and CA2. These data suggest that chronic ethanol self-administration is associated with region- and layer-specific changes in 5-HT1A density in the hippocampus.

0952

SEX DIFFERENCES IN ESTABLISHING DRINKING TO INTOXICATION IN ADOLESCENT RHESUS MONKEYS

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Adolescence is a period of increased vulnerability to the onset of regular heavy drinking, which in turn is associated with lifetime social, emotional, behavioral and health problems. Epidemiological data show male adolescents maintain higher drinking frequency, quantity and volume of consumption compared to female adolescents. Macaque monkeys are particularly useful in addressing drinking patterns in adolescents due to their long life span and the propensity to drink ethanol to intoxication. In comparing young adult onset drinking, male monkeys are proportionally heavier drinkers compared to females. Here we induced ethanol self-administration in mid- to late-adolescent (4–4.5 years) male (n = 8) and female (n = 6) monkeys with a schedule-induced polydipsia procedure in daily sessions. The volume of fluid induced to drink increased every 30 sessions to match a dose of 0.5 g/kg, 1.0 g/kg and 1.5 g/kg ethanol. Data were analyzed from the final 30 sessions of 1.5 g/kg/day because in previous studies the amount of time required to drink this dose and the BEC attained 90 minutes after the start of the session were predictive of future heavy drinking. The preliminary analysis shows the female monkeys drank the 1.5 g/kg dose faster (mean 35 ± 16 min) compared to males (mean 226 ± 50 min) and attained higher BECs (106 ± 21 mg/dl) compared to males (53 ± 24 mg/dl). The induction data suggest that female monkeys introduced to alcohol in mid- to late adolescence may be more similar to young adult males (5.5–6 yrs), compared to adolescent males (4–4.5 yrs), in their risk for future heavy ethanol consumption.

South Carolina

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ALTERED BEHAVIORAL RESPONSE TO ETHANOL IN NR2B NMDA RECEPTOR CONDITIONAL KNOCKOUT MICE

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This study examined behavioral responses to acute ethanol challenge as well as voluntary ethanol drinking in a mouse model with genetic deletion of the NR2B subunit. Since genetic deletion of the NR2B NMDA receptor subunit is lethal, conditional NR2B knockout mice were constructed that delay deletion to test the role of this subunit in ethanol-related behaviors. It was hypothesized that mice with genetic deletion of the NR2B subunit would display less sensitivity to an ethanol challenge, since evidence suggests that pharmacological antagonism of the NR2B subunit results in attenuated responsiveness to acute ethanol (EtOH) exposure. NR2B[f/f] mice carrying the CAMKIIa or CRE transgenes were mated to generate test offspring: NR2B[f/f] wildtype (WT), NR2B[f/f]-CAM (CAM), NR2B[f/f]-CRE (CRE), and NR2B[f/f]-CAM-CRE (KO). A total of 321 mice were used across the 4 Genotypes (WT; CAM; CRE; KO) x 2 Sex (male; female) factorial design of this study. Western blot analyses revealed significant knockdown of NR2B-containing receptors in several brain regions including prefrontal cortex, dorsal and ventral striatum, hippocampus, and amygdala. Results indicated that NR2B KO mice exhibited elevated locomotor activity in a novel open field compared to control littermates (WT, CAM, CRE) following saline injection. Further, NR2B KO mice exhibited an enhanced locomotor stimulant response to a moderate dose of EtOH (1.5 g/kg) as well as an augmented response to sedative/hypnotic doses of EtOH (more suppressed activity following 3.0 g/kg EtOH and longer duration of LORR following a 4.0 g/kg challenge) compared to control littermates. There were no genotypic differences in the amount of ethanol consumed (limited access 2-bottle choice situation), or in the rate of ethanol clearance. Collectively, these results demonstrate a unique behavioral profile both prior to and following EtOH exposure in NR2B deficient (KO) mice. Greater sensitivity to acute low-dose stimulant and high-dose sedative/hypnotic effects of EtOH in NR2B deficient (KO) mice may be due to alterations in ethanol's interaction with NMDA receptor-mediated glutamate transmission within forebrain structures and/or other target neurotransmitter systems. Similar studies are under way using animals in which induction of gene deletion is delayed until adulthood. Supported by grants U01 AA014095, T32 AA007474, and VA Medical Research.

0172

ETHANOL DEPENDENCE–RELATED ALTERATIONS IN CRF EXPRESSION AND BEHAVIORAL COPING IN THE FORCED-SWIM TEST IN C57BL/6J MICE

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We have shown that repeated cycles of chronic intermittent ethanol (CIE) exposure produces escalation of voluntary drinking as well as altered behavioral responsiveness to stress. In this study we sought to investigate whether ethanol dependence-related adaptations in behavioral coping strategy to a stress challenge might be mediated by alterations in brain CRF levels. Adult male C57BL/6J mice received either CIE exposure (16 hr/day for 4 days) via ethanol vapor inhalation for 4 consecutive weekly cycles (EtOH group) or similar handling without ethanol vapor exposure (CTL group). Behavioral responsiveness to an inescapable forced swim test (FST) was then assessed in separate groups of mice 3, 7, or 14 days after the final inhalation treatment. Results indicated that EtOH mice exhibited significantly less immobility than CTL mice when the 10-min FST exposure was given at 3 days into withdrawal. This reduction in immobility was still significant when mice were probed 7 days after CIE exposure, but was no longer significant by the fourteenth day of abstinence. In a second experiment, mice were exposed to the same regimen of CIE exposure and CRF peptide levels measured (ELISA) in the amygdala (AMY) at 3, 7, and 14 days into withdrawal. Similar to the time course of behavioral changes in the FST, CRF peptide levels in the AMY of EtOH mice were elevated 3 and 7 days into withdrawal, but were not different from CTL mice by day 14 of abstinence. Overall, these results confirm our previous findings that EtOH mice exhibit more persistent active escape-like behavior compared to CTL mice in response to the FST stressor, perhaps indicative of a maladaptive response strategy to an inescapable situation. Furthermore, this study suggests that this maladaptive behavioral response to FST as a function of CIE treatment may be related to elevated CRF activity within the AMY. On-going studies are examining alterations in Crf mRNA expression following CIE/FST treatment, as well as possible changes in CRF mRNA/peptide levels in other brain regions (e.g., PFC, BNST). Future studies also will further explore the relationship between ethanol dependence related alterations in coping behavior and brain CRF function via antagonism of CRF activity in mice exposed to the CIE paradigm.

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EFFECTS OF ACUTE AND CHRONIC INTERMITTENT ETHANOL EXPOSURE ON ATTENTION SET-SHIFTING AND NEURON EXCITABILITY IN THE ORBITOFRONTAL CORTEX

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In humans, stroke or trauma-induced damage to the orbitofrontal cortex (OFC) often results in poor judgment and behavioral inflexibility. Human alcoholics also exhibit similar deficits in inhibition and self-control suggesting that OFC neurons are susceptible to alcohol-induced damage or dysfunction. The present experiments investigated this issue by exposing adult male C57BL/6J mice to chronic intermittent ethanol (EtOH) or air vapor inhalation to induce EtOH dependence and examining performance on the attention set-shifting assay. Testing was conducted during the first (days 4–9) or second (days 11–16) week following the last bout of EtOH (or air) inhalation to determine whether EtOH-induced changes in set-shifting are long-lasting. During the first week of EtOH abstinence, EtOH dependent mice showed reduced performance on the reversal learning phase of the task as compared to control mice. These deficits dissipated by the second week of EtOH abstinence suggesting that ethanol-induced changes in OFC function can recover. We used whole-cell patch clamp electrophysiology to investigate whether acute and chronic intermittent EtOH exposure affects the intrinsic excitability and synaptic transmission of OFC neurons. In control mice, a 10 minute bath application of EtOH (11–66 mM) decreased action potential firing induced by direct current injection and caused a small but concentration-dependent decrease in input resistance. Both of thse effects recovered during washout of the EtOH containing solution. Adding the GABAA receptor antagonist picrotoxin to the bath prevented the effects of EtOH on firing and input resistance. Data from preliminary studies suggests that EtOH (33 mM) does not alter the amplitude of stimulus-evoked GABA IPSCs suggesting that EtOH's effect on intrinsic excitability may arise from increases in tonic GABA currents of OFC neurons. Together, these findings suggest that OFC neurons are sensitive to both acute and chronic ethanol exposure and may be a particularly important target for the cognitive impairing actions of EtOH.
Funding: This work was supported by F32 AA019610 (KAB) and the P50 AA010761 (JJW & HCB).

0211

INCREASING GLUTAMATERGIC NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS BY ANTAGONIZING GLUTAMATE REUPTAKE INCREASES ETHANOL DRINKING

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We have shown that chronic intermittent ethanol (CIE) exposure significantly increases ethanol consumption in C57BL/6J mice. Further, using in vivo microdialysis procedures CIE exposure also increases extracellular glutamate (GLU) levels in the nucleus accumbens (NAc). These data suggest that escalated drinking in the CIE model may be linked to increased GLU levels in the NAc. In the present study, we examined the ability of a GLU reuptake blocker, DL-threo-beta-Benzyloxyaspartic acid (TBOA), to alter ethanol drinking in the 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. During the fourth drinking test period, mice were microinjected (0.25 lL/min) with TBOA (0, 250 or 500 lM) 30 min prior to their usual access to ethanol. TBOA dose-dependently increased ethanol drinking in both groups relative to vehicle (main effect of dose, $p < 0.05$). Further, this TBOA-induced increase in drinking was significantly greater in EtOH compared to CTL mice at both doses (main effect of CIE treatment, $p < 0.05$). That is, in CTL mice TBOA produced 22% and 41% increases in drinking, while in EtOH mice TBOA produced 52% and 68% increases in drinking for 250 and 500 lM TBOA, respectively. Finally, in a small, separate study we found that reverse perfusion of TBOA (0 to 500 lM) through a microdialysis probe increased extracelluar GLU in the NAc of ethanol-naive mice. Collectively, these data are consistent with the idea that the hyperglutamatergic state in the NAc produced by CIE exposure drives/promotes escalation of drinking associated with this model of ethanol dependence and relapse. Supported by NIAAA grant P50 AA10761.

0233

CHRONIC INTERMITTENT ETHANOL EXPOSURE ALTERS CRF RELEASE IN THE AMYGDALA AND BED NUCLEUS OF THE STRIA TERMINALIS IN C57BL/6J MICE

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We recently found that repeated cycles of chronic intermittent ethanol (CIE) exposure alters CRF peptide content in amygdala (AMYG) and bed nucleus of the stria terminalis (BNST). In this study we used an ex vivo CRF release assay to investigate CRF function in AMYG and BNST of dependent and nondependent C57BL/6J mice using our CIE model. Brain tissue was collected by rapidly extracting the brain, making 2mm coronal sections on ice and using a tissue punch to isolate AMYG and BNST. Bilateral punches were pooled, briefly homogenized and then incubated at 37_ C in artificial cerebral spinal fluid (aCSF). Samples were briefly centrifuged in 30 min intervals, with the buffer decanted and replaced with fresh aCSF. CRF concentrations in the buffer were determined by ELISA and normalized to total protein. Initial experiments using tissue from nondependent mice showed that after 3 buffer exchanges, CRF release in the AMYG and BNST tissue preparations stabilized. Additionally, we found that increasing potassium (K+; 30, 60 and 120mM) in the aCSF, while maintaining osmolarity, evoked significant concentration-dependent increases in CRF release relative to basal levels (33%, 40% and 70%, respectively, $n = 5$ to 9, $p < 0.05$), with the magnitude of effect similar in AMYG and BNST regions. This K+ evoked CRF release returned to basal levels in control aCSF buffer. Further, K+ stimulated CRF release in AMYG and BNST was reduced by 1mM EGTA to basal levels, consistent with calcium-dependent vesicular release of CRF. In subsequent experiments, we used tissue collected from mice rendered ethanol-dependent by 4 consecutive, weekly cycles of CIE exposure by vapor inhalation (or air for nondependent controls). Tissue samples were harvested 72 hr following final exposure. Again, we found significant concentration-dependent increases in K+ evoked CRF release in AMYG with 30 and 60mM K+ (30% and 43%, respectively, $p < 0.05$) whereas in BNST the increases were 42% and 49% for each concentration, respectively ($p < 0.05$). Preliminary results also suggested a trend for increased sensitivity to K+ stimulated CRF release in dependent compared to nondependent groups. Collectively, these data indicate that CRF release in AMYG and BNST is sensitive to K+ stimulation in a concentration- and calcium-dependent manner, and that capacity for CRF release in these brain regions may be altered as a function of ethanol dependence. Supported by NIAAA grant U01 AA014095 and VA Medical Research.

0747

EFFECTS OF CHRONIC INTERMITTENT ETHANOL EXPOSURE ON VOLUNTARY ETHANOL DRINKING IN BXD STRAINS OF MICE

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The recombinant inbred BXD strains of mice were generated by crossing and inbreeding ethanol-preferring C57BL/6J (B6) and ethanol-avoiding DBA/2J (D2) inbred strains. These mice have been used to map genetic bases of different phenotypes including ethanol intake. This study evaluated voluntary ethanol intake in a panel of 43 BXD strains using a model of dependence and relapse drinking. A total of 121 mice (85 males, 36 females) from BXD lines (n = 1/genotype/sex/group) were evaluated along with C57BL/6J males (n = 6–8/group) that served as positive controls. Mice were tested for baseline ethanol intake using a 2-bottle (15% v/v ethanol vs. water) limited access (2 hr/day) drinking model for 6 weeks (Baseline). Then, mice from each genotype received 4 weekly cycles of chronic intermittent ethanol (CIE) vapor exposure (EtOH group) or air exposure (CTL group) (16 hr/day x 4 days) alternated by 5-day drinking test cycles. Ethanol concentrations in the inhalation chambers were set for all genotypes to yield blood ethanol concentrations (BEC) between 200–300 mg/dl. Results indicated that ethanol intake during baseline varied greatly across genotypes over the 6 weeks. During the last week of baseline, intake ranged from 0.9 g/kg (BXD83) to 5.6 g/kg (BXD81) for males and 1.3 g/kg (BXD71) to 4.3 g/kg (B6) for female mice. As expected, CIE exposure induced a significant increase in ethanol drinking in C57BL/6J mice (2.97–3.88 g/kg) relative to their baseline level (2.44 g/kg) as well as intake in CTL mice that remained relatively stable over the 4 test cycles (_2.60 g/kg). Of significance, voluntary ethanol intake varied greatly among genotypes after CIE exposure. Some strains showed escalation of drinking (e.g., 136% increase; BXD66 male), while others showed a progressive reduction in ethanol intake (e.g., 52% decrease; BXD43 male) from Baseline to Test 4. Importantly, the magnitude and direction of changes in ethanol intake during each test cycle did not relate to BEC recorded in the preceding CIE exposure cycle. Overall, these data indicate a significant genetic influence on the propensity to escalate (or avoid) ethanol drinking as a function CIE exposure. Additionally, this effect seems to be independent from pharmacokinetic factors.
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0971

ALTERATIONS IN HISTONE 3 ACETYLATION IN THE PREFRONTAL CORTEX FOLLOWING ADOLESCENT ALCOHOL EXPOSURE

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Adolescence is a critical period of continued development and refinement of the prefrontal cortex (PFC) and PFC-dependent behaviors such as inhibitory control over risk-taking and impulsivity. It is now clear that during this period, the PFC is particularly vulnerable to environmental influences that may induce functional and morphological changes that persist into adulthood. In particular, repeated binge-like exposure to alcohol during adolescence may cause aberrant maturation of the PFC leading to impairments in higher cognitive. A mechanism by which alcohol exposure could alter PFC development is through epigenetic modifications that impact the refinement of neuronal circuitry in the PFC. Dynamic changes in histone acetylation associated with ethanol exposure and withdrawal together with homeostatic adaptation invoked by these changes may produce aberrant expression patterns of plasticity-related genes during adolescence. Histone deacetylase 2 (HDAC2) dynamically regulates synaptic plasticity by deacetylating the promoter region of plasticity-related genes, and overexpression of HDAC2 reduces synaptic plasticity, decreases formation of dendritic spines and synapses, and inhibits memory formation. Recent studies show that acute ethanol increases acetylated histone 3 (AH3) by inhibiting HDAC2 in amygdala. Because acetylation of Lys9 on histone 3 is highly correlated with regulation of gene expression, one goal of the present study was to determine if acute ethanol induces increases in acetylated Lys9 in mPFC of adolescent rats. Acute ethanol (3.5 g/kg, i.p.) was administered to adolescent rats (35 days old) for a 30-min treatment period. We used double-labeling immunohistochemistry (IHC) and confocal microscopy to examine the effects of acute ethanol exposure on histone acetylation as an epigenetic event that may alter maturation of the mPFC in distinct neuronal populations in adolescent rats. These results demonstrate that acute ethanol exposure increased overall AH3 in the mPFC of adolescent rats, consistent with the suggestion that ethanol may alter the proper development of the PFC during the transition from adolescence to adulthood by inducing epigenetic changes in gene expression in this population of neurons. The results of additional ongoing studies will also be presented examining changes in AH3 after repeated cycles of binge-like episodes of alcohol exposure by vapor inhalation. Supported by NIH grant AA010983 and AA0119967, and AA017922.

0984

CHRONIC INTERMITTENT ETHANOL ALTERS GLUTAMATERGIC SYNAPSES IN MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS CORE NEURONS OF C57BL/6J MICE

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Repeated episodes of heavy alcohol drinking and withdrawal are associated with enhanced relapse vulnerability. Among the various brain structures involved in this response, neurochemical adaptations in the medial prefrontal cortex (mPFC) and its glutamatergic projections to the nucleus accumbens core (NAcc) appear to be particularly important in influencing drinking behavior. In this study, we examined morphological and biochemical alterations of glutamatergic synapses in mPFC and NAc neurons in a chronic intermittent ethanol (CIE) vapor inhalation model that is associated with escalation of drinking in ethanol dependent mice. Adult male C57BL/6J mice were exposed to 4 to 5 weekly cycles of intermittent (14 hr on/10 hr off for 4 days) ethanol vapor inhalation. Mice that served as controls were exposed to air in chambers. The diolistic labeling technique coupled with confocal imaging and 3D image analysis was used to determine CIE-induced changes in dendritic spines in mPFC layer V pyramidal neurons and medium spiny neurons (MSNs) in the NAcc. We also examined alterations in expression of NMDA and AMPA receptor subunits in a PSD enriched fraction following the last CIE vapor inhalation cycle. Chronic intermittent ethanol exposure significantly increased the density of mature, mushroom spines in the basal dendrites of layer V mPFC pyramidal neurons without affecting overall dendritic spine density, length or volume. We also observed a significant elevation in synaptic expression of NR1 and NR2B subunits of the NMDA receptor. In MSNs of the NAcc, CIE exposure significantly increased the density of long, thin spines and the terminal point volume of mature spines. We also observed an increase in mean dendritic shaft diameter of MSNs in CIE-exposed mice. Preliminary evidence suggests that CIE exposure did not alter synaptic expression of NR1 or NR2B subunits in MSNs. The current study demonstrates that CIE exposure causes morphological and neurochemical adaptations in the mPFC and NAcc core. These alterations in the cortico-limbic neurocircuitry could underlie maladaptive behavioral plasticity that contributes to high rates of consumption and enhanced relapse vulnerability in ethanol dependent individuals. Studies are underway to correlate changes in the morphology of dendritic spines with vapor-induced increases in drinking.
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Tennessee

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CONDITIONAL GLUN2B KNOCKOUT MICE REVEAL NMDAR SUBTYPE-DEPENDENT ETOH SENSITIVITY OF *EXCITATORY* TRANSMISSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Acute and chronic ethanol regulate glutamate transmission via actions on NMDA receptors (NMDARs). Understanding the role of NMDAR subunit specificity in these actions is critical to therapeutic development. Pharmacological studies ex vivo do suggest subtype specificity in ethanol actions, while recombinant cell line data largely do not. The extended amygdala, including the bed nucleus of the stria terminalis (BNST), is thought to be a critical region involved in dependence-induced drinking behavior. Work in the extended amygdala using pharmacological approaches has suggested a preferential modulation of the GluN2B NMDAR subunit following ethanol treatments. Thus we set out to determine if GluN2B was also preferentially altered by acute and chronic ethanol in the dorsal lateral (dl) BNST. To do this, we evaluated either acute ethanol effects ex vivo, or two 4-day cycles of chronic intermittent ethanol exposure (CIE) on LTP and NMDAR responses 4–5 hrs into withdrawal in control and conditional GluN2B deletion mice. GluN2B deletion in the conditional knockout line is ~80–90% within the BNST, producing altered synaptic NMDAR currents in accordance with the large reductions in GluN2B and, concomitantly, GluN1, but not GluN2A. Curiously, basal synaptic transmission onto AMPA receptors is upregulated basally in the knockout mice. Additionally in GluN2B knockout mice, LTP could not be elicited suggesting that GluN2B plays an integral role in this form of synaptic plasticity in BNST. Acute ethanol robustly inhibited NMDAR-dependent evoked excitatory postsynaptic currents (EPSCs) in control mice, but this inhibition was absent in the BNST of knockout mice. Chronic ethanol treatment produced enhanced LTP within the dlBNST compared to air-treated controls; however, differences in LTP between air controls and naı¨ve mice suggest that the sham condition may be stressful. We hypothesize that ethanol treatment enhances BNST LTP in a GluN2B selective manner and, therefore, investigated the effects of the GluN2B antagonist Ro 25-6981 (Ro) in these groups. Intriguingly, we observed a selective enhancement of LTP by Ro in ethanol exposed mice but not in naı¨ve or air-controls. This may suggest that extrasynaptic NMDARs tonically suppress LTP induction in ethanol exposed mice.

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INTEGRATIVE GENOMICS OF ALCOHOL USE AND ALCOHOL RESPONSE: FINDING CONVERGENT EVIDENCE ACROSS SPECIES AND EXPERIMENTAL SYSTEMS

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A wealth of genomic technologies have been applied to the study of genes underlying the response to alcohol, alcohol self-administration in animal models, and alcohol use disorders. Each of these studies implicate many genes and gene products in alcohol related phenomena, but few are detected with high stringency in any single analysis. Integrative genomics, applied through our Ontological Discovery Environment web-based software system enables the aggregation of these independent studies through the use of gene homology to provide interpretation and evidentiary support for the numerous results obtained in genomewide studies. In a user guided and data driven fashion, results are brought together to identify genes most highly connected to alcohol related phenotypes, results found in model organisms that are most reflective of results obtained in clinical populations, QTL positional candidates for which there are other genomic evidence sources and phenotypes which most resemble each other on a mechanistic level. Numerous tools are included in the software system and examples of applications from alcohol related genomic analyses from the Integrative Neuroscience Initiative and beyond will be presented. Using over three-thousand genomic data sets from curated literature annotations to Gene Ontology and Mouse Phenome Ontology, positional candidates from QTLs reported in MGI, several hundred differential expression results from the NeuroInformatics Framework Drug Related Gene database and gene expression correlates of behavior generated using GeneNetwork.org, we were able to identify high priority functional candidates for alcohol related phenotypes.
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GENETIC ANALYSIS OF THE microRNA PROCESSING GENES DROSHA AND DICER AND OF THEIR ROLE IN ETHANOL- AND STRESS-RELATED PHENOTYPES

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Over the last few years, it has become clear that genetic differences in ethanol responses are mediated by differences in the expression and functioning of entire molecular network rather than single genes, and thus it is critical to understand network structure, membership, and modulation. Recently a newly defined class of molecules, microRNAs (MIRs), has been implicated in ethanol-related responses. MIRs are short RNA molecules that often bind mRNA and influence translation. The generation of functional MIRs from their precursor transcripts is controlled by several enzymes, including Drosha and Dicer. We have assessed the relationship between expression of these two genes, and evaluated whether one or both modulate ethanol-related phenotypes. We used a systems genetics approach that exploits the BXD family of strains as well as a suite of bioinformatics resources. Levels of Drosha and Dicer mRNA were assessed in hippocampus of 72 of the BXD strains and both parental strains (C57BL/6J and DBA/2J), using the UMUT Affymetrix hippocampal exon array data set. The expression patterns of both genes were well correlated. Both transcripts are associated with high cis eQTLs (expression quantitative trait loci) showing that each gene partly regulates its own expression. Each transcript also was linked to several trans eQTLs, demonstrating that expression is also partly controlled by other genes. Remarkably, both genes share five trans eQTLs showing strong overlap in their genetic control. In the MIR pathway Dicer is upstream of Drosha. Using the correlation function of GeneNetwork (www.genenetwork.org), the relations between expression of Drosha and Dicer and ethanol- and stress-related phenotypes was assessed. Significant correlations exist between expression and voluntary ethanol consumption and with ethanol-induced hypothermia. Baseline traits, such as including locomotor activity, were also correlated with Drosha. These results indicate that Dicer and Drosha work together and share the same gene network, and suggest that both of these polymorphic genes may have roles in mediating genetic differences in ethanol-related phenotypes. This study also suggests a possible means by which differential regulation of microRNAs across different genotypes could occur. Supported by U01AA014425.

Virginia

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DEFINING FYN KINASE-DEPENDENT BASAL AND ETHANOL-RESPONSIVE GENE NETWORKS: IMPLICATIONS FOR ETHANOL BEHAVIORS

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Myelin is an important component of CNS plasticity that is substantially altered in alcohol abuse and dependency. We have previously shown that inbred strains of mice (C57BL/6J vs. DBA/2J, ILS vs. ISS) differing in acute ethanol behavioral phenotypes demonstrate basal and ethanol-evoked differences in myelin gene expression in prefrontal cortex (PFC). Bioinformatic analysis of B6 and D2 expression data implicated the nonreceptor protein tyrosine kinase Fyn as a plausible mediator of differences in B6 and D2 myelin gene expression profiles. Fyn has been previously documented as an important regulator of myelin basic protein (Mbp) expression and acute high-dose behavioral sensitivity to alcohol as determined by the loss of righting reflex (LORR) response. Therefore, we conducted a genomic analysis of Fyn kinase knockout mice to test the hypothesis that ethanol regulation of a Fyn-dependent myelin gene network is an underlying element in the neurobiology of acute ethanol-mediated responses. Microarray analysis results for prefrontal cortex (PFC) and nucleus accumbens (NAC) from Fyn -/- mice were overrepresented for a down-regulation of a myelin gene network (p-value <0.01). Moreover, analysis of basal expression changes showed a previously unappreciated possible role for Fyn in regulation of multiple glutamate, dopamine and potassium channel/ receptor-related signaling genes. Microarray results also showed disruption of select gene networks responding to acute ethanol. Basal isolated myelin gene expression results showed correlation to several ethanol behavioral responses. The results increase our understanding of Fyn signaling networks and suggest a complex impact on ethanol behavioral responses. In particular, our results suggest that Fyn-dependent alterations in myelin gene networks may be an important modulator of ethanol behaviors. Supported by NIAAA grants F31 AA018615 (SPF) and U01AA0116667, U01AA016662, and P20AA017828 to MFM.

0714

INVESTIGATING ALTERATIONS TO THE SYNAPTIC TRANSCRIPTOME IN RESPONSE TO ETHANOL EXPOSURE

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It is suggested that the characteristic behaviors associated with the escalation of drug use are caused by molecular neuro-adaptations precipitated by the drug's continual administration. These lasting activity-dependent changes 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 lead to our hypothesis that behavioral sensitization in response 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 in response to sensitization, we have optimized a protocol for obtaining synaptoneurosomes from the frontal pole of mice treated with repeated ethanol. Characterization of the preparation through western blotting and quantitative PCR indicate synaptic enrichment of the putative synaptoneurosome fraction. Preliminary investigations of the synaptic transcriptome utilizing microarray analysis of tissue from mice chronically exposed to ethanol also revealed an overrepresentation of transcripts denotative of synaptic function in fractions enriched in synaptic elements. These experiments also revealed regulation of synaptic gene expression by ethanol. Genes such as Kcnma1, Rbm9, Gabra2, and Gsk3B were all found to be upregulated in mice chronically consuming ethanol. We therefore conclude that the synaptoneurosome preparation will provide us with samples enriched in synaptically localized mRNAs and proteins that will aid our investigation into the underlying molecular alterations that contribute to behavioral sensitization in response to repeated ethanol. Supported by NIAAA grants P20 AA017828, U01 AA016667 and U01 AA016662 to MFM and by NIDA grant T32DA007027 to MAO.

0716

REGIONAL GENE EXPRESSION CHANGES ASSOCIATED WITH INCREASED VOLUNTARY ETHANOL CONSUMPTION DUE TO DEPRIVATION IN C57BL/6J AND C57BL/6NCRL MICE

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Ethanol interacts with diverse cellular targets to cause changes in gene expression that produce changes in neuronal function and structure, which lead to dependence. Adaptations associated with dependence have been described, including changes in gene expression and protein function, but the transition to dependence is complex and not well characterized. This transition can be modeled using ethanol deprivation of various lengths and schedules, which produces progressive increases in voluntary ethanol consumption over time. This study examined regional changes in gene expression associated with ethanol deprivation using microarrays and quantitative PCR (qPCR) in two closely related strains of mice, and analyzed these changes for gene ontology (GO) terms, canonical signaling pathways, transcription factor binding sites, and coordinately regulated gene networks. Strains used were C57BL/6J and C57BL/6NCrl, which differ in basal and deprivation-induced ethanol intake. Brain regions examined lie along the mesocorticolimbic reward pathway: nucleus accumbens (NAC), prefrontal cortex (PFC), and ventral tegmental area (VTA). Microarray analysis identified nearly 1000 transcripts significantly regulated by one ethanol deprivation across brain regions, with minimal overlap between regions. Regulation of several transcripts was verified by qPCR, including components of voltage-gated calcium channels and glutamate receptors, and regulators of RNA processing and chromatin remodeling. Over-represented GO terms converged on a few areas, including ion transport, gene expression and mRNA processing, and control of cell fate. Several canonical signaling pathways were identified: cAMP-mediated signaling, IGF-1 signaling, CRH signaling, and calcium signaling in NAC, insulin receptor signaling and MAPK signaling in PFC, and EGF signaling in VTA. Binding sites for 3 transcription factors were over-represented across all brain regions: sox17, elf5, and mzf1. Novel gene networks were constructed for NAC and PFC to provide further insight into the effects of deprivation on cell signaling. Finally, genes were ranked based on a protein-protein interaction database to prioritize those with likely biological relevance for further study. Genomic study of regional changes in gene expression associated with ethanol deprivation can identify novel targets for therapies for alcoholism, and illuminate the ways in which substance abuse produces long-lasting changes in behavior.

0985

ETHANOL REGULATION OF SERUM GLUCOCORTICOID KINASE 1 SGK1

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Microarray studies have shown that serum and glucocorticoid-regulated kinase 1, Sgk1, is upregulated by acute ethanol in prefrontal cortex (PFC) of DBA/2J (D2) mice. Sgk1 is a glucocorticoid responsive gene known to modulate synaptic plasticity. Since Sgk1 is a glucocorticoid responsive gene up-regulated by ethanol and ethanol activates the hypothalamic pituitary adrenal (HPA) axis leading to glucocorticoid release, we hypothesized that Sgk1 induction through a HPA axis mediated mechanism may play an important role in modifying behavioral responses to ethanol. Sgk1 mRNA and protein levels were measured using Q-rtPCR and western blotting, respectively. The role of HPA axis signaling in ethanol regulation of Sgk1 was determined through Q-rtPCR comparison of Sgk1 expression in adenalectomized (Adx) vs. sham-treated D2 mice. An adeno-associated virus expressing FLAG-Sgk1, an epitope-tagged version of Sgk1, was used to overexpress Sgk1 in the PFC of D2 mice and determine effects on ethanol locomotor activation and sensitization. We found that Sgk1 mRNA levels were significantly increased 2 and 4 hours post-ethanol treatment but that after ethanol sensitization, Sgk1 expression is blunted. Studies quantifying Sgk1 protein levels and Sgk1 phosphorylation at serine 422 (P-S422), a site known to be involved in the protein's activation, are ongoing. Sgk1 P-S422 is significantly increased 2 hours following acute ethanol treatment. Furthermore, Adx mice showed significantly lower Sgk1 mRNA levels compared to sham mice. Mice overexpressing FLAG-Sgk1 showed a trend towards greater locomotor activation on day 3 of ethanol sensitization studies, but do not sensitize, unlike control mice. Therefore, acute ethanol regulates Sgk1 phosphorylation and mRNA abundance and this may have implications for behavioral responses. Furthermore, Sgk1 induction is possibly due to a HPA axis mediated mechanism. Our results also suggest that the HPA axis may be an important mechanism underlying ethanol regulation of brain gene expression.
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0989

MYELIN GENE EXPRESSION: IMPLICATIONS FOR ALCOHOL ABUSE AND DEPENDENCE

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Prior gene expression studies from our laboratory has demonstrated differential myelin associated gene expression (MAGE) in the medial prefrontal cortex (PFC) as one potential mechanism influencing acute ethanol behaviors between C57BL/6J (B6) and DBA2/J (D2) mice. We and others have also shown that MAGE is reduced in PFC of alcoholics, strongly suggesting that ethanol regulation of MAGE seen in mice may be relevant to behaviors seen in alcoholism. Analysis of basal MAGE across the BXD recombinant inbred (RI) panel, derived from B6 and D2 mice, supported MAGE's impact on ethanol behaviors revealing a densely correlated myelin gene network associated with several ethanol behavioral phenotypes. Literature association tools identified Fyn kinase as potential mechanism regulating MAGE. Fyn knockout mice are known to be more sensitive to the sedative-hypnotic properties of ethanol using the loss of righting reflex (LORE). Therefore we conduced expression profiling across the PFC of Fyn knockout mice to test the hypothesis that MAGE may be an underlying molecular phenotype for the LORE in Fyn null mice. Gene ontology analysis determined a null mutation for Fyn caused a significant decrease in MAGE, suggesting MAGE may be an underlying molecular phenotype for LORE. In support of this premise, microarray analysis of genetic variance in LORE across the Inbred Long Sleep and Inbred Short Sleep mice, as well as congenic mice for the Lore5 quantitative trait locus, also demonstrated an inverse relationship between MAGE and duration of LORE. We further tested this hypothesis using the cuprizone-induced model of reversible demyelination in B6 mice. Cuprizone-treated mice had a significantly greater duration in LORE ($p < 0.01$), demonstrating that myelin is an important contributor to the genetic variance in LORE. Thus, through the use of genetic, genomic, and pharmacological tools we have 'molecular triangulated' a myelin gene network as a contributory element influencing acute ethanol behavioral sensitivity. The ability of myelin to alter acute ethanol sensitivity may warrant a prospective study of myelin in humans as a predictive molecular phenotype for an individual's risk of developing alcohol dependence. Additionally, further genomic dissection of MAGE architecture and associated networks may aid in the development of novel pharmacotherapies for treating and preventing an alcohol use disorder. Supported by NIAAA grants U01 AA016667 to MFM and F31 AA018615 to SPF.

0990

GENETIC DISSECTION OF AN ACUTE ETHANOL-INDUCED ANXIOLYSIS QTL

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Risk for developing alcoholism is determined by a combination of an individual's genetic makeup, environment, and neuroadaptations that occur following acute and chronic exposure to alcohol and there remains a need to understand the biological and genetic bases of risk for developing different stages of the disease. Alcoholics frequently self-report anxiety and stress as motives for drinking and it has been hypothesized that ethanol's ability to relieve stress may contribute to initiation of drinking, development of excessive drinking, and/or its reinforcing effects and relapse. One focus of our laboratory is to elucidate the genetic architecture underlying the acute anxiolytic-like response to ethanol. To do so, we have utilized two parental inbred strains of mice, C57BL/6J (B6) and DBA2/J (D2), BXD (B6 x D2) recombinant inbred (RI) and advanced recombinant inbred (ARI) strains. Following an acute dose of saline or ethanol, mice were assayed in the light-dark box (LDB) model of anxiety and genetic mapping of an acute anxiolytic-like phenotype was performed. Those studies identified and confirmed a quantitative trait locus (QTL) on chromosome 12, deemed Etanq1 (ethanol induced anxiolysis QTL). Using ARI strains, our laboratory has narrowed the support interval for Etanq1 to a 3.4Mb region containing 48 genes. Four genes have putative cis eQTL and functional coding polymorphisms between the B6 and D2 strains, making those our top-priority quantitative trait genes (QTGs). We have performed allele-specific sequencing of one QTG, ninein, in the nucleus accumbens (NAc) of B6D2F1 hybrid mice to confirm the cis eQTL. As expected, NAc mRNA of B6D2F1 mice contains a significantly higher ratio of the D2 sequence versus B6 for ninein. Etanq1 significantly correlates inversely (Pearson's r =)0.678, p = 4.49E-6) to basal NAc expression levels of ninein. An arginine-conjugated 29 amino acid sequence from the rabies virus glycoprotein (RVG-9R) was used to selectively knock down ninein in the central nervous system of D2 mice. We predict that ninein knockdown will cause an increased anxiolytic-like responses to ethanol and preliminary data support this hypothesis. This work will increase our understanding of the anxiolytic-like response to acute ethanol and may lead to new approaches for intervention in alcoholism. Supported by NIAAA P20AA017828 to MFM and NIDA T32DA007027 to WLD.

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INTRA- AND INTERREGIONAL ETHANOL RESPONSIVE GENE NETWORKS OF THE MESOLIMBOCORTICAL DOPAMINE SYSTEM

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The prefrontal cortex (PFC), nucleus accumbens (NAc) and ventral midbrain (VMb) are key brain regions that comprise the mesolimbocortical dopamine system. Previously, we have demonstrated that the transcriptomes of these three regions are robustly altered by exposure to acute ethanol in naive BXD recombinant inbred (RI) strains. As initial level of response (LR) to ethanol is a heritable trait that predicts long term risk for alcohol use disorders (AUDs), acute ethanol induced gene expression changes within these regions may represent key intermediate phenotypes that stand between AUD susceptibility and the causal genetic variants. Here, we attempted to reconstruct the biological pathways underlying LR variability by identifying ethanol responsive gene (ERG) networks within and across the PFC, NAc and VMb. Each ERG network represents a group of genes that exhibit a tightly correlated transcriptional response to acute ethanol across all profiled RI strains. Intra-region ERG networks were significantly enriched for genes involved in nervous system development and synaptic transmission. While characterizing intraregion ERG networks provides valuable insights into region-specific consequences of acute ethanol exposure, many of the key neuroadaptations associated with the development of AUDs and addiction to other drugs of abuse are attributed to changes that alter communication between brain regions. We therefore constructed inter-region ERG networks, composed of genes whose response to acute ethanol is correlated across brain regions, in order to identify ethanol sensitive pathways that connect regions of the mesolimbocortical system. The topology of these interregion ERG networks indicated that the ethanol response of several region-specific gene modules were driven by changes in the expression of highly influential hub genes that originated elsewhere. Furthermore, we found that the frequency of NAc/PFC and NAc/VMb connections were approximately equal, while PFC/VMb connections were the least common. Interestingly, an inordinate number of hub genes originated from the PFC. These results will provide novel information about the molecular pathways that underlie acute ethanol sensitivity and may provide novel AUD susceptibility candidate genes. Supported by NIAAA grants P20 AA017828, U01 AA016667 and U01 AA016662 to MFM and NIMH training grant MH-20030 supporting ARW.