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STRESS

"INIA: STRESS, ANXIETY AND ALCOHOL ABUSE"

Abstracts

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GABAA RECEPTOR PLASTICITY IN THE HIPPOCAMPUS OF SOCIALLY ISOLATED C57BL/6J MICE EXPOSED TO VOLUNTARY ETHANOL CONSUMPTION
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PRENATAL STRESS INCREASES ALCOHOL-SEEKING AND ALTERS GLUTAMATE SIGNALING IN THE NUCLEUS ACCUMBENS
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IDENTIFICATION OF QTLS FOR DEOXYCORTICOSTERONE LEVELS ACROSS THE BXD RI MICE: A PUTATIVE NEUROACTIVE STEROID BIOMARKER FOR ALCOHOL PHENOTYPES

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CHRONIC ETHANOL SELF ADMINISTRATION BUT NOT EARLY LIFE STRESS ALTERS CORTICOTROPIN RELEASING HORMONE RECEPTOR BINDING DENSITY IN MONKEY AMYGDALA

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CHRONIC ETHANOL ALTERS GABAA RECEPTOR AND ALPHA SUBUNIT DENSITY IN THE MONKEY SUBICULAR COMPLEX

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A MONOSODIUM GLUTAMATE-FADE INCREASES VOLUNTARY ETHANOL CONSUMPTION IN THE ETHANOL 'NON-PREFERRING' DBA/2J MOUSE LINE

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EFFECTS OF CHRONIC ETHANOL AND WITHDRAWAL ON FEEDFORWARD AND FEEDBACK GABAERGIC INHIBITION IN THE BASOLATERAL AMYGDALA

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AGE OF ONSET OF ETHANOL SELF-ADMINISTRATION AND RISK FOR HEAVY DRINKING IN MONKEYS
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EFFECTS OF CHRONIC ETHANOL EXPOSURE AND ETHANOL DRINKING ON GLUTAMATE LEVELS IN THE ACCUMBENS OF C57BL/6J MICE

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ETHANOL INCREASES MATRIX METALLOPROTEASE RELEASE IN HIPPOCAMPUS: POSSIBLE ROLE FOR ASTROGLIA IN ETHANOL-INDUCED PLASTICITY OF GLUTAMATERGIC SYNAPSES

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OF MICE AND MONKEYS – EXPRESSION NETWORKS CORRELATING WITH ETHANOL DRINKING BEHAVIOR ACROSS SPECIES
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OVER-EXPRESSION OF GSK-3b IN PFC INCREASES VOLUNTARY ALCOHOL DRINKING AND WITHDRAW-INDUCED ANXIETY IN MICE
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MICROARRAY ANALYSIS OF ACUTE ETHANOL ACTION IN THE MESOCORTICOLIMBIC SYTEM OF FYN KINASE KNOCKOUT MICE
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ETHANOL REGULATION OF SERUM GLUCOCORTICOID KINASE 1 (SGK1)
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Italy

GABAA RECEPTOR PLASTICITY IN THE HIPPOCAMPUS OF SOCIALLY ISOLATED C57BL/6J MICE EXPOSED TO VOLUNTARY ETHANOL CONSUMPTION

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Social isolation (SI) is a model of prolonged mild stress that has been shown to be associated, in the rat, with marked behavioral alterations, a decrease in brain and plasma concentrations of neuroactive steroids, and an abnormal response to acute stressful stimuli as well as an increased neurosteroidogenic effect induced by the acute administration of ethanol (EtOH). We here used social isolation in the C57BL/6J mouse strain to investigate the effect EtOH in the free-choice drinking paradigm on gene expression and function of GABAA receptor (GABAAR) in the hippocampus. Socially isolated (SI) and group-housed (GH) mice were exposed for 6 weeks to the two-bottle choice (EtOH vs. water). Both groups of animals were given, during the whole period of isolation free access to EtOH for 2 hour in their home cage, beginning at 0.5 hour prior the start of the dark cycle. Mice from both experimental groups were individually housed for the 2-h procedure. Specific GABAAR subunits expression were measured by RNase protection assay and immunohistochemistry. GABAAR function was evaluated by conventional whole-cell patch clamp recording in brain slices. We found a significant increase in the abundance of both a4 and d subunits of the GABAAR in the hippocampus of SI mice (+20 and 26% respectively $p < 0.001$) compared to GH animals. On the contrary the abundance of the a1 subunit mRNA was unchanged in SI mice as compared to GH mice. Voluntary EtOH drinking resulted in a marked increase (89%, $p < 0.01$) in d subunit mRNA levels in GH mice, whereas in SI animals, it completely abolished the increase in a4 subunit mRNA but did not alter that of the d subunit with respect to the SI mice. Parallel changes in a4 and d subunit peptides were observed by immunohistochemistry. Patch clamp recording in dentate gyrus granule cells obtained from SI mice revealed a greater enhancement of tonic currents induced by THIP compared to that in GH animals. Voluntary EtOH consumption reduced the increase in tonic current associated with social isolation. These results suggest that voluntary EtOH drinking in SI mice has a selective influence on a4 subunit since blocks its enhanced expression but fails to alter the up-regulation of d subunit.

CHANGES IN GABA-A RECEPTOR GENE EXPRESSION AND FUNCTION IN THE CENTRAL AMYGDALA OF SOCIALLY ISOLATED C57BL/6J MICE: EFFECT OF VOLUNTARY ETOH DRINKING

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In the central amygdala (CeA) the inhibitory GABAergic transmission has been suggested to play a role in the expression of emotionality, including behavioral states of fear and anxiety, as well as in mediating certain behavioral actions of acute and chronic ethanol (EtOH) administration. Because stress reduction has long been considered to contribute to EtOHseeking behavior in humans, it was hypothesized that the CeA and its neuronal connections might be sites for the GABA-like effects of EtOH to mediate EtOH self administration. We examined in C57Bl/6J mice that underwent to social isolation for 6 weeks the interaction between stress, GABAergic system and EtOH. Specific immunostaining for the a4 subunit of the GABA-A receptor was significantly reduced (21%, $p < 0.05$) in the CeA of socially isolated mice compared to group-housed controls. The expression of the c2 subunit did not change following social isolation. Socially isolated and group-housed animals were also exposed to 6 weeks to the two-bottle choice (EtOH/water) paradigm. Both groups of mice were given, during the whole period of isolation free access to EtOH/water for 2 hours in their home cage, starting at 0.5 hour prior the beginning of the dark phase. Socially isolated mice consumed greater amounts of EtOH and preferred EtOH over water compared to group-housed animals. The expression of the a4 subunit in the CeA of socially isolated mice that consumed EtOH resulted reduced (15%, $p < 0.05$) compared to group-housed animals exposed to EtOH. The expression of the c2 subunit was significantly reduced by 22% in both groups of animals exposed to EtOH. Whole-cell patch clamp recording of GABAergic spontaneous IPSCs (sIPSCs) in CeA neurons revealed that while the basal values of amplitude, rise time, and decay time of these currents were not altered by social isolation or voluntary EtOH drinking, the frequency of sIPSCs resulted markedly enhanced in socially isolated mice that were exposed to EtOH compared to group-housed. These results suggest that the stress and EtOH self-administration can alter the gene expression the of GABA-A receptor and synaptic function in the CeA.

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California

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Early environmental insults can impact neurodevelopment, resulting in the elevation of an individual's predisposition to a wide range of neuropsychiatric disorders. Preclinical studies have demonstrated that prenatal stress (PNS) has been shown to increase adult responsiveness to stressors and psychomotor stimulant drugs. Here, we examine the impact of PNS on alcohol seeking behavior and consumption and on glutamate neurotransmission within the nucleus accumbens (NAC). Pregnant C57Bl/6J dams were subjected to repeated restraint stress (3 · 1 hour/day) from E14 until birth (PNS) or left undisturbed (control). At 8 weeks of age, 2 male and 2 female offspring were selected from each litter for testing under operant access to alcohol. Mice were initially trained to press a lever for oral sucrose reinforcement (20 ll of 15% w/v sucrose in water) followed by sucrose fading until unsweetened ethanol (20 ll of 10% ethanol in water) served as the final reinforcer. Reinforcement was initially delivered under a fixed ratio 1 schedule that was incrementally increased to FR4. During ethanol reinforcement, there was a significant interaction between PNS and sex with PNS males exhibiting increased responding and consumption compared to control males and PNS females exhibiting similar responding and consumption to control females. We have previously found that PNS induces alterations in basal and drug-stimulated dopamine and glutamate neurotransmission in the NAC and, thus, we also investigated the impact of PNS on glutamate receptors and related scaffolding proteins in this brain region. PNS increased mGluR1, mGluR5, and PKC_ in males and females, whereas, PNS males, but not females, exhibited increases in specific NMDA receptor subtypes and PNS females, but not males, exhibited reduced Homer 1b/c. Given the critical role of Homer scaffolding proteins to integrate glutamate signaling between ionotropic and metabotropic receptors, our combined behavioral and molecular results suggest that PNS increases alcohol seeking in males by increasing glutamate receptor and intracellular signaling molecules whereas PNS in females fails to increase alcohol seeking because increased receptor and signaling molecules are accompanied by a compensatory down-regulation of Homer proteins. These findings indicate that PNS modulates glutamate signaling in a sex-dependent fashion and suggest that this alteration in glutamate function contributes to the vulnerability to alcoholism.

PRENATAL STRESS PRODUCES SEX-SPECIFIC EFFECTS ON ALCOHOL REINFORCEMENT AND INTAKE IN C57BL/6J MICE

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Early environmental trauma can change the course of neurodevelopment, resulting in elevated risks for neuropsychiatric disorders. Prenatal stress (PNS) exposure has been shown to increase adult responsiveness to stressors and stimulant drugs. In this study, we examined the impact of PNS on alcohol seeking behavior and intake. For PNS groups, C57Bl/6J dams underwent repeated restraint stress (3 · 1 h/d) from E14 until birth, while controls were left undisturbed. At 8 weeks of age, 2 male and 2 female pups were selected from each litter, and single housed with ad libitum food and water. Alcohol consumption was first examined under continuous 2-bottle access, with 1 bottle of water and one bottle of 15% alcohol available in the homecage. Mice were also tested under limited access conditions, in which a bottle containing 20% alcohol replaced the homecage water bottle for 2 hour per day, 3 hour into the dark cycle. No significant differences were found between PNS and control mice in either homecage condition, and overall females consumed more alcohol. Mice were also examined under alcohol reinforcement of operant behavior. Initially, they were trained to lever press for an oral sucrose reinforcer (20 ll of 15% w/v sucrose in water) during daily 15 minute sessions. This was followed by sucrose fading until unsweetened ethanol (20 ll of 10% ethanol) served as the final reinforcer. Then, the fixed ratio schedule of reinforcement was incrementally increased from FR1 to FR4, followed by extinction of operant responding. Overall, on the FR1 schedule, PNS mice consumed significantly more sucrose and ethanol reinforcers than controls. Female PNS mice had increased active lever responding compared to female controls during sucrose reinforcement, while male PNS mice had increased responding compared to male controls during FR1 ethanol reinforcement. On an FR4 schedule, PNS males exhibited both greater active lever responding and greater ethanol consumption than control males, but no differences were apparent in females. During the first 2-day bin of extinction, there was a significant interaction between sex and PNS status, with PNS males and control females responding more on the active lever than other groups. These findings indicate that PNS affects the motivation for alcohol in a sex-specific fashion, highlights how task demands impact alcohol consumption, and provides support for the idea that early stressors may contribute to the development of alcohol use disorders.

Maryland

CHRONIC EXPOSURE TO ETHANOL ALTERS NEUROTRANSMISSION ONTO MEDIUM SPINY NEURONS OF THE MONKEY AND MOUSE STRIATUM

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The dorsal striatum plays an important role in the execution of movement and cognitive functions, such as associative and habit learning. It receives convergent glutamatergic afferents from the cortex and thalamus as well as dopaminergic afferents from the substantia nigra. GABAergic medium-sized spiny neurons (MSNs) constitute 95% of the total striatal neurons and are the principal output neurons of the striatum. Although 20% of striatal synapses are GABAergic, these synapses play a large role in controlling the effects of the excitatory innervation and therefore shape the output of the striatum. The dorsal striatum is likely to be an important site of alcohol actions leading to alcohol dependence, tolerance, and relapse after withdrawal. Extensive evidence suggests that chronic exposure to ethanol (EtOH) alters the balance of inhibitory and excitatory neurotransmission in areas of the brain such as the cortex, hippocampus and cerebellum; however knowledge of its effect in the striatum is lacking. Therefore we hypothesized that acute and chronic EtOH alters both GABAergic and glutamatergic neurotransmission onto MSNs of the striatum in both rodent and nonhuman primate. To test our hypothesis, we used whole-cell patch clamp electrophysiology in acute brain slices containing the striatum obtained from either adult male C57Bl/6J mice (~20 weeks old) or cynomolgus monkeys (7–9 years old) to examine the neuroadaptive changes onto MSNs of the dorsal striatum. In a series of current-clamp experiments, hyperpolarizing and depolarizing current steps were used to investigate intrinsic physiological properties of MSNs. Membrane properties of MSNs are comparable across species with similar resting membrane potential and action potential frequency. However in MSNs recorded from chronic EtOH exposed animals, the resting membrane potential and the voltage threshold to elicit an action potential were more depolarized in MSNs recorded from their control counterparts. The frequency of action potentials was not affected by chronic EtOH. Analysis of sEPSCs suggests that the glutamatergic system is largely unaffected by chronic EtOH exposure. On the other hand, chronic EtOH exposure decreases GABAergic transmission, most likely via a decrease in GABA release manifested as decreased sIPSC frequency. We are currently examining if chronic exposure to EtOH alters the effects of acute EtOH or produces changes in the cannabinoid modulation of neurotransmission.

PRENATAL ETHANOL EXPOSURE DISRUPTS SUBSEQUENT HABIT FORMATION IN ADULT MICE

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Prenatal ethanol exposure-induced changes in brain developmental processes contribute to learning deficits in adulthood. Our preliminary work suggests that prenatal exposure to moderate doses of ethanol alters dorsal striatum cannabinoid (CB1) receptor function. Interestingly, both dorsal striatal function and CB1 receptors are necessary for habit formation (Yin et al., 2004; Hila´ rio et al., 2009). Previous studies show that while ratio schedules of reinforcement predispose goal-directed actions which are sensitive to changes in response outcome contingency and expected outcome value, habit formation is more readily seen under interval reinforcement schedules and is less sensitive to alterations in outcome value. In the current experiments, we examined whether prenatal ethanol exposure would alter goaldirected or habitual behaviors. C57Bl/6J mice were either exposed to air or ethanol (approximately 190 mg/dl) vapor throughout gestation till P7. In adulthood, we trained the same subjects in an instrumental lever-pressing task using ratio and interval schedules in different contexts, so that schedules were distinguished by different contextual cues. Mice were trained to press a single lever for a reinforcer (pellets or sucrose), with the remaining reinforcer freely available in the home cage. Every day mice were trained in both schedules: upon completion of one schedule, mice were immediately trained in the remaining schedule, with schedule order counterbalanced across subjects. Following acquisition, mice were given a devaluation test (2 days) where either the reinforcer earned from lever pressing or the home-cage reinforcer was devalued via ad libitum exposure immediately before serial extinction tests in each context. Following devaluation treatment, when tested in the ratiotrained context prenatal ethanol exposure did not alter sensitivity to changes in the value of the earned reinforcer, as both groups showed goal-directed behavior. However, when tested in the interval-trained context that produced habitual responding in controls, prenatal ethanolexposed mice exhibited goal-directed behavior. These results suggest that prenatal ethanol exposure induces changes that may alter dorsal striatal circuits mediating habit and goal learning perhaps through a CB1 receptor mechanism. We are currently examining whether there are prenatal ethanol exposure-induced changes in dorsal striatum neural activity that underlie the shift from habitual to goal-directed behaviors.

PRENATAL ETHANOL EXPOSURE CAUSES DYNAMIC CHANGES IN THE GABAERGIC SYSTEM THROUGHOUT DEVELOPMENT OF THE MOUSE STRIATUM

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Ethanol is the most frequently used drug whose abuse during pregnancy leads to birth defects, as well as physical, mental, and behavioral disabilities, referred collectively as Fetal Alcohol Spectrum Disorder (FASD). Since the dorsal striatum plays a large role in the execution of movements as well as associative and habit learning, disruptions during development may lead to the behavioral abnormalities associated with FASD. There is extensive evidence demonstrating the effects of prenatal ethanol exposure on brain development specifically in the cortex, hippocampus and the cerebellum. However effects of fetal alcohol exposure on striatal development are not well characterized. We hypothesized that chronic ethanol exposure alters the development of the GABAergic system of the dorsal striatum. To test this we employed transgenic mice in which eGFP is under the control of the promoter for the 65-kDa isoform of glutamic acid decarboxylase (GAD65), one of the enzymes responsible for synthesizing GABA. In the striatum of this mouse line, eGFP expression is limited to medium-sized GABAergic spiny neurons (MSNs). Dams were exposed to either air or ethanol vapor from embryonic day 0.5 - postnatal day (P)10. Offspring were sacrificed at ages ranging from P0-P30. Data obtained from whole-cell patch clamp electrophysiology of mechanically dissociated MSNs from the dorsal striatum suggests an ethanol-induced developmental shift in the expression of GABAA receptors. At P0, ethanol exposure leads to an increase in apparent GABA efficacy, suggesting increased receptor number. However after P7, GABA concentration-response curves derived from MSNs of ethanol-exposed pups reveal decreases in apparent efficacy and affinity compared to controls, suggesting a decrease in the expression of functional GABAA receptors of the same isoform, different isoforms, or both. MSNs recorded in acute brain slices of ethanol-exposed animals display decreased frequency and amplitude of sIPSCs, suggesting a decrease in presynaptic GABA release and postsynaptic receptors, respectively. Acute exposure to 50 mM EtOH decreased the amplitude and frequency of sIPSC recorded in control MSNs. This effect was eliminated in MSNs recorded from slices of prenatal ethanol-exposed pups. Ongoing experiments are designed to assess whether chronic ethanol exposure alters the developmental distribution of subsets of striatal neurons and the expression of GABAA receptor isoforms.

LONG-LASTING ETHANOL POTENTIATION OF GABAERGIC SYNAPTIC TRANSMISSION MODULATED BY 5-HT3 RECEPTORS IN MECHANICALLY ISOLATED HIPPOCAMPAL CA1 NEURONS

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The neurotransmitter serotonin (5-HT) has been implicated in the neural actions of EtOH and in modulation of GABA release from presynaptic terminals. In the present study, we investigated EtOH modulation of GABA release induced by 5-HT3-R activation. Mechanically isolated neuron/bouton preparations from rat (postnatal days 13–17) CA1 hippocampal subregion were used to record spontaneous GABAergic inhibitory postsynaptic currents (sIPSCs) in the presence and absence of EtOH. Immunocytochemistry of the isolated neuron/bouton showed that fluorescent puncta immunopositive for the presynaptic protein synapsin I were observed apposed to the soma and dendrites of these neurons, and were found to colocalize with glutamic acid decarboxylase 65/67 (GAD 65/67) and GABA transporter 1 (GAT1). In the majority of cells, 5-HT transiently increased the frequency of sIPSCs, but not the amplitude, indicating a presynaptic action of 5-HT. This effect was blocked by the selective 5-HT3 receptor antagonist MDL72222 and mimicked by a selective 5-HT3 receptor agonist, m-chlorophenylbiguanide (mCPBG). Preincubation in EtOH (80 mM) produced >3 · increases in the frequency and the charge transfer of IPSCs in the presence of mCPBG compared with initial responses to mCPBG alone. Interestingly, this potentiation of the response to mCPBG was maintained even after washout of EtOH. This effect was seen at ethanol concentrations as low as 10 mM. Calcium imaging was performed to monitor the change of Ca2 + concentration during the 5-HT3-R activation and/or EtOH exposure. After loading Ca2 + indicator (Fluo-4AM) into the isolated neuron/bouton preparation, the postsynaptic cell was patched to dilute dye in the postsynaptic cytosol as well as for recording sIPSCs. The dilution in the postsynaptic cell enabled selective visualization presynaptic puncta. Fluorescence was monitored using an EMCCD (electron multiplying charge-coupled device) camera mounted on an inverted microscope with a 60 · 1.4 N.A. objective. In a subpopulation of puncta, spontaneous increases in fluorescence intensity were observed, indicating Ca2 + entry into the presynaptic terminals. The number and the fluorescence intensity of puncta increased during EtOH (80 mM) exposure. These findings indicate that ethanol can potentiate 5-HT3-induced GABA release, perhaps by modulating calcium entry into presynaptic terminals.

ADOLESCENT EXPOSURE TO RITALIN MODIFIES THE STRIATAL NEURONAL RESPONSE TO ETHANOL IN ADULT RAT

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Ritalin (methylphenidate, MPH) is a psychostimulant frequently prescribed to children for the treatment of ADHD. The aim of this study is to elucidate how chronic adolescent administration of MPH may affect synaptic transmission and future susceptibility to alcohol addiction in adulthood. Field potential recordings in dorsolateral striatum (DLS) were carried out to examine the effects of ethanol on synaptic efficacy in MPH treated rats. This striatal subregion has important roles in habit formation and drug addiction. Field potential recordings estimate relative synaptic efficacy by measuring the amplitude of the population spike (PS). Rats were administered 2 mg/kg MPH orally for 28 days (PD30), mimicking consumption in humans (Kuczenski & Segal, 2005). One week after the completion of MPH administration, 350 lM coronal slices of the DLS were obtained. Striatal field PS recordings were elicited using single stimulus pulses (intensities of 0.1–1.5 mA) 1-ms duration, every 30 seconds. Input/Output curves, designed to show the efficacy of synaptic responding, showed differences between groups. MPH rats showed a decreased PS in response to stimulation, indicating that chronic blockade of the dopamine transporter may result in long-term changes of striatum neurons. For EtOH experiments, following 10 minute of stable baseline recording EtOH (50 mM) was applied to slices for 20 minutes. Comparison of MPH and saline treated rats show that MPH treatment had clear effects on the depression of PS amplitude produced by EtOH. In saline treated rats EtOH did not produce a significant change from baseline PS amplitude, as seen previously in other studies (Adermark, 2009). However, in MPH treated animals, EtOH induced a significant decrease in PS amplitude suggesting alterations in synaptic transmission. The EtOH effect was most evident approximately 40 minutes after beginning EtOH application. The effect was reversible during washout in saline rats only. Upcoming experiments are designed to examine if alterations in striatal physiology and pharmacology have behavioral correlates. Briefly, 2-bottle choice EtOH drinking and EtOH conditioned place preference tests will be conducted to assess whether chronic MPH treatment results in altered preference for the drug. Findings to date indicate that adolescent MPH exposure produces a neurodevelopmental change in striatal physiology and response to EtOH. The precise nature of these modifications deserves further investigation

GABRG1 AND GABRA2 MARKERS MODERATE THE CARDIOVASCULAR EFFECTS OF ALCOHOL

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Risk of alcohol dependence (AD) has a substantial genetic component. Alcohol exerts many of its effects via interactions with gamma-aminobutyric acid (GABA) receptors. AD is associated with markers in the GABAA c-1 receptor subunit gene (GABRG1) and the adjacent gene encoding the GABAA a-2 receptor subunit (GABRA2). Because increases in cardiovascular reactivity to alcohol are hypothesized to be associated with the reinforcing effects of alcohol, we determined if cardiovascular response to acute alcohol administration is related to variation in GABAA-receptor subunit genes.

Methods: 31 healthy social drinkers [71% males, 87% Caucasian, 23.9 years (SD = 2.8)] participated in an alcohol sensitivity challenge session. Subjects received a placebo drink at time 0, followed by three equal alcohol doses at 45 minute intervals, achieving a mean peak blood alcohol concentration (BAL) of 105 mg/dL (SD = 25). The major outcomes were: heart rate (HR), which was continuously recorded during the session; systolic (SBP) and diastolic blood pressure (DBP) which were obtained every 5 minutes for the session duration. Genotype effects were analyzed using longitudinal analysis by GEE methods, controlling for body mass index, time and BAL.

Results: HR: subjects homozygous for the AD associated allele for single nucleotide polymorphisms (SNPs) in the GABRG1 and intergenic region demonstrated on average significantly higher HR response (rs1497577 $p = 0.04$; rs7654165 $p = 0.04$) compared to those heterozygous and homozygous for the common allele. In addition, subjects homozygous and heterozygous for the minor allele for SNPs in the GABRA2 region showed on average higher HR response compared to those homozygous for the common allele (rs3849591 $p < 0.01$). SBP: subjects homozygous and heterozygous for the AD associated and minor allele for SNPs in the GABRA2 region demonstrated on average increased SBP effects (rs279858 $p = 0.04$; rs1025852 $p = 0.02$) compared to those homozygous for the common allele. DBP: subjects heterozygous for the minor allele for SNPs in the intergenic region 5¢ GABRG1–3¢ GABRA2 showed on average higher DBP responses compared to those homozygous for the common allele (rs17536530 $p < 0.01$). Conclusion: Our results suggest that the proposed variations modulate cardiovascular responses to alcohol and thus may enhance susceptibility to AD.

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EFFECTS OF NALTREXONE AND BACLOFEN ON EXTINCTION OF ALCOHOL SEEKING RESPONDING IN BABOONS

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The extent to which stimuli paired with alcohol consumption come to control behaviors previously associated with alcohol (alcohol-seeking) can be used to as indices of ‘‘craving’’ and relapse. The treatments for alcohol dependence and relapse would ideally include drugs that facilitate extinction of these responses. In the present study, baboons responded according to a chained schedule of alcohol reinforcement consisting of three separate ‘‘linked’’ components, each associated with distinct stimuli and different behavioral contingencies leading to the opportunity to self-administer alcohol (4% w/v). After alcohol selfadministration
had been maintained and stable, test sessions preceded by acute
administration of naltrexone (NTX; 0.32 – 3.2 mg/kg, IM, 5 minute pretreatment), baclofen (BAC; 0.1 – 2.4 mg/kg, IM, 60 minute pretreatment) and their vehicles were conducted to determine if behaviors associated with gaining access to alcohol (seeking) were altered by NTX and BAC. During test sessions, extinction was in effect in the seeking component and responding did not result in access to alcohol; sessions terminated after 30 minutes elapsed during which there was no responding. During the NTX experiment, the mean number of extinction responses during vehicle administrations ranged from 771.3 – 4163.3 across baboons (N = 4). Acute administration of NTX reduced the number of extinction responses. Paired t tests of the completed doses (0.32 – 1.0 mg/kg) confirmed that administration of 1.0 mg/kg NTX significantly decreased extinction responding compared to vehicle ($t = 3.921$; $df = 3$; $p < 0.03$). Preliminary results suggest that BAC does not alter extinction responding. These results indicate that pretreatment with NTX, but not BAC, facilitates extinction of seeking responding in the context of ongoing alcohol self-administration. (Supported by NIAAA R01AA015971 and the Peter F. McManus Trust)

STRIATAL DOPAMINE RELEASE IS COMPROMISED IN ALCOHOL-DEPENDENT SUBJECTS

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Dopamine is a major neurotransmitter in the response to addictive drugs, and dopamine function may be compromised after extended drug use. We investigated the role of the dopamine as a neurochemical correlate in alcohol dependency. PET measurements of dopamine release were carried out in 19 subjects by using 11C raclopride displacement via an intravenous amphetamine challenge. The primary subjects were mostly male, all with a DSMIV diagnosis of alcohol dependence (AD). They were compared to 15 age-matched healthy controls. Binding potentials were obtained by both the simplified tissue method (SRTM) and the Logan Reference Tissue Graphical Method (RTGA). We found significant decreases in amphetamine-induced dopamine release in our AD subjects compared to the control subjects, which ranged from 30% less release in putamen and ventral striatum to 89% less release in posterior caudate using RTGA. Binding potential baseline (using RTGA) was significantly decreased in all striatal subdivisions in the AD subjects by 14–19% as compared to controls. With SRTM, baseline BPND decreased by 10–16%. The reduction in binding potential was highest in the posterior caudate. We conclude that the dopamine system is highly compromised in AD. At risk AD subjects and DA release progress will also be discussed.

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North Carolina

CHRONIC ALCOHOL EXPOSURE IN MONKEYS, RATS AND MICE REDUCES DOPAMINE SIGNALING IN STRIATUM

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Existing data indicate that alcohol affects DA neurotransmission, however, questions remain about the effects of chronic exposure on the delicate equilibrium between such neurochemical processes as DA release and uptake. Dysregulation of these processes in the mesolimbic and nigrostriatal systems after chronic alcohol exposure could be a neuroadaptation contributing to dependence. We have examined the effects of chronic alcohol administration on striatal DA dynamics in monkeys, rats and mice. DA release, uptake, and activity of D2-type presynaptic autoreceptors were measured using voltammetry in nucleus accumbens and dorsal striatum in all 3 species. Cynomolgus macaques voluntarily drank alcohol for more than 12 months in Dr. Kathleen Grant's laboratory, and for the rat and mouse studies, we employed a chronic intermittent alcohol vapor inhalation model of alcohol administration. We found that chronic alcohol exposure enhanced DA uptake rates, decreased release and increased the sensitivity of D2 autoreceptors. Although the exact DA changes were variable between the different species studied, all appear aimed at reducing DA signaling. This is in opposition to the acute DA-elevating effects of alcohol and would serve to limit excess DA in the extracellular space.

IDENTIFICATION OF QTLS FOR DEOXYCORTICOSTERONE LEVELS ACROSS THE BXD RI MICE: A PUTATIVE NEUROACTIVE STEROID BIOMARKER FOR ALCOHOL PHENOTYPES

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GABAergic neuroactive steroids contribute to ethanol actions and regulate stress homeostasis in the central nervous system. Systemic ethanol administration increases plasma and cerebral cortical levels of deoxycorticosterone (DOC) in rodents. Furthermore, dexamethasone suppression of plasma DOC is predictive of subsequent voluntary alcohol consumption in ethanol-naı¨ve cynomolgus monkeys. That is, ethanol-naı¨ve monkeys that are insensitive to dexamethasone drink the most alcohol in a two bottle self-administration paradigm. In addition, dexamethasone suppression of plasma and cerebral cortex DOC levels is blunted in C57BL/6J compared to DBA/2J mice, and may correspond to higher ethanol intake in C57BL/6J mice. DOC levels and DOC sensitivity to dexamethasone were tested across the BXD recombinant inbred (RI) mice in order to model the genetic variability known to exist in the human population. Mice were injected with 0.075 mg/kg dexamethasone sodium salt or saline at 8:00 am and were sacrificed 6 hours later. DOC levels were measured in plasma and cerebral cortex by radioimmunoassay. Basal cerebral cortical DOC levels across 42 BXD strains and the parental strains range between 1.4 and 12.2 ng/g, resulting in a 8.7-fold genetic variation [$F(43,246)=4.33$, $p < .0001$]. Basal plasma DOC levels across 47 BXD strains and the parental strains range between 2.8 and 12.1 ng/ml resulting in a 4.3-fold genetic variation [$F(48,282)=3.69$, $p < .0001$]. Quantitative trait loci (QTLs) for basal DOC levels were identified on chromosomes 4 and 14, respectively in cerebral cortex and plasma. Further, basal DOC levels were correlated with several behavioral measures of alcohol sensitivity across the BXD mice. The dexamethasone-induced changes in DOC levels showed a 4.4-fold variation in cerebral cortex and a 4.1-fold variation in plasma. DOC sensitivity to dexamethasone also correlated with some behavioral measures of alcohol sensitivity across the BXD mice. In summary, this study identified two significant QTLs, associated with basal cerebral cortical and plasma DOC levels across the BXD mice. Furthermore, basal DOC levels were positively correlated with ethanol sensitivity, suggesting that the neuroactive steroid DOC may be a putative biomarker for alcohol responses. Supported by INIA-NIAAA AA013614 (PP), AA016672 and AA010564 (ALM), AA016662 (MFM, EJC), AA13499 and AA017590 (RWW).

DISRUPTIONS IN SEROTONERGIC REGULATION OF CORTICAL GLUTAMATE RELEASE IN PRIMATE INSULAR CORTEX IN RESPONSE TO CHRONIC ETHANOL AND EARLY LIFE STRESS

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Altered activity within the anterior insular cortex (AIC) has been implicated in several nervous system disorders, such as anxiety and addiction. In primates, an increased susceptibility to these pathological states has been shown in a model of early-life stress (ES) that is induced through maternal separation (peer-rearing). Because disruptions in the serotonin system also occur in these disorders, we chose to investigate the expression and function of serotonin receptors in the AIC within the context of ES and chronic alcohol consumption. We found that both ES in peer-reared (PR) monkeys and chronic alcohol consumption in mother-reared (MR; non-stressed) cohorts led to enhanced glutamatergic activity within the AIC. This hyperactivity in MR, alcohol-consuming monkeys was accompanied by an increased regulation of glutamate release through presynaptic 5-HT1A receptors that was not apparent in ethanol-naı¨ve, MR cohorts. In contrast, while chronic alcohol increased the relative expression of 5-HT2A receptors in MR monkeys, the observed AIC hyperactivity mediated solely by early life stress in PR cohorts was accompanied by a decrease in expression of 5-HT1A and 5-HT2A receptors. Finally, the interaction between ES and chronic alcohol consumption resulted in a synergistic enhancement of both 5-HT1A and 5-HT2A receptor mRNA levels such that the decreases in expression between ethanol-naı¨ve, PR and MR monkeys were no longer apparent following chronic alcohol consumption. Our data indicate that chronic alcoholism is partially sustained by increases in AIC activity. In MR animals, this increased AIC activity may be compensated by upregulation of presynaptic 5-HT1A receptors, but in PR animals this mechanism may be less effective. Our results suggest possible mechanisms for increased addiction potential that include disruptions in both AIC activity and serotonin system dynamics resulting from early life stress.

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CHRONIC ETHANOL SELF ADMINISTRATION BUT NOT EARLY LIFE STRESS ALTERS CORTICOTROPIN RELEASING HORMONE RECEPTOR BINDING DENSITY IN MONKEY AMYGDALA

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Early childhood stress can alter brain and neuroendocrine development leading to a higher risk of alcohol abuse and addiction. Corticotropin releasing hormone (CRH), a primary mediator of the stress response in the brain, also plays a significant role in chronic ethanol consumption. The amygdala is involved in the behavioral response to alcohol and may modulate interactions between chronic stress and drug-reinforcement. The goal of this study was to identify how early life stress and ethanol drinking affected CRH receptor function in the amygdala. Using a schedule-induced polydipsia technique, male rhesus macaques [motherreared (MR, n = 4) or peer-reared (PR, n = 4)] were trained to self-administer ethanol (4% w/v) or an isocaloric maltose-dextrin solution [MR (n = 5) or PR (n = 5)]. After induction, the monkeys self-administered ethanol or maltose-dextrin along with concurrently available water during daily 22 hour sessions in their home cages for at least 12 months. At necropsy, brains were processed for in vitro receptor autoradiography. [125I] Sauvagine was used to determine total CRH receptor binding; Astressin and Stressin were utilized as blockers to measure CRH receptor type 1 (CRH-R1) and type 2 (CRH-R2), respectively. Binding was analyzed in the five regions with the densest CRH binding: the magnocellular portions of the basal and accessory basal nuclei, the parvocellular portion of the accessory basal (ABpc) nucleus, the lateral nucleus and periamygdaloid cortex (PAC). Preliminary analysis revealed no significant difference between rearing groups. There was, however, significantly ($p < 0.001$) less dense CRH-R1 binding in ethanol drinking animals. The most robust changes were the significant ($p < 0.001$) decreases in CRH-R1 binding density in the ABpc (49%), lateral nucleus (50%) and PAC (37%). There was no difference of CRH-R2 binding. CRH-R1 is the predominant receptor subtype in the amygdala, and CRH-R1 binding is 50% denser than CRH-R2 binding in control animals. These results indicate that this peer-rearing model of early life stress did not significantly affect CRH receptor binding in the amygdala, though chronic ethanol self administration was shown to lower CRH-R1 binding density in both rearing groups. This study was supported by AA014106, AA015568 and AA007565 (DPF).

CHRONIC ETHANOL ALTERS GABAA RECEPTOR AND ALPHA SUBUNIT DENSITY IN THE MONKEY SUBICULAR COMPLEX

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Chronic ethanol consumption potentiates the effects of GABAergic transmission and has been shown to alter the subunit composition of receptors along with their functional properties in brain regions such as the hippocampus. The present study examines the changes in total GABAA and a1 and 4 subunit containing receptors in the entorhinal cortex and subicular complex 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 8 months without an operant panel or ethanol. At necropsy, brains were processed for in vitro receptor autoradiography. The GABAA receptor agonist [3H]RO15-4513 was used to measure total GABAA receptor density; coincubation in the presence of 75 nM Zolpidem or 100 nM Diazepam allowed us to quantify the densities of the a1or 4 subunits, respectively. Binding was measured in the entorhinal cortex, subiculum, presubiculum and parasubiculum. Two-way ANOVA did not reveal significant differences in GABAA receptor densities between ethanol drinkers and controls in the entorhinal cortex. The subicular complex, however, showed significant differences between ethanol drinkers and controls. There was a main treatment effect in total GABAA receptors, with a lower density in the ethanol group. The a4 subunit also showed a significant treatment effect, with a significantly lower density of receptors in the ethanol group, due largely to a significant difference in the parasubiculum layers I–IV. The a1 subunit shows a significant differences in parasubiculum layers I – IV, with a greater receptor density in the ethanol group. The parasubiculum has reciprocal projections to the anterior nuclei of the thalamus, which are implicated in memory function. It also projects to the entorhinal cortex and could modify hippocampal input. Alterations in inhibitory tone in the parasubiculum could alter hippocampal inputs and disrupt memory processing.

VERVET MONKEYS WITH A LOW BEHAVIORAL AND PHYSIOLOGICAL STRESS RESPONSE TO NOVELTY EXHIBIT HPA AXIS HYPORESPONSIVITY AND INCREASED ALCOHOL CONSUMPTION

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Stressful life events are commonly associated with alcohol abuse and dependence. The relationship between stress and drinking is unclear but research suggests that HPA axis dysregulation may be involved. HPA axis hyporesponsivity has been reported in actively drinking and recently abstinent alcoholics. Because family history positive (FH+) individuals are also hyporesponsive, some of the differences observed in HPA axis function in alcoholics may represent risk factors for abuse and dependence. Nonhuman primates provide unique research opportunities because they faithfully model human drinking, physiology and brain organization allowing for a more direct translation of findings to the human condition. HPA axis function was assessed in 10 ethanol naı¨ve vervet monkeys behaviorally and physiologically characterized for stress response to novelty [high cortisol/high reactivity (HR, n = 5); low cortisol/low reactivity (LR, n = 5)]. Each challenge was administered at least 5 days apart to all monkeys while they were comfortably seated in a primate chair. Three doses of ethanol (0.5, 1.0, 1.5 g/kg) were administered nasogastrically to assess the stimulatory effect of acute ethanol on the HPA axis. Intravenous administration of 1 lg/kg ovine corticotropin releasing hormone (CRH) was used to assess pituitary responsivity and 2 doses (125, 375 lg/kg) of naloxone were used to assess inhibitory opioid input on hypothalamic CRH neurons. Using a 2 · 2 design, half of the monkeys were subsequently trained to self-administer ethanol using a schedule induced polydypsia technique [HR (n = 3), LR (n = 2)]. Acute ethanol administration resulted in a significantly attenuated cortisol response in low compared to high responders at all 3 doses (p £ 0.01). No between group differences were observed in response to CRH or low dose nalxone. The high dose of naloxone, however, resulted in an enhanced cortisol response in LR vs HR animals (p £ 0.01). Over 7 months of voluntary ethanol self-administration, low responders exhibited greater average monthly intake than the high group (p £ 0.01). These preliminary data suggest that monkeys with a low behavioral and physiological stress response to novelty exhibit HPA axis hyporesponsivity and increased ethanol intake similar to that reported in FH+ individuals who are at greater risk for alcohol dependence. These findings suggest that this model may be useful in determining the mechanistic relationships among stress, HPA axis dysfunction and drinking.

A MONOSODIUM GLUTAMATE-FADE INCREASES VOLUNTARY ETHANOL CONSUMPTION IN THE ETHANOL 'NON-PREFERRING' DBA/2J MOUSE LINE

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Voluntary oral ethanol consumption in outbred rodents and non-preferring lines can be initiated by first combining ethanol with a sweetener, typically sucrose or saccharine, and then slowly 'fading' away the sweetener. However, this approach is not as useful for some inbred strains of mice like DBA/2J. Importantly, DBA/2J mice carry a polymorphism in a subunit of the 'sweet' taste receptor which might help explain the contrasting weak voluntary oral ethanol consumption and strong ethanol conditioned place preference in this strain. As an alternative to sucrose, we used a fade from an alternative tastant, monosodium glutamate (MSG). Using a two-bottle home-cage continuous access model, we first defined the most preferred concentration of MSG (100 mM). We next used a single-bottle 'drinking in the dark' protocol to compare the efficacy of MSG-fade to sucrose-fade to initiate voluntary consumption of ethanol across a range of concentrations. We found that, while MSG- and sucrose-fades engendered similar levels of consumption of low ethanol concentrations (5%), MSG-history DBA/2J mice drank significantly more ethanol than sucrose-history mice at higher ethanol concentrations (10% and 15%). For example, MSG-DBA mice drank 1.5±0.2 g/kg/2 hour while sucrose-DBA mice drank 0.7±0.1 g/kg/2 hour ($p < 0.001$, t-test). These findings suggest the potential utility of the MSG-fade to establish stable voluntary oral ethanol consumption in mice, including ethanol 'non-drinking' mice like the DBA2/J strain. Preliminary studies measuring ethanol drinking following physical dependence in these support this conclusion.

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AFFERENT-SPECIFIC ALTERAITONS OF AMYGDALA PRESYNAPTIC GLUTAMATERGIC FUNCTION BY CHRONIC 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 2 major pathways differentiated by their anatomical arrangement and the upstream brain areas giving rise to the pathways. The external capsule (EC) borders the lateral edge of the BLA and brings primarily cortical information from lateral brain areas. Conversely, the stria terminalis/longitudinal association bundle (‘‘medial’’ input) is found along the medial border of the BLA and 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. We were therefore led to investigate glutamate synaptic signaling mediated by the EC and medial glutamatergic afferents following chronic ethanol and withdrawal. Using BLA brain slices and whole cell ‘‘blind’’ patch clamp electrophysiology, this study focused on putative presynaptic measures of glutamate release as represented by synaptic responses to paired electrical stimuli. The preliminary data indicate that alterations in presynaptic glutamate release by withdrawal from chronic ethanol were localized to the medial input only. These data indicate that multiple afferent pathways into the BLA are differentially altered by chronic ethanol and withdrawal. These data also lay the foundation to investigate the mechanisms responsible for this presynaptic alteration. Ultimately, this work highlights the importance of upstream brain regions as they likely impact BLA function during chronic ethanol and withdrawal. This work was supported by NIH grant #AA014445.

EFFECTS OF CHRONIC ETHANOL AND WITHDRAWAL ON FEEDFORWARD AND FEEDBACK GABAERGIC INHIBITION IN THE BASOLATERAL AMYGDALA

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Alcohol withdrawal-induced anxiety is partly mediated by the basolateral amygdala (BLA), the primary input of the anxiety circuit. Within the BLA, GABAergic and glutamatergic systems interact to regulate anxiety-like behaviors. The GABAergic system consists of 2 populations of interneurons: local (feed-back type) and lateral paracapsular cells mass (LPCs; feed-forward type) neurons. Given that principal neuron activity within the BLA is governed by relative contributions from each of these 2 populations, we hypothesize that chronic ethanol and withdrawal may disrupt these interneurons and ultimately alter the balance between the excitatory and inhibitory systems. To test this, animals were exposed to intermittent ethanol vapor inhalation (12 hour on/off) for 10 consecutive days (CIE) or the CIE treatment followed by 24 hours room air alone (WD). Electrophysiological recordings were then performed on BLA principal neurons within brain slices prepared from CIE and WD animals. We found a decreased probability of release (increased paired-pulse ratio) at LPC GABAergic synapses, but not the local GABA synapses, during CIE and WD. Interestingly, LPC GABAergic synapses become less sensitive to the effects of zolpidem, an a1-specific modulator, during CIE and WD. Consistent with the zolpidem experiment, total a1 protein expression measured on western blots is also decreased in CIE and WD animals. Furthermore, while total a4 protein levels remain unaffected by CIE and WD, electrophysiological data suggests decreased benzodiazepine sensitivity at local synapses, but not at LPCs, consistent with increased a4-like contributions. While these data suggest that pre- and post-synaptic changes in BLA GABAergic transmission occur in response to CIE and WD, further investigation of potential mechanisms underlying the observed changes is warranted (i.e. GABAB contributions on release probability at LPC synapses, changes in surface/internal expression of subunits). Nevertheless, it is evident that the 2 BLA interneuron populations are differentially modulated by the CIE exposure and subsequent withdrawal. Supported by AA014445, AA014445 S1, and F31AA017576.

ETHANOL-INDUCED INCREASED EXPRESSION OF a4 GABAA RECEPTOR SUBUNITS IS DEPENDENT UPON PKCc IN CULTURED CEREBRAL CORTICAL NEURONS

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Ethanol exposure and dependence are known to result in elevated expression of a4 GABAA receptor subunits both in vivo and in vitro, but the mechanisms of this regulation remain unknown. To explore the mechanisms of ethanol regulation, we investigated its effects in cultured cerebral cortical neurons, where similar effects are observed. 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 4 hours and a4 subunit expression was assessed following subcellular fractionation. Miniature inhibitory postsynaptic currents (mIPSCs) were assessed using whole cell patch clamp recordings. Ethanol exposure increased a4 subunit expression by 89±14% ($p < 0.01$), which was accompanied by reductions in the mean mIPSC decay time constant 42±15% ($p < 0.05$). Exposure to the general PKC activator phorbol 12, 13-dibutyrate (PDBu) increased a4 expression similar to ethanol, while the general PKC inhibitor calphostin C prevented ethanol-induced increases in a4 expression. In contrast, PKA inhibition using Rp-cAMP did not alter the ethanol-induced increase in a4 subunit expression. To determine which PKC isozymes were involved, we assessed the effect of PKCc and PKCb inhibition. We found that ethanol-induced increases in a4 subunit expression were ablated in cells transfected with siRNA for PKCc compared to cells transfected with scrambled siRNA. However, inhibition of PKCb with PKCb pseudosubstrate did not alter ethanol-induced increases in a4 subunit expression. Overall, these data suggest that PKCc may regulate ethanol-induced increases in a4 subunit expression in cultured cerebral cortical neurons.

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DEVELOPMENT OF AN ADENO-ASSOCIATED VIRAL VECTOR TO INCREASE NEUROACTIVE STEROID PRODUCTION IN SPECIFIC BRAIN REGIONS

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Neuroactive steroids are endogenous neuromodulators synthesized in the brain, adrenal glands, and gonads. Systemic administration of neuroactive steroids can alter alcohol reinforcement and consumption in rodents. Neuroactive steroids that positively modulate c-aminobutyric acid type A receptors produce central nervous system inhibition and anxiolytic effects, which may be therapeutic during alcohol withdrawal. Pregnenolone is the precursor of all other neuroactive steroids. Recent data from our laboratories has demonstrated that systemic administration of pregnenolone decreases alcohol self-administration in alcoholpreferring (P) rats. To produce long term increases in neuroactive steroids an adenoassociated serotype 2 viral vector (AAV2) expressing the mitochondrial cytochrome P450 side chain cleavage (P450scc) enzyme was developed. P450scc initiates the neuroactive steroid synthetic pathway by converting cholesterol to pregnenolone. Overexpression of P450scc should allow us to investigate how sustained increases in steroidogenesis isolated to specific brain regions affect alcohol-related behaviors. In vitro analysis confirmed that cultured cerebral cortical neurons infected with the AAV2-P450scc vector displayed an approximate 94% increase in P450scc mRNA expression and pregnenolone levels were elevated by approximately 530% in the cell media compared to cells infected with a control vector expressing green fluorescent protein (GFP). To confirm AAV2 vector P450scc gene transduction in vivo, we stereotaxically microinjected the AAV2-P450scc or AAV2-GFP vectors bilaterally into the nucleus accumbens shell of wistar rats at a volume of 2 or 4 lL/ hemisphere. One week post-injection animals were sacrificed and the nucleus accumbens was dissected to determine P450scc mRNA and protein expression. The AAV2-P450scc vector increased both P450scc mRNA and protein levels in the nucleus accumbens shell in a vector concentration dependent manner. The maximal increase in P450scc mRNA levels was 573 ± 126% ($p < 0.01$) while P450scc protein was increased by 172 ± 69% ($p < 0.05$). Studies are currently in progress to determine if the AAV2-P450scc vector alters operant ethanol self-administration, general locomotor activity, and anxiety-like behaviors. These experiments may lead to the development of new therapeutic strategies for treating alcoholism.

IDENTIFICATION OF QTLS FOR DEOXYCORTICOSTERONE LEVELS ACROSS THE BXD RI MICE: A PUTATIVE NEUROACTIVE STEROID BIOMARKER FOR ALCOHOL PHENOTYPES

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GABAergic neuroactive steroids contribute to ethanol actions and regulate stress homeostasis in the central nervous system. Systemic ethanol administration increases plasma and cerebral cortical levels of deoxycorticosterone (DOC) in rodents. Furthermore, dexamethasone suppression of plasma DOC is predictive of subsequent voluntary alcohol consumption in ethanol-naı¨ve cynomolgus monkeys. That is, ethanol-naı¨ve monkeys that are insensitive to dexamethasone drink the most alcohol in a two bottle self-administration paradigm. In addition, dexamethasone suppression of plasma and cerebral cortex DOC levels is blunted in C57BL/6J compared to DBA/2J mice, and may correspond to higher ethanol intake in C57BL/6J mice. DOC levels and DOC sensitivity to dexamethasone were tested across the BXD recombinant inbred (RI) mice in order to model the genetic variability known to exist in the human population. Mice were injected with 0.075 mg/kg dexamethasone sodium salt or saline at 8:00 am and were sacrificed 6 hours later. DOC levels were measured in plasma and cerebral cortex by radioimmunoassay. Basal cerebral cortical DOC levels across 42 BXD strains and the parental strains range between 1.4 and 12.2 ng/g, resulting in a 8.7-fold genetic variation [$F(43,246)=4.33$, $p < .0001$]. Basal plasma DOC levels across 47 BXD strains and the parental strains range between 2.8 and 12.1 ng/ml resulting in a 4.3-fold genetic variation [$F(48,282)=3.69$, $p < .0001$]. Quantitative trait loci (QTLs) for basal DOC levels were identified on chromosomes 4 and 14, respectively in cerebral cortex and plasma. Further, basal DOC levels were correlated with several behavioral measures of alcohol sensitivity across the BXD mice. The dexamethasone-induced changes in DOC levels showed a 4.4-fold variation in cerebral cortex and a 4.1-fold variation in plasma. DOC sensitivity to dexamethasone also correlated with some behavioral measures of alcohol sensitivity across the BXD mice. In summary, this study identified two significant QTLs, associated with basal cerebral cortical and plasma DOC levels across the BXD mice. Furthermore, basal DOC levels were positively correlated with ethanol sensitivity, suggesting that the neuroactive steroid DOC may be a putative biomarker for alcohol responses. Supported by INIA-NIAAA AA013614 (PP), AA016672 and AA010564 (ALM), AA016662 (MFM, EJC), AA13499 and AA017590 (RWW).

MODULATION OF GABAA RECEPTOR TRANSMISSION IN RAT CEREBRAL CORTICAL CULTURED NEURONS BY THE ANTI-INFLAMMATORY CYTOKINE IL-10

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The effects of cytokines on synaptic function and receptor trafficking have been the topic of investigation by various laboratories. Both acute and chronic ethanol exposure cause alterations in levels of pro- and anti-inflammatory cytokines. It has been shown that production of IL-10, an anti-inflammatory cytokine, by human monocytes is increased after acute ethanol treatment. In addition, i.c.v. administration of IL-10 causes inhibition of sleep in rats. Administration of IL-10 also reverses the depressive-like phenotype of IL-10 knockout mice. The aim of this study was to examine the effects of IL-10 on GABAA receptor function and expression in cultures of rat cerebral cortex after 18–24 days in vitro. First, we examined if there was any endogenous IL-10 produced in these cultures. In preliminary ELISA studies, we detected ~82 pg/ml of endogenous IL-10 in cultured cortical neurons. Ethanol exposure (50 mM, 4 hour) increased IL-10 levels to ~175 pg/ml in these cultures. This endogenous IL-10 was significantly attenuated after incubation with a rat IL-10 neutralizing antibody. We then investigated the effects of IL-10 application on pharmacologically isolated miniature inhibitory postsynaptic currents (mIPSCs) using whole-cell patch clamp recordings. Application of 5 ng/ml rat recombinant IL-10 caused an outward shift in the holding current ($p = 0.018$, one-way ANOVA). Application of IL-10 (5–50 ng/ml) dose-dependently decreased the total charge transfer of mIPSCs ($p = 0.018$, one-way ANOVA). Furthermore, a 4–8 hour incubation of cultures with rat IL-10 neutralizing antibody caused a 2.3 fold increase in mIPSC frequency ($p = 0.0076$, Student's t-test) as compared to untreated sister cultures. In parallel experiments, IL-10 (50 ng/ml, 30 minute) caused a 22 ± 7% decrease in the expression of alpha1 GABAA receptor subunits, as measured by Western blot analysis ($p = 0.04$, Student's t-test). Studies to examine the effects of IL-10 on GABAA receptor currents in slice preparations from naı¨ve rats are ongoing. These data suggest that the anti-inflammatory cytokine IL-10 modulates GABAergic transmission via pre- and post-synaptic mechanisms.

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CHARACTERIZATION OF A NOVEL POPULATION OF DOPAMINE NEURONS THAT INNERVATE THE EXTENDED AMYGDALA

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The extended amygdala, including the bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA) receives substantial dopaminergic innervation, and studies suggest that dopamine signaling in these regions is important in stress and addiction-related behaviors. Interestingly, studies in rats indicate a major portion of the dopaminergic innervation of this region originates from the A10Dc group of dopamine neurons in the ventral lateral periaqueductal gray (vlPAG), a region strongly implicated in emotional behavior. The goal of this study was to document the location of DA neurons that project to the dorsal lateral BNST (dBNST) in a transgenic mouse strain that expresses eGFP as a marker of catecholaminergic neurons. Further, we used this anatomical data as a guide to characterizing some basic membrane properties of A10dc eGFP positive neurons in sub regions of the VTA/A10dc continuum, with a focus on areas that showed the highest number of eGFP positive neurons that were both tyrosine hydroxylase positive and projected to the dBNST. We used acute brain slice preparations from appropriate coronal levels of the transgenic mouse to find and record activity from eGFP positive neurons. Using whole patch recordings, we compared the basic membrane properties of VTA DA neurons with eGFP positive, presumed DA neurons, from select areas of the PAG and A10dc that have a high number of neurons that project to the dBNST. We found that the A10dc/PAG in mice contribute a large portion of the DA neurons that project to the dBNST and are easily identified in brain slice preparations from a transgenic mouse line that expresses eGFP in a subset of TH+ neurons. In particular, the caudal part of the dorsal raphe nucleus and the rostral linear raphe nucleus contain most of the DA afferent neurons that are found outside of the VTA. These data agree with previously published data from rats. Selective recording from eGFP+ neurons from these mice will enable understanding of their role in drug use/ abstinence/craving/relapse series of behaviors.

KAPPA OPIOID RECEPTOR MODULATION OF INHIBITORY TRANSMISSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Emotional behavior is regulated by a host of chemicals, including neurotransmitters and neuromodulators, acting on specific circuits within the brain. There is strong evidence for the existence of both endogenous stress and anti-stress systems. Chronic exposure to alcohol and stress are hypothesized to modulate the relative balance of activity of these systems within key circuitry in the brain leading to dysregulated emotional behavior, including increased anxiety and alcohol self-administration. The kappa opioid receptor and its endogenous agonist, dynorphin, are one such ‘‘stress’’ system. Specifically, activation of kappa opioid receptors is associated with both the dysphoric component of stress and dependence-induced alcohol self-administration. The bed nucleus of the stria terminalis (BNST) is a brain region associated with anxiety and stress. Interestingly, dynorphin is expressed in the cell bodies and terminals of the BNST, raising the possibility that kappa opioid receptor activation in this region is involved in regulation of emotional behavior. However, the impact of kappa opioid receptor activation on synaptic transmission in this region is not well known. Using whole-cell voltage clamp recordings in an ex vivo slice preparation, we investigated the effect of kappa receptor activation on inhibitory transmission in the BNST. We found that activation of kappa opioid receptors lead to a significant decrease in evoked inhibitory post-synaptic currents. Using multiple approaches, our results support the possibility that this inhibition is mediated via altering neurotransmitter release. Further, we found evidence for alterations in the kappa opioid receptor system following chronic stress exposure. Taken together these findings suggest a mechanism by which alterations in kappa opioid receptor systems can modulates output from the BNST, leading to altered recruitment of targets of the BNST, such as the lateral hypothalamus, the paraventricular nucleus of the hypothalamus and the ventral tegmental area.

Oregon

PRIMATE CEREBELLAR GRANULE CELLS EXHIBIT A TONIC GABAA CONDUCTANCE THAT IS NOT AFFECTED BY ALCOHOL

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In rodents, alcohol can increase vesicular release of GABA and can directly potentiate high affinity, extrasynaptic GABAA receptors that sense ambient levels of extracellular GABA to generate tonic inhibition. A neglected issue in translating rodent literature to humans is that phylogenetic differences may alter the actions of alcohol. Poignantly, tonic inhibition is regulated by astrocytic GABA transporters, and the density of astrocytic processes relative to neuronal processes increases with phylogenetic advancement. In fact, it is not known if primate neurons exhibit tonic GABAA inhibition. To address this issue we made voltage-clamp recordings from granule cells (GCs) in cerebellar slices from the non-human primate, Macaca Fascicularis. We found that similar to rodents, primate GCs exhibit a tonic conductance (32±8 pA with ECl-=0 mV and Vh=-60 mV) that is generated by a6d subunit containing GABAA receptors, as evidenced by its block by the GABAA antagonist, GABAzine (10 lM), its inhibition by the a6 selective antagonist, furosemide (100 lM), and its enhancement by THDOC (10–20 nM). Despite exhibiting a pharmacologically similar tonic GABAA current, in contrast to rodent GCs, in most primate GCs (~65%), acute application of EtOH (25–105 mM) did not increase sIPSC frequency or the amplitude of the tonic current. The lack of affect of EtOH was not due to a lack of functional Golgi cell inputs to GCs, because in all EtOHinsensitive GCs tested, the tonic GABAA current was reduced by blocking action potentials with tetrodotoxin (500 nM). Thus, most primate GCs are contacted by functional Golgi cells that are insensitive to physiological concentrations of EtOH. In a minority of cells (~35%), EtOH did cause an increase in sIPSC frequency that was accompanied by a small increase in the amplitude of the tonic current. The magnitude of the EtOH-induced increase in tonic GABAA current was significantly smaller in primates than in rodents, possibly due to stronger regulation of spillover by GABA transporters in primates. Conclusions: EtOH does not directly modulate a6d subunit GABAA receptors in primates. Instead, EtOH enhanced GABAergic transmission is mediated by enhanced GABA release. Rodent cerebellar GC responses to alcohol are only representative of a small subpopulation of primate GCs. This suggests that the impact of EtOH on primate cerebellar physiology will be reduced compared to rodents, and will likely have different computational and behavioral consequences.

ETHANOL DISCRIMINATION IN AGED CYNOMOLGUS MONKEYS AND ITS GABAA RECEPTOR MEDIATION

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Excessive alcohol consumption is less common in aged compared to young adult humans. Aged individuals show greater sensitivity to many behavioral effects of ethanol. We tested the hypothesis that ethanol has strong discriminative stimulus properties in aged primates by training male (n = 2, mean ± SEM: 19.5 ± 1.5 years of age) and female (n = 6, 17.7 ± 1.1 years of age) cynomolgus monkeys to discriminate 1.0 g/kg ethanol from water with a 60-minutes pre-treatment interval. Previously, 5 to 6 year-old cynomolgus monkeys acquired discrimination of 1.0 g/kg ethanol from water in (mean ± SD) 137 ± 77 sessions (Grant et al., 2000, Psychopharmacology 152). Of the four naı¨ve aged monkeys, two acquired criterion discrimination (89 to 247 sessions). However, stimulus control remained weak and few discrimination tests were conducted. Blood-ethanol concentration 60 minutes after administration of 1.0 g/kg ethanol was greater in aged female monkeys (mean ± SEM, 113 ± 5.9 mg/dl) compared to published data from young adult female monkeys (82 ± 5 mg/dl), although elimination rate was similar (aged, 40 ± 2 mg/dl/h; young, 34 ± 2 mg/dl/h; Green et al., 1999, ACER 23). The three monkeys trained to discriminate 1.0 g/kg ethanol from water about a decade earlier reacquired discrimination (18 to 166 sessions). In contrast, a female monkey previously trained to discriminate 2.0 g/kg ethanol did not acquire discrimination of 1.0 g/kg ethanol after 240 sessions. Substitution tests with pentobarbital, midazolam, allopregnanolone, pregnanolone and androsterone indicated that GABAA receptors continued to mediate discrimination of 1.0 g/kg ethanol from young adulthood to middle-age. There were wide individual differences in substitution potency shifts. Previous studies showed that aged monkeys were not impaired at discrimination learning in general (Voytko et al., 1999, Neurobiol Aging 20), so the data suggest that 1.0 g/kg ethanol, the equivalent of about 4 drinks, may be a weak discriminative stimulus in aged monkeys despite higher blood-ethanol concentration, compared to young adult monkeys. Exposure to ethanol in young adulthood may facilitate behavioral control by ethanol-like discriminative stimulus effects in middle age.

AGE OF ONSET OF ETHANOL SELF-ADMINISTRATION AND RISK FOR HEAVY DRINKING IN MONKEYS

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Epidemiological studies suggest that age of onset of regular alcohol drinking is highly predictive of future diagnosis of Alcohol Use Disorder and alcoholism. One difficulty with epidemiological data is that people are not randomized to drink or abstain from alcohol, and therefore self-selection can confound causal inferences of age as a risk factor. Animal models help address these concerns by allowing randomization of subjects to treatment as well as allow accurate measures of daily and like time intakes. Macaque monkeys are particularly useful due to their long life span and the propensity to drink ethanol to intoxication. In this study 19 male rhesus monkeys ranging in age from 5.5 (young adult) to 9.5 (adult) years of age were subjected to a standard procedure for inducing alcohol self-administration. Adolescence in rhesus monkeys is reported to be anywhere from 2 to 5 years, with sexual maturity at 3 to 4 years of age. Following induction, all monkeys were given daily 22 hour sessions where ethanol (4% w/v in water) and water were concurrently available and food was delivered in meals. After 3 months (91 sessions), there was a strong negative correlation ($r =)0.64$, $p < 0.003$) between the age at inducing ethanol self-administration and average daily ethanol intakes. Only the monkeys that started drinking alcohol under 6 years of age had average daily intakes of >3.0 g/kg, indicative of a chronic heavy drinker ($n = 5$). Further, only the monkeys that started drinking alcohol over 9 years of age had average daily intakes of <1.0 g/kg, indicative of a low alcohol drinker. The data suggest strongly support the human epidemiological findings that early adulthood is a particularly vulnerable time to regularly begin drinking alcohol in terms of future classification of a chronic heavy drinker.

IDENTIFICATION OF SYUA AND TPH2 POLYMORPHISMS ASSOCIATED WITH HPA AXIS FUNCTION IN RHESUS MACAQUES

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The hypothalamic-pituitary-adrenal (HPA) axis orchestrates the physiological response to, and recovery from, acute stress. A blunted HPA axis response has been associated with alcohol abuse and increased risk of relapse among alcoholic men. To investigate the role of the HPA axis in alcohol consumption in rhesus macaques, and to explore the genetic contributions to HPA axis function, we selected a cohort of 60 unrelated, 5–8 year old rhesus males for study. HPA axis function was assessed by measuring circulating ACTH levels 12 hours before and after the administration a low dose (0.13 mg/kg) of dexamethasone. The ACTH suppression levels of the cohort were normally distributed over a range of)21% of 66%. Promoter, exonic and 3' UTR sequences from selected candidate genes were then sequenced and analyzed in this HPA axis study group. Significant allelic associations with ACTH suppression level were identified within two genes, TPH2 (encoding tryptophan hydroxylase 2) and SNCA (encoding alpha-synuclein). Analysis of a 3' UTR polymorphism in TPH2 (A2051C) identified an allele dosage correlation, with each copy of the ''C'' allele associated with a suppression increase of 12.5 units, accounting for 18.4% of the observed variation in ACTH suppression. Analysis of the SNCA gene identified a haplotype spanning from intron 2 through exon 4 that was associated with a 17.7 unit decrease in ACTH suppression, accounting for 14.4% of the observed variation. The TPH2 association supports the role of the serotonergic pathway in establishing HPA axis functional variation in rhesus macaques. SNCA variants may modulate HPA axis function through multiple pathways, since alpha-synuclein is involved in the trafficking of serotonin, dopamine and norepinephrine transporters to and from the plasma membrane and may also bind these transporters directly, further decreasing their activities.
(This work supported by grants AA13510and RR00163)

ANALYSIS OF PREFRONTAL AND ORBITOFRONTAL TRANSCRIPTOMES IN CYNOMOLGUS MACAQUE MONKEYS AFTER SELF-ADMINISTRATION OF ETHANOL

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Similar to human populations, Cynomolgus monkeys (Macaca fascicularis) display high individual differences in ethanol self-administration, with intakes ranging from <1.0 up to 4.0 g/kg/day after induction, even with strict environment control. Thus, these monkeys are an ideal population for translational research investigating genetic risk factors of alcohol selfadministration and abuse. Here, we present the first transcriptome-wide sequence analysis (RNA-Seq) of Cynomolgus macaques using Next Generation Sequencing (NGS). Adult female monkeys were induced to drink 4% ethanol (w/v in water) over 120 days, and then allowed 22-hour/day access to the 4% ethanol for self-administration over 12 months with continuous access to food. In a pilot study, RNA-Seq data from prefrontal cortex samples (areas 24, 25, and 32 combined) of 1 'low' and 1 'high' drinker (0.16 and 3.45 g/kg/day average consumption, respectively) was generated, yielding over 40 million 50 bp reads per sample. These reads were mapped to available Cynomolgus cDNA sequences (~9700 sequences; NCBI) as well as the latest genome assembly for rhesus macaque (Ensembl 56; Mmul_1). Roughly 15% mapped to the Cynomolgus cDNAs and more than 60% of the reads mapped to the genome assembly, querying more than 19,500 of the 28,000 annotated rhesus genes. Reads per kilobase of exon per million mapped reads (RPKMs) were calculated across genes for each sample to determine gene expression levels. Differences in gene expression levels up to 4-fold between the high and low drinker were detected, including genes involved in oxidative stress reponse and GABAergic- and glutamatergic-related neurotransmission. Additional RNA-Seq data are currently being produced for orbitofrontal cortex (area 13) samples harvested from these animals as well as additional low and high drinkers (<2 and ‡3.7 g/kg/day average consumption, respectively). Altogether, this data provides further insight into gene expression changes in brain regions involved in impulsivity and other behaviors that are associated with alcohol addiction. Supported by: NIAAA, NIDA, and VA.

South Carolina

BEHAVIORAL CHARACTERIZATION AND ETHANOL SENSITIVITY IN NR2B NMDA RECEPTOR KNOCKOUT MOUSE

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It is well established that NMDA glutamate receptors play a significant role in mediating a wide variety of pharmacological effects of acute and chronic ethanol. The subunit composition of NMDA receptors is known to influence their pharmacological properties and it has been postulated that the NR2B subunit confers greater sensitivity to the modulatory effects of ethanol. This study examined behavioral responses to acute ethanol in a mouse model with genetic deletion of the NR2B subunit. Mice lacking the NR2B gene (KO) were produced by mating NR2B[f/f] mice with CAMKIIa-drive tTA transgenic mice and the tetO-CRE transgenic mice. Adult male and female offspring representing each of the resultant genotypes (KO, CAM, CRE, and wild type (WT) mice) were tested for basal anxiety behavior using the light/ dark test, baseline (saline) locomotor activity in an open field, and open field locomotor activity following acute low dose (1.5 g/kg) and high dose (3.0 g/kg) ethanol challenge. Findings indicate that male and female mice lacking the NR2B subunit are more anxious than the other (control) genotypes, as evidenced by significantly less amount of time spent on the light side of the apparatus. Further, KO mice exhibited greater overall activity in comparison to other genotypes during the anxiety test session, and this effect was replicated in analysis of saline-induced locomotor activity in an open field arena. All genotypes showed increased locomotor activity following exposure to a low dose of EtOH (1.5 g/kg, ip) and reduced activity following administration of a high dose of EtOH (3.0 g/kg, ip). However, NR2B KO mice exhibited a significantly exaggerated stimulant response to low dose (1.5 g/kg) EtOH challenge. This difference was also noted even when differences in baseline (saline) activity was factored into the analysis. Preliminary Western blot analyses confirmed significant reduction in NR2B expression in cortex and striatum in KO mice. Together, these data indicate the NR2B subunit of the NMDA receptor is involved in regulating anxiety-related behavior, general locomotor activity, and low-dose stimulant effects of ethanol. Future studies will examine ethanol self-administration, sensitivity to the anxiolytic effects of ethanol, stress responsiveness, and behavioral responses following chronic ethanol exposure in this unique mouse model. Supported by grants U01 AA014095, T32 AA007474, and VA Medical Research.

EFFECTS OF CHRONIC ETHANOL EXPOSURE AND ETHANOL DRINKING ON GLUTAMATE LEVELS IN THE ACCUMBENS OF C57BL/6J MICE

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We have shown that chronic intermittent ethanol exposure increases voluntary ethanol drinking and extracellular glutamate (GLU) levels in the nucleus accumbens (NAc). In the present study, we examined the role that access to ethanol drinking plays in modulating GLU in the NAc using this model of ethanol dependence and relapse. After implanting guide cannulae and establishing stable baseline ethanol (15% v/v) intake, mice received 4 weekly cycles of chronic intermittent exposure (16 hour/day for 4 day) 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 mice compared to CTL mice (3.5 ± 0.5 vs 2.2 ± 0.4 g/kg). At the end of the 4th drinking test period (7 days post-chamber), microdialysis procedures indicated that baseline GLU values in the NAc were significantly greater in the EtOH compared to CTL mice (0.95 ± 0.3 vs. 0.56 ± 0.4 lM). Separate groups of EtOH and CTL mice were similarly treated but were allowed to continue daily limited access drinking sessions for an additional week prior to dialysis while others were left undisturbed (drinking access terminated) before dialysis. During this extended week of drinking (after the 4th exposure cycle), EtOH mice continued to exhibit increased ethanol intake compared to CTL mice (2.9 ± 0.3 vs. 2.2 ± 0.2 g/kg) and analysis of dialysate samples indicated increased baseline GLU levels in the EtOH compared to CTL mice (0.80 ± 0.1 vs. 0.40 ± 0.1 lM). However, this baseline difference in extracellular GLU levels was no longer evident in EtOH mice that were not given an opportunity to continue drinking (0.56 ± 0.16 vs. 0.65 ± 0.17 lM for EtOH and CTL groups, respectively). Together with our previous data, these data indicate that repeated cycles of chronic intermittent ethanol exposure results in a hyper-glutamatergic state in the NAc which is maintained for at least 2 weeks following final chronic ethanol exposure by daily opportunity to self-administer ethanol, but normalizes when limited access drinking sessions are terminated. Ongoing studies are examining possible mechanisms underlying this hyper-glutamatergic state in the NAc that is associated with escalated drinking in ethanol dependent mice. Supported by NIAAA grant P50 AA10761 and VA Medical Research.

EFFECTS OF ETHANOL DEPENDENCE ON ETHANOL INTAKE AND BEHAVIOR IN THE FORCED SWIM TEST IN MALE C57BL/6J MICE

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We previously have shown that repeated cycles of chronic intermittent ethanol (CIE) exposure produces escalation of voluntary ethanol drinking when access to ethanol was provided in between each cycle of CIE exposure. The current study examined whether increased ethanol consumption would also be observed if animals were not given the opportunity to drink until after CIE exposure. Additionally, the forced swim test (FST) was used to investigate potential changes in stress effects on drinking and stress responsiveness in dependent versus nondependent mice. Once stable baseline ethanol (15% v/v) intake was established in adult male C57BL/6J mice using a 2-bottle choice limited access (2 hour/day) procedure, mice received 4 weekly cycles of CIE (or air) exposure in inhalation chambers. Each cycle consisted of exposure (16 hour/day · 4 days) to ethanol vapor (EtOH group) or air (CTL group), followed by 72 hour undisturbed in the home cage. Starting at 72 hour after the final (fourth) cycle, some of the EtOH and CTL mice were tested for voluntary drinking (daily 2 hour limited access sessions) for 2 weeks, while other mice had delayed access to ethanol (access only during the second week). An additional group of EtOH and CTL mice were tested for ethanol consumption for 2 weeks, but were also stressed in the FST prior to drinking sessions during the first week of testing. Results showed that ethanol-dependent mice exhibited significantly greater ethanol intake compared to CTL mice after 4 cycles of CIE exposure. However, ethanol-dependent mice that had delayed access to ethanol did not exhibit significant elevations in intake compared to CTL mice. Repeated FST stress did not significantly alter intake in EtOH or CTL groups. Finally, immobility measured in the FST was significantly decreased in EtOH compared to CTL mice on the first day of stress testing. Overall, these results indicate that repeated cycles of CIE exposure produces a significant increase in voluntary ethanol drinking after stable baseline intake is established, even if access to ethanol is withheld until after inhalation treatment. However, this effect is not observed if access is delayed for a longer interval. Results also suggest that mice with an established history of ethanol dependence unexpectedly exhibit significant decreases in immobility in the FST. Supported by grants U01 AA014095, T32 AA007474, and VA Medical Research.

ETHANOL INTAKE, PLASMA CORTICOSTERONE LEVELS AND BRAIN REGIONAL CRF LEVELS IN ETHANOL-DEPENDENT C57BL/6J MICE

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Repeated cycles of chronic intermittent ethanol (CIE) exposure produces escalation of voluntary ethanol drinking in C57BL/6J mice. This study examined changes in plasma corticosterone (CORT) levels and brain regional changes in corticotrophin releasing factor (CRF) peptide expression in relation to this model. Adult male mice were trained to drink 15% ethanol (vs. water) in a 2-bottle limited access (2 hour/day) procedure. Once stable baseline intake was achieved, mice received 2 or 5 cycles of chronic intermittent exposure (16 hour/ day for 4 days) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers, with each exposure cycle followed by 72 hour abstinence and then 5 days limited access drinking test sessions. EtOH and CTL mice were sacrificed immediately upon final withdrawal (HR-0), or at later times following withdrawal (HR-8 and HR-72). Blood and micropunched brain samples were collected for analysis of plasma CORT levels (by RIA) and CRF peptide content (by ELISA). Results showed that after 2 or 5 cycles of CIE exposure EtOH mice consumed significantly more ethanol (~3.5–4.1 g/kg) compared to CTL mice (~2.7 g/kg) during the final testing period and at baseline (~2.6 g/kg). Overall, CORT levels were significantly higher in EtOH mice compared to CTL mice following 2 or 5 CIE exposure cycles, especially at peak withdrawal (HR-8). However, CORT was significantly greater after 2 compared to 5 cycles even though blood ethanol levels were similar for the groups at time of withdrawal. This suggests blunted HPA axis activation during withdrawal following multiple (5) CIE exposure cycles. CRF peptide levels after 2 cycles of exposure were 29% higher in the amygdala (AMY) of EtOH compared to CTL mice at HR-8 while CRF content in the bed nucleus of the stria terminalis (BNST) was 28% lower in EtOH mice compared to CTL mice at 72 hour into withdrawal. A different pattern of results was obtained, after 5 CIE cycles: CRF content in AMY was significantly lower (-40%) at HR-0 but higher (44%) than CTL levels at HR-8 while CRF in the BNST was higher than CTL (37%) at HR-0. These results indicate that increased number of CIE exposure cycles (5 vs. 2 cycles) produces greater escalation of ethanol drinking, possible blunting of HPA axis activation (reduced plasma CORT levels at peak withdrawal), and augmented changes in CRF peptide content in extra-hypothalamic brain structures. Supported by NIAAA grant U01 AA014095 and VA Medical Research.

ETHANOL-DEPENDENT C57BL/6J MICE SHOW INCREASED ETHANOL INTAKE AND TOLERANCE TO ETHANOL'S AVERSIVE EFFECTS BUT NOT METABOLIC TOLERANCE TO ETHANOL

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We evaluated whether the increase in voluntary drinking following repeated cycles of chronic intermittent ethanol (CIE) exposure relates to the development of tolerance to ethanol's aversive effects or the development of metabolic tolerance. Adult male C57BL/6J mice were trained to drink 15% ethanol (vs. water) in a limited access (2 h/d) procedure. After stable baseline intake was achieved (2.8 g/kg), mice were exposed to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers (16 h/d for 4 days). At 72 hour following CIE (or air) exposure, ethanol drinking resumed for 5 days. This pattern was repeated for three cycles. Consistent with previous studies, repeated cycles of CIE exposure increased ethanol drinking in EtOH mice while drinking in CTL mice remained stable over time (3.7 vs. 3.1 g/kg for EtOH and CTL mice after the 3rd CIE cycle, respectively). At 72 hour after a fourth CIE cycle, the aversive effects of ethanol were evaluated in EtOH and CTL mice using a conditioned taste aversion (CTA) paradigm. Saccharin (1% w/v) was the conditioned stimulus (CS) paired with an IP injection of vehicle (0.9% saline), or ethanol (1, 2, or 3 g/kg), which served as the unconditioned stimulus (US). CS intake was evaluated 24 hour later. The lowest ethanol dose (1 g/kg) did not induce CTA but the highest dose (3 g/kg) produced robust CTA in both groups (~50% reduction in CS intake). The 2 g/kg dose induced a significant learned aversion in CTL mice (49% reduction) but not in EtOH mice (20% increase), indicating tolerance to ethanol's aversive effects. Additionally, when LiCl (0.4 M) served as the US, a similar reduction in CS intake was observed in both CTL and EtOH mice (~55%). This suggests that tolerance to the aversive effects of a moderate (2 g/kg) ethanol dose in EtOH mice is not related to a general learning deficit. After a fifth CIE cycle, mice were injected IP with a 2 g/kg ethanol dose. Blood and brain ethanol peaked at the same level and the elimination rate was similar for both groups. Taken together, these data indicate that tolerance to ethanol's aversive effects but not metabolic tolerance may play a role in promoting escalation of ethanol drinking in dependent mice. Ongoing studies are examining adaptations in glutamatergic neurotransmission in the basolateral amygdala, a brain region critical for CTA, in the development of tolerance to ethanol's aversive effects.

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CHRONIC ETHANOL EXPOSURE ENHANCES BACKPROPAGATING ACTION POTENTIAL-INDUCED CALCIUM TRANSIENTS IN DISTAL APICAL DENDRITES OF CA1 PYRAMIDAL NEURONS

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Backpropagating action potentials (bAPs) in apical dendrites of hippocampal neurons are hypothesized to provide distance-dependent signals that modulate dendritic and synaptic plasticity. Electrophysiological recordings from apical dendrites of CA1 pyramidal neurons have demonstrated that bAP amplitudes decrease with distance from the soma. This reduction in bAP amplitude is thought to reflect an increase in the density of A-type K+ channels in distal dendrites. Chronic ethanol-induced neuroadaptive plasticity of glutamatergic synapses is well documented; however, it is unknown if prolonged ethanol exposure affects dendritic plasticity. Here we examined how chronic ethanol exposure affects the efficacy of action potential backpropagation by imaging Ca2+transients evoked by bAPs in distal apical dendrites of CA1 pyramidal neurons in organotypic hippocampal slices treated chronically (7–9 days) with ethanol. Cells were loaded with the Ca2+-sensitive dye Oregon Green 488 BAPTA-1 (OGB-1) during whole-cell patch-clamp electrophysiology and bAPs were evoked by brief somatic current injection. Ca2+ transients from OGB-1 were imaged at multiple locations along the apical dendrite using fast confocal line-scans. Chronic ethanol exposure increased bAP-induced Ca2+ transients, and the magnitude of the potentiation increased with distance from soma. Because Kv4.2 channels underlie the transient A-type K+ current in hippocampal dendrites where they control the amplitude of bAPs, we next examined the effect of chronic ethanol exposure on expression of Kv4.2 channels. Cross-linking analysis revealed that chronic ethanol exposure significantly reduced surface expression of Kv4.2 channels. The reduction in surface expression was associated with an increase in phosphorylation of Kv4.2 and a significant decrease in expression of Kv channel interacting protein auxiliary subunits. These data suggest that decreased surface expression of Kv4.2 channels contributes to the increase in bAP-associated Ca2+ transients in distal apical dendrites of CA1 pyramidal neurons following chronic ethanol exposure. Moreover, ethanol-induced enhancement of bAPs may affect dendritic metaplasticity and signal integration in apical dendrites of hippocampal neurons.

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ETHANOL INCREASES MATRIX METALLOPROTEASE RELEASE IN HIPPOCAMPUS: POSSIBLE ROLE FOR ASTROGLIA IN ETHANOL-INDUCED PLASTICITY OF GLUTAMATERGIC SYNAPSES

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Astroglia dynamically regulate synaptic plasticity through the secretion of neurotrophic factors and gliotransmitters. Astroglial regulation of synaptic activity and plasticity may occur through remodeling of the synapse via changes in the actin cytoskeleton of dendritic spines or degradation of the extracellular matrix (ECM). Astroglial fine processes are present at the axonal-spine interface (i.e., tripartite synapses) where astroglial release of matrix metalloproteases (MMPs) can degrade the ECM and allow remodeling of the microenvironment that surrounds dendritic spines. Recent evidence suggests that chronic ethanol may affect dendritic spine number and morphology in key brain regions involved in addictive processes. Thus, astrocytes may contribute to the adaptive structural and morphological plasticity of dendritic spines associated with chronic ethanol exposure. To begin to test this hypothesis, we used in situ slice zymography using FITC-conjugated DQ gelatin for visualization of MMP activity and 3D imaging of tripartite synapse using diolistic loading techniques. As expected, pro-inflammatory cytokine TNF-a (100 ng/ml) and endotoxin LPS (100 ng/ml) treatment for 24 hour induced extracellular MMP proteolytic activity in the stratum radiatum of the CA1 region in organotypic hippocampal slices. Acute ethanol (50 mM) treatment also markedly enhanced extracellular MMP activity in the stratum radiatum. Consistent with previous reports, we found that approximately 55% of dendritic spines were closely associated with astrocytic fine processes, and morphological classification revealed that mature, mushroom spines had a higher rate of association at perisynaptic regions than immature, stubby spines. Together, these data suggest that astroglia contribute to ethanolinduced remodeling of glutamatergic synapses possibly through degradation of the ECM surrounding dendritic spines or physical contact at perisynaptic regions.

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Tennessee

GRAPH THEORETIC ANALYSIS OF BXD MOUSE MRNA EXPRESSION ETHANOL AND PHENOTYPE DATA. C.A. Phillips; A.R. Wolen; M.F. Miles; M.A. Langston. University of Tennessee, Department of Electrical Engineering and Computer Science, Knoxville, TN, 37996; Virginia Commonwealth University, Richmond, VA, 23284.

We used graph-theoretic algorithms in a combined analysis of a BXD microarray expression dataset and BXD phenotype data. The microarray expression dataset consisted of 37 recombinant inbred BXD strains. It contained a control group, which was given saline, and a test group, which was administered ethanol (1.8 g/kg). Affymetrix M430A 2.0 microarrays were used to measure mRNA expression in the prefrontal cortex at four hours post-treatment. The dataset was normalized using both RMA and S-scores. The phenotype dataset consisted of 2137 phenotypes, each with quantitative measurements on up to 92 BXD strains, the BL6 and D2 parental strains, and the two F1 strains.

Combining the 22626 probesets in the Affymetrix microarray with the 2137 phenotypes, we calculated all pairwise Pearson correlations. Since the phenotypes typically did not have measurements for all strains, we translated the Pearson correlation into correlation p-values. The result was represented by a weighted graph in which each vertex is either a probeset or a phenotype and the weight of each edge is the correlation p-value. We then constructed an unweighted graph by retaining only those edges with weight below some threshold *t*. To maintain similar edge density between probes and phenotypes, we used contrasting values of *t* for phenotype-phenotype, probeset-phenotype, and probeset-probeset thresholds.

A highly natural grouping of vertices is by cliques (fully connected subgraphs). Finding the maximum clique is a computationally intractable problem. Nevertheless, the sparsity of biological graphs coupled with state-of-the-art algorithms and hardware implementations puts the solution within reach. Thus we iteratively extract maximum cliques and account for noise with the paraclique method. Each paraclique forms a putative biological network along with potentially associated phenotypes. For example, our analysis produced one paraclique containing a number of cocaine phenotypes, which suggests a common biological mechanism between ethanol networks and cocaine phenotypes.

STRAIN-SPECIFIC STRESS-INDUCED MODIFICATION OF GENE EXPRESSION: IS DNA METHYLATION INVOLVED?

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Stress and alcoholism are multifactorial traits that have reciprocal interactions with stress affecting rates of alcohol consumption and alcoholism contributing to stress responses. Additionally, it is likely that these traits are, at least partially, mediated by sequence variants in a host of genes. To begin to identify both what these genes are and to assess the potential role of epigenetic modifications in this regulation, we compared changes in gene expression with the regions that showed epigenetic modifications following exposure to stress. Of greatest interest in this analysis are the genes that show both significant alterations in expression and that show a concomitant epigenetic modification. Adult female mice were examined from C57BL/6J and DBA/2J strains with restraint used as the stressor. Three conditions were examined: control (no stress), acute and chronic stress using three biological replicates for each strain/stress combination. Analysis of gene expression was conducted using Illumina microarrays while DNA methylation of CpG islands was examined using methylation microarrays. Hundreds of genomic regions were shown to be differentially methylated across strains and across stress levels. The known genes at these regions were identified and their expression levels were compared using the microarray data. Comparisons of gene expressions with DNA methylation identified a set of genes where expression was negatively correlated with DNA methylation suggesting that expression changes of these genes are potentially regulated by epigenetic mechanisms. Interestingly, the majority of genes identified after acute stress were distinct from those identified following chronic stress providing evidence of differential regulation of the 2 processes. Moreover, there are strainspecific differences in the genes that exhibit both changes in expression and in DNA methylation providing genetic pathways that could underlie strain-specific differences in stress responses. Further analysis can now be conducted to determine if any of these pathways are modulated by ethanol exposure and could thus be a molecular mechanism for the interaction of these 2 traits. Support: U01-AA014425.

REPEATED CHRONIC INTERMITTENT ETHANOL VAPOR EXPOSURE ALTERS LONG-TERM POTENTIATION IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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One behavior that modulates relapse in dependent subjects during withdrawal is anxiety. A region that is particularly involved in the modulation of anxiety and stress is the bed nucleus of the stria terminalis (BNST). This region has been shown to modulate responses to anxiogenic stimuli and stress-induced relapse to drug taking and, therefore, is thought to be critical to the understanding of ethanol dependence. We previously reported that in the dorsal lateral (dl)BNST, acute in vitro ethanol administration was able to reduce an early portion of NMDA receptor-dependent LTP. Further, we previously demonstrated that one cycle (4-days) of chronic intermittent ethanol (CIE) vapor exposure was able to produce anxiety-like behavior 4 to 6 hours into the final withdrawal. The current work evaluated a more prolonged CIE paradigm on LTP in the dlBNST 4 to 5 hours into withdrawal. 7 week old C57 male mice were exposed to two 4-day cycles of either ethanol vapor or air (16 hours in chambers and 8 hours in home cage daily) and cycles were separated by 3 days in the home cage. A separate cohort of age-matched control mice (naı¨ve mice) were maintained under standard housing conditions for the duration of the experiment. LTP was induced with 2 trains of 100 Hz, 1 second tetanus delivered with a 20 second intertrain interval at the same intensity as baseline test pulses. There were no significant difference in the early phase of LTP (0 to 5 minutes after tetanus) between naı¨ve, air-controls, or chronic ethanol exposed mice. However, significant differences were found between groups during the late phase of LTP (55 to 60 minutes after tetanus). This difference was produced from a reduction of LTP in air exposed control mice compared to both naı¨ve and chronic ethanol exposed mice. No differences in late phase LTP were found between chronic ethanol exposed and naı¨ve mice. These results suggest that the stress from daily injections, cage changes, and/or vapor chamber exposure produced a blunting of LTP in air-exposed mice. However, the effects from these potential stressors were either prevented by chronic ethanol treatment or ethanol treatment resulted in an enhancement of LTP that compensated for the reduced LTP caused by stress. Additional work will use pharmacological and genetic approaches to determine the mechanisms behind these effects.

ALTERED ANXIETY-LIKE BEHAVIOR IN ADULT MICE FIRST EXPOSED TO CHRONIC UNPREDICTABLE STRESS AND ETHANOL IN ADOLESCENCE

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Background: Chronic exposure to stressors and alcohol, especially in vulnerable adolescence, can lead to an increased risk of developing alcohol use disorders (AUDs). To date, however, the potential inter-dependency and long-term effects of chronic intermittent and unpredictable stress (CUS) and ethanol (CUSE) exposure during adolescence on brain function and anxiety-like behavior during adulthood has not been thoroughly investigated.
Methods: In the present study, adolescent and adult mice were exposed to a regimen of chronic and unpredictable stressors and EtOH (or air; CUSE and CUSA, respectively) for 8–10 weeks and then tested in adulthood for altered anxiety-like behavior [elevated plus maze (EPM) and social interaction (SI) test]. Studies suggest that the bed nucleus of the stria terminalis (BNST) is a key site of convergence for alcohol and anxiety-related behaviors. Thus, following behavioral testing, mice were re-exposed to CUSE (and CUSA for controls) for an additional 3 sessions. 4–6 hours following the final EtOH (or air) exposure, field potential recordings of the dorsal-lateral (dl)BNST were performed.
Results: Mice first exposed during adolescence to CUSE displayed lower levels of anxietylike behavior on the EPM compared to mice first exposed to CUSE during adulthood and control mice only exposed to CUSA, regardless of whether their first air exposure occurred during adolescence or adulthood. However, mice first exposed to CUSE during adulthood displayed higher levels of social interaction on the SI test compared to those first exposed during adolescence and control CUSA mice. In addition, mice exposed to CUSE, also regardless of age of first exposure, displayed blunted LTP in the dlBNST compared to those exposed only to CUSA.
Conclusions: This study shows that age of first exposure to CUSE is an important determinant for anxiety-like behaviors during adulthood and that CUSE exposure for mice first exposed as adolescence as well as those exposed as adults, results in blunted plasticity in the adult dlBNST.

CHRONIC ETHANOL EXPOSURE MODULATES A1-ADRENERGIC RECEPTOR INDUCED SYNAPTIC PLASTICITY IN THE EXTENDED AMYGDALA

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The a1-adrenergic receptor antagonist prazosin has recently been shown to have potential in treating alcoholism in both animal models and humans. Because prazosin preferentially inhibits dependence-enhanced alcohol intake, it has been postulated to work through modulation of a1-adrenergic receptors (ARs) in the extended amygdala. We have previously reported that a1-AR activation in the bed nucleus of the stria terminalis (BNST), a component of the extended amygdala, results in a long-term depression (LTD) of excitatory transmission in this region. LTD is an important synaptic mechanism for limiting excitatory influence over circuits subserving cognitive and emotional behavior. A major means of LTD induction is through the recruitment of signaling via Gq-linked receptors like the a1-AR. These receptors have been proposed to converge on a common postsynaptic LTD maintenance mechanism, such that hetero- and homosynaptic induction produce similar alterations in glutamate synapse efficacy. We find that in BNST, recruitment of Gq-linked receptors by glutamate or norepinephrine initiates mechanistically distinct forms of postsynaptically-maintained LTD, and these LTDs are differentially regulated by stress exposure. In particular, we show that while both mGluR5- and a1-AR-dependent LTDs involve postsynaptic endocytosis, the a1-ARinitiated LTD exclusively involves modulation of signaling through calcium-permeable AMPA receptors. Further, a1-AR- but not mGluR5- dependent LTD is disrupted by restraint stress. a1-AR-LTD is also impaired in mice chronically exposed to ethanol. These data thus suggest that in the BNST, norepinephrine- and glutamate-activated Gq-linked signaling pathways differentially tune glutamate synapse efficacy in response to stress.

Texas

THE CONTROVERSIAL EVIDENCE THAT STRESS INDUCES DRINKING: RESULTS FROM NATURALISTIC AND EPIDEMIOLOGIC STUDIES

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Exposure to potentially traumatic events in childhood (e.g. sexual or physical abuse or neglect) is associated with increased risk of use and misuse of alcohol. Whether stress in adulthood results in alcohol use/misuse, however, remains as equivocal in humans as it is in the preclinical literature. This talk will discuss the areas of uncertainty remaining in the field, with particular attention given to specific stress-alcohol associations and the relevance of environmental, population, and individual confounds, including the type of stress experienced (e.g. trauma vs. non-trauma, acute vs. chronic); whether alcohol use is assessed in a general population, heavy drinkers, or alcohol-dependent patients; individual differences in response to stress (due to coping style, gender, race, co-existing psychopathology, stress sensitivity); and the temporal association between stressors and alcohol use in ecological momentary assessment studies.

NEUROPEPTIDE Y (NPY) AND BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) RESPONSIVITY AFTER ACUTE STRESS IN ALCOHOL-DEPENDENT AND CONTROL SUBJECTS

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NPY and BDNF serve protective and neuroadaptive functions within the central nervous system and modulatory functions in the peripheral system. As such, both NPY and BDNF have been associated with physiological challenges such as stress and substance abuse. Previous studies have found correlations between increased NPY and BDNF expression and alcohol dependence, as well as with chronic and episodic stress. In this study, we examined the effect of acute stress upon both peptides in alcohol-dependent and healthy control populations.

Methods: Four to 6-week abstinent male alcohol-dependent patients were recruited from residential treatment programs and race- and age-matched controls were recruited from the general public. The Trier Social Stress Test (TSST), a public speaking task, was administered at 7 PM. Serum basal samples were obtained 15 and 5 minutes prior to the stressor. Two additional serum samples were collected 5 and 15 minutes after the stressor. Basal measures were averaged and peptide response was determined by the net maximal response following the stressor (aˆ peak). NPY was obtained in 9 controls and 11 patients; BDNF in 7 controls and 10 patients.

Results: There were no significant basal group differences in NPY. aˆ peak increases in NPY were observed in both groups (controls: $p < 0.10$, patients: $p = 0.11$). aˆ peak were not significantly different between groups. A basal group difference was observed in BDNF ($p < 0.09$). aˆ peak increases in BDNF were observed in both groups (controls: $p = 0.11$, patients: $p = 0.16$). aˆ peak were not significantly different between groups.

Conclusion: Non-statistically significant increases in both serum NPY and BDNF followed a behavioral stressor. Group trend differences were limited to higher basal BDNF levels in patients. These findings were limited by small group sizes and marked intra- and inter-subject NPY and BDNF variability. Despite recent headway in elucidating the roles of these two peptides in stress and alcoholism, the high variability in these human populations suggests a complex interplay between NPY, BDNF, and other unknown factors that must be investigated further.

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BRAIN ACTIVATION ELICITED BY ANTICIPATORY ANXIETY IN ABSTINENT ALCOHOL-DEPENDENT SUBJECTS

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Purpose: Exposure to negative affect stimuli increases alcohol craving in alcohol-dependent individuals. In order to explore the mechanism in which negative emotion induces relapse in this population, we studied the neural response of anticipatory anxiety in 2 to 5 week abstinent alcohol-dependent subjects with functional magnetic resonance imaging (fMRI).
Methods: Twelve male alcohol-dependent subjects and nine male healthy age- and racematched subjects participated in the study. A thermal stimulus was used as the unconditioned stimulus (US). Subject's heat threshold to the US was determined prior to fMRI and administered accordingly. During scanning, a very hot or warm thermal stimulus was applied to the subject's left wrist with a thermode for 8 seconds. Prior to each US, a conditioned stimulus (CS) lasting 10 to 18 seconds signaled the US. A triangle CS preceded a warm US; a square CS signaled either a warm or hot US. During the first 4 seconds of the CS, subjects rated their anxiety. There were 40 trials (20 triangle and 20 square), each lasting 19 to 29 seconds. fMRI was performed on a 3T scanner equipped with 12-channel head coil. fMRI data was analyzed with FSL FEAT v5.98.
Results: There were no group differences between controls and patients in subjective anxiety to the CS (square vs. triangle, $p = 0.327$) or the thermal CS experienced ($p = 0.112$). The bilateral insula, bilateral caudate, and anterior cingulate cortex (ACC) were all showed increased BOLD activity during square vs. triangle CS in healthy controls. Only the left caudate showed increased BOLD response in the corresponding period in alcohol-dependent patients. However, patients exhibited a decreased BOLD response in the medial OFC and posterior cingulate.
Conclusion: Control subjects show marked increased limbic activation during aversive conditioned stimuli, whereas alcohol-dependent subjects did not. These findings may have relevance to the experience of anxiety that portends relapse following negative events.
This work was funded by the INIAStress U01AA13515 and supported by the Dept of Veterans Affairs.

CHILDHOOD TRAUMA AS A PREDICTOR OF ADULT PSYCHOSOCIAL STRESS IN ALCOHOL-DEPENDENT MEN

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The relationship between psychosocial stress and alcohol dependence has been widely researched. However, the literature offers conflicting reports as to the direction of the relationship: does alcohol use increase stress or does stress increase alcohol use? The relationship between psychosocial stress and alcohol dependence is further complicated when the experience of childhood trauma is considered. Whereas previous studies support a strong relationship between the experience of childhood trauma and future alcohol abuse, it is unclear whether childhood trauma is also associated with adult levels of psychosocial stress. In this study, we hypothesized that childhood trauma would predict adult psychosocial stress in alcohol-dependent subjects and, in turn, that relationship would be mediated by alcohol use. Methods:

Alcohol dependent males (N = 41) were assessed while in residential substance abuse treatment. Participants completed the Childhood Trauma Questionnaire (CTQ) to assess childhood trauma, the UCLA Life Stress Interview to assess chronic psychosocial stress over the 6 months prior to admission, and the Timeline Follow-Back (TLFB) to quantify lifetime alcohol consumption. Statistical analyses were conducted using the CTQ total raw score and the 5 CTQ raw domain scores (i.e., physical abuse and neglect, emotional abuse and neglect, and sexual abuse). Linear regression models were used to determine if alcohol use mediated the relationship between CTQ and UCLA chronic stress.

Results: Neither total nor domain measures of childhood trauma predicted adult psychosocial stress. However, significant positive associations were present between lifetime total number of drinks and CTQ total score ($p < 0.05$ as well as the domain scores for sexual abuse ($p = 0.001$), physical abuse ($p < 0.02$), and physical neglect ($p < 0.001$).

Discussion: While the expected relationship between childhood trauma and recent stress was not demonstrated, strong associations were identified between the experience of childhood trauma and total alcohol consumption. These findings are consistent with previous reports of an association between childhood trauma and alcohol abuse, but suggest that the relationship may be strongest for specific types of childhood experiences.

This work was funded by the INIAStress U01AA13515 and the Department of Veterans Affairs.

ANTISOCIAL PERSONALITY CHARACTERISTICS AND ALCOHOL USE AS PREDICTORS OF CORTISOL REACTIVITY TO A BEHAVIORAL STRESSOR

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Introduction: The hypothalamic-pituitary-adrenal (HPA) axis is a primary stress response system. Previous studies suggest that antisocial behaviors are related to a muted cortisol response to stress challenges. Other studies reveal that prolonged alcohol use also blunts cortisol reactivity. It is not known whether the effects of antisocial behaviors and alcohol use are independent or mutually dependent predictors of cortisol reactivity. We predicted that cortisol and ACTH reactivity to a stressor would negatively correlate with antisocial behaviors in an alcohol-dependent population and that this relationship would be mediated by lifetime alcohol use.

Methods: Thirty-three alcohol-dependent men were studied at 4–6 weeks abstinence. Childhood and adulthood antisocial behaviors were assessed with the Conduct Disorder (CD) and Antisocial Personality Disorder (ASPD) sections of the Diagnostic Interview Schedule (DIS). Serum ACTH and cortisol concentrations were assessed before (T=)15 and)5 minutes) and after (T=10, 20, 30, 40, 50, and 60 minutes) the Trier Social Stress Test, a behavioral stressor. Independent measures were CD and ASPD item counts, dependent measures were ACTH and cortisol net peak and net total integrated (AUC) response, and the mediator was total lifetime drinks. Linear regression models were used to determine if alcohol use mediated the relationship between CD/ASPD counts and the ACTH/cortisol response to the TSST.

Results: Neither CD nor ASP counts significantly predicted ACTH or cortisol responses to the stressor. Neither CD nor ASPD counts correlated with lifetime alcohol use.

Conclusions: The absence of support for the hypothesized relationships between CD/ASP behaviors and pituitary-adrenal reactivity was unexpected. Use of continuous measures of CD/ASP behaviors within a single diagnostic (alcohol-dependent) group rather than dichotomous, diagnostic measures, our measures of alcohol use, and the stressor utilized may all account for the unexpected findings.

This work was funded by the INIA Stress U01AA13515 and the Department of Veterans Affairs.

RELATIONSHIP OF SELF-REPORTED CHILDHOOD TRAUMA TO PERSONALITY CHARACTERISTICS IN ALCOHOL-DEPENDENT MEN

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Trauma victims show significantly higher scores on the Neuroticism domain of the NEO-PI-R and lower scores on the Agreeableness and Extroversion domains. Similar personality characteristics are also reported in substance abusers. However, literature is scarce regarding substance abusers with a co-morbid trauma history. This study assessed whether alcoholdependent subjects with high levels of childhood trauma would have higher scores for the Neuroticism domain, and lower scores in the Extroversion and Agreeableness domain, regardless of level of alcohol use.

Methods: Forty-one male alcohol-dependent subjects (age=43±10 y/o) were assessed during participation in a residential treatment program. Subjects completed the NEO-PI-R to assess personality characteristics and the Childhood Trauma Questionnaire (CTQ) to assess childhood trauma. Total raw score of the CTQ was used in the analyses. The Timeline Follow Back (TLFB) was administered to assess lifetime number of drinks. To explore if alcohol use mediates the relationship between personality dimensions and childhood trauma, separate linear regression models were conducted using lifetime drinks as a mediator of CTQ scores and the personality characteristics of Extroversion (E), Agreeableness (A), or Neuroticism (N).

Results: Higher childhood trauma scores predicted lower E total score ($p < .05$) and lower scores on the Warmth facet of the E domain ($p < .05$). Childhood trauma predicted greater lifetime alcohol use ($p = .005$), but alcohol use did not mediate the relationship between childhood trauma and E total score. Although childhood trauma was not associated with total N or A scores, high trauma scores predicted high scores on the Vulnerability facet of the N domain ($p = .005$), whereas trauma scores were inversely correlated with the Trust ($p < .02$) and Altruism ($p < .02$) facets of the A domain. Lifetime alcohol use was not shown to be a mediator between childhood trauma and scores on the NEO-PI-R.

Discussion: In an alcohol-dependent population, childhood trauma significantly predicted personality dimensions of Neuroticism, Agreeableness, and Extroversion as assessed by the NEO-PI-R. As these relationships were not mediated by alcohol use, it is important to consider childhood trauma when assessing personality characteristics in persons with substance use disorders.

This work was funded by the INIA Stress U01AA13515 and the Department of Veterans Affairs.

INNOVATIVE APPLICATIONS OF OCULOMOTOR PLANT METRICS AS PREDICTORS OF SOCIAL DRINKING LEVELS AND ATTENTIONAL BIASES TO ALCOHOL-RELATED STIMULI

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Previous research has linked decrements of neurophsyiological functioning, including subtle oculomotor dysfunction, to chronicity of alcohol consumption among problem drinkers. However, the relationship between basic oculomotor functioning and perceptual/attentional responses to alcohol-related stimuli has not been adequately examined to date. In the current study, basic measures of oculomotor function (e.g., oculomotor plant metrics) were examined, along with visual measures of attentional bias to alcohol-related images, to determine (i) if oculomotor plant metrics are related to quantity/frequency of alcohol consumption among young social drinkers, (ii) which measure (oculomotor plant metrics vs. attentional bias) better predicts quantity/frequency of alcohol consumption and (iii) whether or not oculomotor plant metrics correlate with attentional bias measures among young social drinkers. Participants (N = 29) completed questionnaires assessing basic demographics and alcohol consumption characteristics. Prior to questionnaire completion, participants completed 2 ocular imaging tasks: (i) an attentional task in which they viewed photographs of alcohol-related scenes, household objects or a combination of these items, and (ii) a task including saccadic stimuli designed to elicit oculomotor plant metrics. Attentional measures were better predictors of quantity/frequency of alcohol consumption. Although not predictive of QFI, a number of oculomotor plant metrics were correlated with attentional measures of dwell time on alcoholrelated images and point of initial fixation (alcohol vs. control images). Taken together, these results suggest that, even among young social drinkers, individuals with subtle decrements of oculomotor functions may exhibit enhanced attentional bias for alcohol-related images. This quality could be considered an early risk factor for the development of alcohol abuse or dependence, as attentional bias to alcohol-related images may induce craving, which may lead to excessive alcohol use.

ALCOHOL EXPECTANCIES AND THEIR DIFFERENTIAL RELATIONSHIP TO MEASURES OF STRESS AND ATTENTIONAL BIAS AMONG ALCOHOL ABUSERS IN TREATMENT

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For many alcohol dependent individuals, stress may be a precipitating factor for relapse. However, few studies have examined physiological measures of stress among detoxified, abstinent alcohol abusers, particularly as they relate to self-reported reasons for drinking and behavioral responses to alcohol cues. The current study examined the relationship between drinking expectancies, alcohol-related attentional bias, perceived stress, and salivary cortisol in 25 inpatients undergoing treatment for alcohol abuse/dependence. Self-report measures included the Comprehensive Effects of Alcohol Questionnaire (CEAQ; Fromme et al., 2003) and the Perceived Stress Questionnaire (Cohen et al., 1983). Attentional bias to alcohol cues was measured via reaction times to a computerized dot probe task, administered to a subset of 13 participants. Saliva samples were processed via enzyme immunoassay. For statistical analyses, perceived stress, reaction time change scores (incongruent alcohol trials—congruent alcohol trials) and cortisol levels were entered into separate stepwise linear regressions with CEAQ subscales as predictors. Participants' responses to the ''liquid courage'' subscale of the CEAQ negatively predicted cortisol levels (b =)0.54, t =)2.80, p = 0.01). The ''sexuality'' subscale positively predicted perceived stress levels (b = 0.60, t = 2.65, p = 0.02). The ''sociability'' and ''liquid courage'' subscales positively predicted attentional bias to alcohol cues (b = 1.28, t = 3.95, p = 0.006 and b = 3.33, t = 4.28, p = 0.004, respectively); whereas, ''sexuality'' and ''risk and aggression'' subscales negatively predicted alcohol cues (b =)2.82, t =)4.87, p = 0.002 and b =)2.22, t =)4.15, p = 0.004, respectively). The most intriguing finding of the current study is that physiological and selfreport measures of stress during treatment for alcohol abuse appear to be differentially related to alcohol use expectancies. Further, participants' expectancies about alcohol may influence attentional bias to alcohol cues during alcohol treatment. These results represent a first step toward a better understanding of the relationship between stress, alcohol expectancies and response to alcohol cues during the crucial first period of abstinence during in-patient treatment. Longitudinal studies are currently underway to determine the predictive utility of these findings as they relate to relapse and recovery.

STRESS-RELATED ANXIETY IS MODERATED BY SALIVARY CORTISOL, DRINKING EXPERIENCE AND ALCOHOL EXPECTANCIES IN SOCIAL DRINKERS

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Alcohol expectancies may be an important determinant in social drinkers' decisions to consume alcohol. For instance, many individuals endorse the notion that alcohol relieves stress and increases well-being. To date, few studies have examined the relationship between alcohol-related cognition and stress response among social drinkers. The current project examined the relationship between changes in state anxiety due to an acute stressor (Paced Auditory Serial Addition Task; PASAT, Gronwall, 1977) and drinking expectancies, drinking history, trait anxiety, perceived stress and salivary levels of cortisol in a group of 37 male and female social drinkers. Self-report measures included the Spielberger State and Trait Anxiety inventories (Spielberger, 1983), Perceived Stress Questionnaire (Cohen et al., 1983) and the Comprehensive Effects of Alcohol Questionnaire (CEAQ; Fromme et al., 2003). Saliva samples were processed via enzyme immunoassay. For statistical analyses, changes in state anxiety due to the PASAT and current drinking patterns were entered into separate stepwise linear regressions with CEAQ positive and negative subscales, baseline cortisol changes and cortisol changes due to the PASAT stressor as predictors (and age as a covariate). Changes in baseline levels of cortisol were significantly negatively related to changes in state anxiety due to the PASAT ($b =)0.30$, $t =)2.10$, $p = 0.04$); in other words, larger drops in cortisol levels during the baseline period were associated with smaller subsequent changes in state anxiety due to the PASAT. Positive expectancies regarding alcohol and number of years drinking ($b =)0.57$, $t =)4.07$, $p < 0.0001$, and $b =)0.46$, $t =)2.44$, $p = 0.02$, respectively) were also negatively related to increases in state anxiety due to acute stress. Self-reported recovery of anxiety after the stressor was significantly positively related to state anxiety recovery after stress, with larger recovery associated with more positive alcohol expectancies ($b = 0.42$, $t = 2.67$, $p = 0.01$). These findings could have implications for the understanding of the cognitive processes involved with stress and coping among social drinkers. This project was funded by a pilot grant from INIA-Stress/NIAAA.

ERP CORRELATES OF ATTENTIONAL CAPTURE BY ALCOHOL-RELATED IMAGES IN SOCIAL DRINKERS

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The use of alcohol to cope with stress has significant health implications, yet the exact neurophysiological mechanisms underlying this tendency remain elusive. We examined the effects of an acute stressor (Paced Auditory Serial Addition Task; PASAT, Gronwall, 1977) on attention-sensitive event-related potentials (ERPs) elicited by images of alcohol and objects using a 3-stimulus oddball paradigm. Seventy-five participants were randomly assigned to either an alcohol target (respond to alcohol pictures, ignore other objects or nonsense shapes) or an object target condition (respond to object pictures) while event-related potentials (ERPs) were measured before and after either stress induction or a non-stressful control task. Analyses focused on the peak latencies of 2 prominent ERP components: a frontocentral negativity (the N3) and a centroparietal positivity (the P3). Two separate ANOVAs conducted for the peak latencies of each component with stimulus type (alcohol vs. object) and time (pre- vs. post-task) as within subjects factors and task (PASAT vs. control) and target condition (alcohol as target vs. nontarget) as between-subjects variables. Analyses revealed that stress significantly decreased the latencies of both components beyond that of mere repetition. Follow-up analyses indicated that latency changes to alcohol targets for both the N3 and P3 after the control task were equivalent to those to targets (both alcohol and object) after the PASAT. These results suggest that alcohol images capture attention automatically in social drinkers in a way that is robust to state-related changes in anxiety, as indexed by similar repetition effects over time in the absence of stress. These results and their implications for alcohol abuse disorders are discussed in the context of the Elaborated Intrusion Theory of Desire (Kavanaugh, Andrade, & May, 2005), wherein intrusive thoughts about appetitive targets like alcohol images can be triggered automatically by external cues.

Virginia

OF MICE AND MONKEYS – EXPRESSION NETWORKS CORRELATING WITH ETHANOL DRINKING BEHAVIOR ACROSS SPECIES

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Genome-wide expression approaches have produced prodigious lists of genes regulated by ethanol in brain or showing expression correlations with given ethanol behavioral traits. Identifying single genes or gene networks having functional roles for ethanol phenotypes within such datasets has been a considerable challenge. To address this issue we have compared brain region expression profiling across multiple species. Here we describe brain regional expression networks correlated with ethanol drinking behavior in both mouse and primate models. We profiled (Affymetrix microarrays) medial prefrontal cortex, nucleus accumbens, hippocampus, and amygdala in a long-term ethanol drinking model in Macaca fasicularis (Cynomolgus macaque). Female cynomolgus monkeys (n = 12) were induced to drink ethanol using a schedule-induced polydipsia paradigm. A total of 12 months of freechoice ethanol (4% w/v) self-administration followed the induction period. Food and water were concurrently available during 12 months of free access to ethanol. This model produced a high proportion of heavy drinking monkeys (67%) consuming more than 3 g/kg of ethanol daily. Controls (n = 4) were treated by a similar induction protocol with diets isocaloric to the ethanol group, followed by only water drinking. Expression arrays were also performed across medial prefrontal cortex, nucleus accumbens and ventral tegmental area from individual C57BL/6 mice consuming ethanol by 2-bottle choice drinking for 6 weeks. Ethanol drinking showed a striking correlation with an extended dopamine signaling expression network in monkey nucleus accumbens. This network included a number of genes known to modulate ethanol drinking behavior. A novel set of 49 genes had nucleus accumbens expression correlated with drinking behavior in both mice and monkeys. Of considerable interest, our results point to vesicle trafficking, actin cytoskeleton regulation and mGluR type I signaling as important molecular components underlying variance in ethanol drinking behavior. Additional bioinformatics and network analysis has identified potential hub genes for validation by gene targeting approaches. These results could provide novel insight into gene networks regulating ethanol drinking behavior across multiple animal models. Supported by NIAAA INIA grants AA013641 (KAG) AA016667 (MFM) and AA016662 (MFM).

ELUCIDATION OF ETHANOL RESPONSIVE GENE NETWORKS IN BXD RECOMBINANT INBRED STRAINS

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Initial responses to acute ethanol in humans have proven to be heritable and highly informative predictors of future drinking behavior and long term risk for developing alcoholism. A similar inverse relationship between acute ethanol sensitivity and drinking behavior also exists in mice. For example, DBA/2J (D2) inbred mice are highly sensitive to ethanol but generally avoid consuming it in 2 bottle choice studies. Conversely, C57BL6/J (B6) inbred mice are less sensitive to ethanol but consume it in relatively large volumes. Our laboratory previously reported that the divergent ethanol responses exhibited by B6 and D2 mice are accompanied by significant disparities in their neurogenomic response to the drug. While ethanol induced robust changes in gene expression across multiple brain regions in both strains, there were marked differences in the pattern of altered expression between B6 and D2 mice, which may contribute to the observed differences in their ethanol behaviors. In order to identify and characterize the biological pathways that are perturbed by acute ethanol, we profiled the prefrontal cortex (PFC) transcriptome of 27 B6 · D2 (BXD) recombinant inbred mice 4 h after receiving IP injections of either saline or ethanol. We then used the S-score algorithm to measure the significance of each gene's change in expression between treatment groups. We then applied a novel graph-theoretical algorithm to this data in order to construct genetic networks comprised of genes exhibiting highly similar responses to ethanol across the BXD panel. A permutation based S-score analysis identified a subset of these networks that were highly sensitive to ethanol. We screened these ethanol sensitive gene networks for enrichment in functional categories and found an over representation of genes involved in nervous system development and synaptic transmission. These networks are also significantly correlated with several responses to other drugs of abuse as well as neurotransmitter systems known to modulate ethanol responses. Expression quantitative trait locus (eQTL) mapping revealed that several gene networks contain large trans bands, indicating their ethanol response is largely modulated by a small number of genetic loci. Genes with cis eQTL that coincide with trans bands are candidate regulators of these networks. These results may identify novel mechanisms underlying acute ethanol sensitivity and provide new candidate genes for alcoholism susceptibility.

OVER-EXPRESSION OF GSK-3b IN PFC INCREASES VOLUNTARY ALCOHOL DRINKING AND WITHDRAW-INDUCED ANXIETY IN MICE

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Alcohol dependence and abuse are important public health issues that cause substantial morbidity and exacerbate a wide range of additional medical and psychiatric conditions. However, molecular mechanisms underlying alcohol dependence and abuse have not been fully elucidated. Previous DNA microarray studies by our laboratory showed that glycogen synthase kinase 3b (GSK-3b) expression was increased in mouse prefrontal cortexes (PFC) after acute ethanol treatment. In the present study, we hypothesize that GSK-3b may be involved in ethanol drinking and other ethanol-related behaviors. To test this hypothesis, recombinant adeno-associated virus (AAV) vectors containing GSK-3b cDNA (AAV.GSK), dominant-negative GSK-3b cDNA (AAV.DN-GSK), or control AAV virus (AAV.IRES) were microinjected into the PFC of C57BL/6 mice to upregulate or suppress GSK-3b function. We first confirmed by q-rtPCR that GSK-3b mRNA expression was increased in PFC after acute ethanol treatment (i.p., 2 g/kg). Western blotting also showed ethanol-induced increased phosphorylation of GSK-3b. In viral vector studies, mice receiving AAV.GSK injections had significantly increased ethanol drinking compared with those injected with AAV.DN-GSK or AAV.IRES alone. Twenty-four hours after cessation of prolonged ethanol drinking, mice injected with AAV.GSK showed decreased time/distance traveled in the light compartment of a light-dark box assay for anxiety-like behavior. This suggests increased anxiety-like behavior on ethanol withdrawal compared to control mice and might contribute to increased ethanol drinking behavior seen with AAV.GSK injected animals. These results indicate that GSK-3b has a crucial role in modulating ethanol drinking behavior and ethanol withdraw-induced anxiety. This work may aid in the development of new pharmacotherapies for the treatment of alcohol abuse and alcoholism. Supported by NIAAA Grants R01 AA014717 and U01 AA016667 to MFM.

MICROARRAY ANALYSIS OF ACUTE ETHANOL ACTION IN THE MESOCORTICOLIMBIC SYTEM OF FYN KINASE KNOCKOUT MICE

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Acute behavioral responses to ethanol in human and animal models have predictive validity for determining an individual's risk of long-term drinking behavior. Although the neurobiology of alcohol addiction is extremely complex, prior microarray studies from our laboratory have demonstrated that brain region specific variation in gene expression is one potential factor influencing acute ethanol behavioral responses. For example, we have previously shown that inbred strains of mice (C57BL/6J and DBA/2J) differing in acute ethanol behavioral phenotypes demonstrate basal and ethanol-evoked differences in gene expression across the mesocorticolimbic system, including myelin-associated gene expression. Myelin is an important component of CNS plasticity that is substantially altered in alcoholics. Bioinformatics analysis of B6 and D2 expression data implicated Fyn kinase as a likely mediator of differences in B6 and D2 myelin gene expression profiles. Fyn has been previously shown to be an important regulator of myelin basic protein (Mbp) expression and a subset of acute ethanol behavioral phenotypes. Therefore, we conducted a genomic analysis of Fyn kinase knockout mice to test the hypothesis that a myelin gene network is an underlying element in the neurobiology of acute ethanol-mediated responses. In addition, we have identified ethanol-responsive gene networks related to Fyn within the mesocorticolimbic system. The prefrontal cortex (PFC), nucleus accumbens (NAC), and ventral tegmental area (VTA) all exhibited a decrease in Mbp expression; however, the NAC revealed a down-regulation in an entire myelin gene network (p-value<0.01). Given the essential role of myelin in synaptic transmission, its altered expression in our previous findings, and those herein, myelin gene expression should be considered a critical underlying aspect of both acute and long-term ethanol exposure. Additional overrepresented functional categories included genes related to potassium ion channels, GABA-A receptor activity, regulation of G-protein signaling, and cytoskeletal organization. Ongoing molecular studies will seek to further characterize mechanisms underlying Fyn effect on acute ethanol behavioral phenotypes, and may aid in the development of new, promising pharmacotherapies for alcohol abuse and alcoholism.
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ETHANOL REGULATION OF SERUM GLUCOCORTICOID KINASE 1 (SGK1)

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Previous microarray studies in our laboratory have shown that serum and glucocorticoidregulated kinase 1, Sgk1, is prominently up-regulated by acute ethanol (2 g/kg) in prefrontal cortex (PFC) of DBA/2J mice. Strikingly, Sgk1 was accompanied by a highly correlated group of genes, many of which are also known to respond to glucocorticoids or various types of cellular stress. This suggests that stress-related signaling events might play an important role in ethanol regulation of the Sgk1 gene network. Functionally, Sgk1 is an important focal point of intracellular signaling cross-talk through which the cell surface receptors such as insulinlike growth or neurotrophins, nuclear receptors, and cellular stress pathways converge to control many cellular processes, including receptor or ion channel trafficking, cell proliferation and/or apoptotic responses. Prior work by others also shows that Sgk1 can play an important role modulating neural pathway synaptic plasticity occurring in memory. Together, these findings suggest that Sgk1 induction might play an important role in modifying behavioral responses to ethanol. To test this hypothesis, we chose a gene delivery approach since specific pharmacological agents for modifying Sgk1 function do not exist. We have successfully generated and purified high-titer adeno-associated virus (AAV) expressing FLAGSgk1, an epitope-tagged version of Sgk1, and a K127M kinase dead mutant version of FLAGSgk1. Both viruses were successfully delivered into the prefrontal cortex of DBA/2J mouse brain using stereotaxic microinjection techniques. Following stereotaxic microinjection, we measured behavioral responses (locomotor activation, anxiety or sleep time) to both an acute locomotor-activating dose of ethanol (2 g/kg) and an acute sedative dose of ethanol (3.8 g/kg). Viral expression and placement in mouse brain was verified by immunohistochemistry. No significant acute behavioral differences were found between viral treatment groups. Ongoing studies will determine possible Sgk1 effects on locomotor sensitization and acute withdrawal as well as ethanol 2-bottle choice drinking (in C57BL/6J mice). Together, these studies will provide a robust test for the potential role of Sgk1 in modulating ethanol behaviors. Our results could provide a novel target for development of new pharmacotherapies for alcoholism. Supported by NIAAA Grants R01 AA014717, U01 AA016667 and P20 AA017828 to MFM and NIDA Grant T32 DA007027 to BC.