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STRESS

"INIA: STRESS, ANXIETY AND ALCOHOL ABUSE"

Abstracts

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California

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J.C. Campbell, C.P. Dunn, R.R. Nayak, R.J. Carnatean, T.E. Kippin
University of California, Santa Barbara, Psychology Department, Santa Barbara, CA 93106

Maryland

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Marguerite C. Camp, Jessica L. Ihne, Andrew Holmes
Section on Behavioral Science and Genetics, Laboratory for Integrative Neuroscience,
National Institute on Alcoholism and Alcohol Abuse, NIH, Rockville, MD, USA

GLUTAMATERGIC MEDIATION OF ACUTE INTOXICATING EFFECTS OF ETHANOL IN MICE
A. Holmes, NIAAA
NIAAA, Rockville, MD, USA

ETHANOL INTERACTIONS WITH GI/O-COUPLED G-PROTEINS IN GABAERGIC TERMINALS
David M. Lovinger, Giuseppe Talani
Section on Synaptic Pharmacology, Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism/National Institutes of Health, Bethesda, MD 20892

ETHANOL-ENDOCANNABINOID INTERACTIONS AT GABAERGIC SYNAPSES IN THE BASOLATERAL AMYGDALA
G. Talani, D.M. Lovinger
Section on Synaptic Pharmacology, Laboratory for Integrative Neuroscience, NIAAA/NIH,
Bethesda, MD 20892, USA

New York

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H. Bach-Mizrachi, V. Arango, J.Z. Davis, S.A. Kassir, J.J. Mann, M.D. Underwood
New York State Psychiatric Institute, Columbia University, New York, N.Y. 10032

North Carolina

ETHANOL METABOLISM IN VERVET MONKEYS (CERCOPITHECUS AETHIOPS)
EJ Burnett, AT Davenport, VM Maxey, EP Dolson, DP Friedman
Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

EFFECT OF ORAL ADOLESCENT METHYLPHENIDATE TREATMENT AND WITHDRAWAL ON ETHANOL DRINKING AND LOCOMOTOR RESPONSE TO PSYCHOSTIMULANTS

E.S. Carter, A.M. Chappell, S.R. Jones, J.L. Weiner
Department of Physiology and Pharmacology, Translational Center for the Neurobehavioral Study of Alcohol, Wake Forest University School of Medicine, Winston-Salem, NC, 27157

EFFECT OF CHRONIC ETHANOL ON 5HT1A DENSITIES AND SEROTONIN TRANSPORTER LEVELS IN THE DORSAL RAPHE OF MOTHER-REARED VS NURSERYREARED MACAQUES.
D.P. Friedman, E.P. Dolson, V.M. Maxey, E.G. Burnett, J.B. Daunais, A.T. Davenport
Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

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T.A. Mathews, J. Locke, D.P. Friedman, S.R. Jones
Dept. of Physiology and Pharmacology, Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA

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B.A. McCool, A.E. Chappell
Wake Forest University School of Medicine and the Translational Center for Neurobehavioral Studies on Alcohol, Winston-Salem NC 27157; Dept. Physiology & Pharmacology, Wake Forest Univ. School of Medicine, Winston-Salem, NC, 27157

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T.E. Spence, D.T. Christian, M.R. Diaz, B.A. McCool
Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

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K.A. Grant, M. Odagiri, A. Rau, J. Basile, K. Nusser
Oregon National Primate Research Center, OHSU, Beaverton, OR 97006

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C.D. Kroenke, G.S. Flory, K.A. Grant
Oregon National Primate Research Center, Oregon Health and Science University, Portland, OR 97239

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T. Moschak, C.M. Helms, A. Buckert, K.A. Grant
Oregon Health & Science University, Portland, OR 97239

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M. Odagiri, A. Rau, J Basile, K.A. Grant

Department of Behavioral Neuroscience, Oregon National Primate Research Center, OHSU, Portland, OR 97239 USA

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A. Rau, B Ferguson, J. Bahk, M. Odagiri, J. Basile, K.A. Grant
Department of Behavioral Neuroscience, Oregon National Primate Research Center, OHSU, Portland, OR 97239 USA

South Carolina

ALTERED ETHANOL DISCRIMINABILITY IN NR2A DEFICIENT MICE
H.C. Becker, M. Cunningham, M. Overstreet, A. Holmes, D. Lovinger
Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425

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J. E. Grisel, J. Crawford, M.E. Locklear, T. E. Spence, N.y J. Anderson, B. A. McCool
Furman University, Greenville, SC; Wake Forest University, Winston Salem, NC

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M.F. Lopez, W.C. Griffin III, M.P. Overstreet, H.C. Becker
Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425

Tennessee

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K.M. Hamre, G. Mittleman, M. Cook, C.D. Blaha, T. Ansah, V.M. Philip, E.J. Chesler, D. Goldowitz
Dept. Anat. & Neurobiol., UTHSC, Memphis TN 38163; Dept. Psych., Univ. Memphis; Meharry Med. Coll; Sys. Gen. Group, Bio. Div., ORNL & Gen. Sci. & Tech. Prog., Univ. Tenn.; CMMT, UBC.

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T.L. Kash, A.J. Baucum, K.L. Conrad, R.J. Colbran, D.G. Winder
Department of Molecular Biology and Biophysics, Vanderbilt University School of Medicine

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V. M. Philip, M. Cook, K. Hamre, G. Mittleman, T. Ansah, C. Blaha, D. Goldowitz, E. J. Chesler
Oak Ridge Natl Lab. and U. Tenn, Oak Ridge, TN, 37831; U. Memphis, TN, 38152; U. Tenn Hlth Sci Ctr, Memphis, TN, 38163; Meharry Med Coll, Nashville, TN, 37208; U. British Columbia, V6T 1Z4

Texas

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N. Ceballos, R. Guiliano, N. Y. Y. Wicha, R. Graham

Department of Psychology, Texas State University, San Marcos, TX 78666.; Department of Psychology, Texas State University, 601 University Drive, San Marcos, Texas, 78666

Virginia

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N.A. Bruce1, J. T. Wolstenholme1, J. B. Daunais3, A. Rau3, C. Keily3, K. A. Grant2, M. F. Miles1
1Depts of Pharmacology/ Toxicology & Neurology, Virginia Commonwealth Univ. 2Dept of Behav Neuroscience, Oregon Health & Sciences Univ. 3Dept of Physiology & Pharmacology, Wake Forest Univ. School of Med; 1217 E. Marshall Street, Medical Sciences Building, Room 630, Richmond, VA, 23298

ACUTE ETHANOL-RESPONSIVE GENE EXPRESSION IN FYN KINASE KNOCKOUT MOUSE PREFRONTAL CORTEX
S.P. Farris, N.A. Bruce, M.F. Miles
Department of Pharmacology and Toxicology, Virginia Commonwealth University, School of Medicine, Richmond, VA 23298

GENE EXPRESSION NETWORKS IN PREFRONTAL CORTEX CONTRIBUTING TO ETHANOL ANXIOLYTIC EFFECTS
M.F. Miles, A. Wolen, A. Putman, N. Bruce
Dept. of Pharmacology and Toxicology, Virginia Commonwealth University

FINE MAPPING A QUANTITATIVE TRAIT LOCUS FOR THE ANXIOLYTIC-LIKE RESPONSE TO ACUTE ETHANOL IN BXD RECOMBINANT INBRED STRAINS
A.R. Wolen, A.H. Putman, N.A. Bruce, M.F. Miles
Virginia Commonwealth University, School of Medicine, Richmond, VA 23290

California

PRENATAL STRESS INCREASES OPERANT RESPONDING AND ALCOHOL INTAKE DURING ALCOHOL REINFORCEMENT IN ADULT C57BL/6J MICE

J.C. Campbell, C.P. Dunn, R.R. Nayak, R.J. Carnatean, T.E. Kippin
University of California, Santa Barbara, Psychology Department, Santa Barbara, CA 93106

Early environmental insults can alter neurodevelopment, predisposing individuals to neuropsychiatric disorders, including substance abuse. Prenatal exposure to stress has previously been shown to produce disruptions in nervous system development, and an adult behavioral phenotype which is hyperresponsive to stress and psychomotor stimulant drugs. This study examined the impact of prenatal stress (PNS) on the motivation to seek and consume alcohol. Timed pregnant C57Bl/6J dams were subjected to repeated restraint stress from E14 until delivery (1 hr, 3 times daily), while control dams were left undisturbed during gestation. After birth, all groups were left undisturbed until weaning at 21 days into same-sex groups. At 8 weeks of age, 2 male pups were selected from each litter for testing and single housed. All mice were maintained under standard conditions with ad libitum food access. In a two-bottle choice task, mice were allowed continuous access to one bottle of water and one bottle of 15% alcohol in water. No significant differences were found in the two-bottle choice alcohol consumption between PNS and control mice. Next, mice were examined under operant alcohol access conditions. Mice were initially trained to press a lever for oral sucrose reinforcers (20ll volume of 15% w/v sucrose in water) during 15 minute daily sessions. This was followed by a standard sucrose fading procedure, with an unsweetened ethanol solution (20ll of 10% ethanol in water) as the final reinforcer. The PNS and control groups were not significantly different in either operant responding rate or volume consumed during sucrose reinforcement. During ethanol reinforcement, PNS and control groups were significantly different on both measures, with the PNS group showing increased responding on the active lever and increased alcohol consumption compared to controls. These findings indicate PNS increases the motivation for alcohol and that early environmental factors are likely to play a role in the development of alcoholism.

Maryland

INTERMITTENT VAPOR EXPOSURE AS A MODEL FOR EXAMINING WITHDRAWALINDUCED ANXIETY, TOLERANCE AND INCREASED ETHANOL SELF-ADMINISTRATION IN MICE

Marguerite C. Camp, Jessica L. Ihne, Andrew Holmes
Section on Behavioral Science and Genetics, Laboratory for Integrative Neuroscience,
National Institute on Alcoholism and Alcohol Abuse, NIH, Rockville, MD, USA

Rodent models of ethanol dependence are imperative for investigating both the underlying neurobiology of this disease and post-ethanol-dependent behaviors. Recent studies in rodents have established that intermittent exposure to ethanol vapor induces an increase in ethanol drinking (Becker and Lopez, 2004; Finn et al., 2007; Hansson et al., 2007; Lopez and Becker, 2005). The present experiments used a chronic intermittent ethanol vapor exposure model to examine key features of ethanol dependence; namely, acute withdrawal-induced anxiety, tolerance to the ataxic and sedative/hypnotic effects of ethanol, and increased ethanol drinking in mice. Male C57BL/6J mice were exposed to ethanol vapor or air for 16 hours/day for 4 days. Separate groups of mice underwent 2, 3 or 4 weeks of the 4-day cycles. Two days following the last cycle, acute withdrawal-induced anxiety was measured using the dark-light emergence test. Tolerance to ethanol-induced ataxia, using the accelerating rotarod test, and ethanol induced sedation/hypnosis, were tested 3 and 4 days after the final cycle, respectively. Ethanol self administration was examined using a 24 hour 2-bottle continuous access paradigm. Preliminary results showed that mice exposed to intermittent ethanol vapor exhibited anxiety-like behavior, as measured by lesser exploration of the light compartment of the emergence test, as compared to the air-exposed controls, although this appears to depend on cycle number. Preliminary data also show that ethanol exposed mice exhibit reduced ataxic and sedative/hypnotic responses to an ethanol challenge, relative to air-exposed controls, again in a cycle-dependent manner. Finally, replicating earlier findings, 4-cycle ethanol-exposed mice showed an increase in ethanol drinking relative to both their pre-ethanol exposed levels and air-exposed controls. Collectively, these data further validate the chronic intermittent vapor method as a procedure to model multiple key symptomatic features of alcohol dependence. Ongoing studies are utilizing this method to elucidate the role of the glutamatergic system in the development and expression of ethanol-dependent behaviors.
Research supported by the National Institute on Alcohol Abuse and Alcoholism Intramural Research Program.

GLUTAMATERGIC MEDIATION OF ACUTE INTOXICATING EFFECTS OF ETHANOL IN MICE

A. Holmes, NIAAA
NIAAA, Rockville, MD, USA

Compounds with anti-glutamatergic properties currently in clinical use for various indications (eg Alzheimer's disease, epilepsy, psychosis, mood disorders) have potential utility as novel treatments for alcoholism. Enhanced sensitivity to certain acute intoxicating effects (ataxia, sedative) of alcohol may be one mechanism by which anti-glutamatergic drugs modulate alcohol use. We have found that one N-methyl-D-aspartate receptor (NMDAR) antagonist, memantine, but not another, dextromethorphan, potentiated the ataxic but not hypothermic or sedative/hypnotic effects of ethanol in C57BL/6J mice. In addition, lamotrigine accentuated ethanol-induced sedation/hypnosis, whereas oxcarbazepine was without effect. Topiramate was without effect per se under baseline conditions in C57BL/6J, but had a synergistic effect with MK-801 on ethanol-induced sedation/hypnosis. Comparing inbred strains, topiramate was found to significantly potentiate ethanol's sedative/hypnotic effects in BALB/cJ, but not 129S1, C57BL/6J, or DBA/2J strains. Topiramate also increased ethanol-induced sedation/hypnosis in C57BL/6J after exposure to chronic stress exposure. Data will be discussed in terms of the possibility that topiramate and possibly other anti-glutamatergic drugs could promote the acute intoxicating effects of ethanol in specific subpopulations defined by genetics or life history.

ETHANOL INTERACTIONS WITH GI/O-COUPLED G-PROTEINS IN GABAERGIC TERMINALS

David M. Lovinger, Giuseppe Talani
Section on Synaptic Pharmacology, Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism/National Institutes of Health, Bethesda, MD 20892

Ethanol potentiates GABAergic synaptic transmission, and this acute action is thought to contribute to intoxication. Increased release of GABA from presynaptic terminals contributes to the EtOH enhancement of GABAergic synaptic inhibition. However, the molecular mechanisms that contribute to, and modulate, this presynaptic enhancement are not yet understood. Evidence from several laboratories indicates that presynaptic G-protein-coupled receptors (GPCRs) that activate Gi/o proteins can counteract the effects of ethanol. We have observed that ethanol potentiates GABAergic transmission at synapses onto principal neurons in the basolateral amygdala in vibrodissociated neurons and brain slices. Ethanol (50-80 mM) increases the frequency and amplitude of spontaneous GABAA receptor-mediated inhibitory postsynaptic currents (sIPSCS), but only increases the frequency of miniature IPSCs, indicating a presynaptic locus of EtOH potentiation. In the isolated neuron preparation the effect of ethanol desensitizes, and this desensitization is prevented by a GABAB receptor antagonist. In brain slices, EtOH potentiation is prevented by agonists or antagonists of the CB1 cannabinoid receptor. The CB1 agonist effect is similar to the effects of GABAB receptor activation described by Ariwodola and Weiner (J. Neurosci., 2004), in that both compounds work through Gi/o-coupled receptors to inhibit GABAergic transmission and prevent potentiation by EtOH. Gi/o-coupled GPCRs are known to inhibit adenylyl cyclase activity, while EtOH has been shown to potentiate the function of certain adenylyl cyclase subtypes. Thus, the opposing effects of GPCRs and EtOH on presynaptic function may involve this enzyme or other molecules that regulate presynaptic function.

ETHANOL-ENDOCANNABINOID INTERACTIONS AT GABAERGIC SYNAPSES IN THE BASOLATERAL AMYGDALA

G. Talani, D.M. Lovinger
Section on Synaptic Pharmacology, Laboratory for Integrative Neuroscience, NIAAA/NIH, Bethesda, MD 20892, USA

The basolateral amygdala (BLA) plays crucial roles in drug and alcohol dependence. Activation of type 1 cannabinoid receptors (CB1) in the BLA inhibits neurotransmiter release. Strong activation of BLA pyramidal neurons can produce endocannabinoids (eCBs) that travel retrogradely at synapses and transiently suppress GABA release; a phenomenon called depolarization induced suppression of inhibition (DSI). Ethanol (EtOH) can increase GABA release in BLA. Thus, there may be EtOH-eCB interactions at BLA GABAergic synapses. Using rat BLA coronal slices from young animals (p14-p19) we performed whole cell patch clamp recordings of spontaneous IPSCs from BLA pyramidal neurons. Similar to previous findings, we observed that perfusion of EtOH 80mM increased both amplitude and frequency of sIPSCs (Amp: 132±1.2, Freq: 138±3.8 vs. control). DSI could be activated by a 4 sec depolarization of the postsynaptic neuron from -60 to 0 mV. After this step we observed a decrease in sIPSC frequency without a change in amplitude (Amp: 102.9±2.7, Freq: 76.2±1.5 vs. control values before dep. step).When EtOH (50-80mM but not 10mM) was added in the bath DSI was eliminated, with a partial recovery after 5 min EtOH wash-out. The magnitude and duration of DSI was increased by loading the postsynaptic neuron with anandamide (50 lM) via the patch pipette, and EtOH 80mM still prevented DSI under this condition. These findings indicate that EtOH likely prevents DSI at a step downstream from eCB production. To determine if EtOH interacts with CB1 receptors we co-applied CB1 agonist or antagonist with EtOH and examined sIPSCs. The CB1 agonistWIN55 212-2 (5lM) reduced GABAergic transmission and blocked EtOH potentiation of sIPSCs (Amp: 100.7±1.7, Freq: 97.5±2). Surprisingly, application of the CB1 antagonist SR141716A (1lM) also decreased EtOH potentiation of sIPSCs. However, when a high concentration of EtOH (150mM) was used, SR141716A was ineffective in antagonizing EtOH actions. The effect of EtOH was reduced in CB1 KO and heterozygote mice (change in sIPSC freq inWT: 129.8±5.7, KO: 110.8±5.5*, hetero: 98±4* vs control). These data suggest that CB1 receptors also influence EtOH potentiation of GABA release, and that EtOH does not prevent synaptic inhibition by CB1 activation. These findings could help to explain the strong relationship between the eCB system and the neural effects of EtOH.

New York

REGULATION OF NEURONAL TRYPTOPHAN HYDROXYLASE (TPH2) BY PROTEIN KINASE A (PKA) AND EFFECTS ON GLYCOGEN SYNTHASE KINASE 3 BETA (GSK3B) IN ALCOHOLISM

H. Bach-Mizrachi, V. Arango, J.Z. Davis, S.A. Kassir, J.J. Mann, M.D. Underwood
New York State Psychiatric Institute, Columbia University, New York, N.Y. 10032

Low serotonin (5-HT) is associated with alcoholism. Paradoxically, we find higher immunoreactivity for TPH2, the rate-limiting 5-HT biosynthetic enzyme, in alcoholics. More TPH2 protein could reflect a compensatory response to less brain 5-HT release by the brainstem raphe nuclei, which include the dorsal (DRN) and median (MRN) raphe nuclei. TPH2 activity is regulated by PKA and GSK3b. To explain the findings of more protein but less product, we hypothesize that in alcoholics, PKA, a TPH2 activity-enhancing kinase, will be lower, while GSK3b, a kinase exhibiting higher activity in mutant mice with low TPH2 activity, will be higher, representing an indirect measure of lower TPH2 function. Western blots were used to measure TPH2, PKA and GSK3b protein in sections of brainstem containing DRN and MRN, from five pairs of age and sex matched alcoholics and controls. The housekeeping protein GAPDH was used to control for inter-blot variability. Blots were quantified using densitometry measured by computer assisted image analysis. We detected no differences between groups in any of the three proteins measured. However, a negative correlation between GSK3b and TPH2 protein levels ($r=-0.883$, $p=0.04$) and between PKA and TPH2 protein levels ($r=-0.820$, $p=0.024$) was found in alcoholics where more GSK3b protein was associated with less TPH2 protein. These correlations were not present in controls ($p>0.05$). GSK3b was negatively correlated with duration of alcohol abuse ($r=-0.80$, $p=0.056$) and with age in alcoholics ($r=-0.710$, $p=0.07$). There were no significant correlations with age in controls ($p>0.05$). A negative correlation between PKA and TPH2 in alcoholics suggests a failure to enhance catalytic activity. Deficits in TPH2 catalytic activity are further indicated by the inverse relationship between TPH2 and GSK3b, where GSK3b is an index of TPH2 activity. These results are consistent with reports in mice where regulation of GSK3b is indicative of 5-HT level and TPH2 catalytic activity. Our findings indicate that higher GSK3b protein levels are correlated with shorter duration of alcohol abuse. The negative correlation with age in alcoholics and not controls suggests that this is not an age-dependent effect. However, studies with larger sample sizes will determine whether there are confounding effects of age. Supported by AA09004, AA11293, MH40210, MH79439, MH62185, INIA-NIAAA Pilot Project and the Dianne Goldberg Foundation.

North Carolina

ETHANOL METABOLISM IN VERVET MONKEYS (CERCOPITHECUS AETHIOPS)

EJ Burnett, AT Davenport, VM Maxey, EP Dolson, DP Friedman
Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Alcohol abuse and dependence are widespread disorders that constitute a significant public health concern. Nonhuman primates provide unique research models to study alcohol use disorders since they are phylogenetically close to humans, have significant physiological similarities and extensive capacities for complex social and cognitive behaviors which parallel human development. These, combined with the neuroanatomical similarities observed, help to facilitate the translation of findings from these animal models to the human condition. We have begun to examine the influence of behavioral and physiological characteristics related to stress and response to novelty on ethanol consumption in vervet monkeys. Vervets have been reported to self-administer significantly greater amounts of ethanol than other monkey species. Despite these reports, there are inadequate data available regarding potential species differences in ethanol metabolism. Ten ethanol naïve vervet monkeys [high cortisol/high reactivity (n=5); low cortisol/low reactivity (n=5)] were used to examine ethanol metabolism over a five hour window following an acute bolus dose of ethanol. Three doses of ethanol (30% w/v; 0.5, 1.0, 1.5 g/kg) were administered nasogastrically three weeks apart to awake monkeys while comfortably seated in primate chairs. Blood samples were obtained at baseline, 15, 30, 60, 90, 120, 180, 240 and 300 minutes following ethanol administration. Samples were analyzed for blood ethanol concentration (BEC) and blood acetaldehyde concentration using a gas chromatograph. Blood acetaldehyde concentrations increased immediately following ethanol administration for all three doses and returned to zero by 240 minutes post-ethanol for the 0.5 g/kg dose but remained elevated out to 300 minutes for the two higher doses. BEC values increased as a function of dose. At the 0.5g/kg dose, peak BECs were obtained between 30 and 60 minutes post-ethanol (25.6±6.12 mg% and 25.9±4.15 mg% respectively). Higher doses of ethanol (1.0 and 1.5 g/kg) produced peak BECs at 120 minutes (85.37±17.71 mg% and 132.11±21.34 mg% respectively). No differences in metabolism were observed between high and low cortisol/reactivity groups. These BEC values correspond well with previous values we have obtained in a similar study using rhesus macaques. The data presented here does not suggest that a difference in ethanol metabolism accounts for the difference in ethanol consumption between species.

EFFECT OF ORAL ADOLESCENT METHYLPHENIDATE TREATMENT AND WITHDRAWAL ON ETHANOL DRINKING AND LOCOMOTOR RESPONSE TO PSYCHOSTIMULANTS

E.S. Carter, A.M. Chappell, S.R. Jones, J.L. Weiner
Department of Physiology and Pharmacology, Translational Center for the Neurobehavioral Study of Alcohol, Wake Forest University School of Medicine, Winston-Salem, NC, 27157

Methylphenidate (MPH) is widely used in the treatment of attention deficit hyperactivity disorder (ADHD). Although MPH and other stimulants are effective ADHD treatments, some studies have suggested that chronic childhood exposure to these drugs may increase risk of substance abuse in adulthood. Our recent data revealed that rats given daily MPH injections (5 mg/kg) during adolescence (PD 28-50) exhibited transient increases in anxiety and sustained increases in the rate of ethanol (EtOH) drinking in an operant procedure. A significant increase in locomotor response to acute cocaine injection was also observed four months after MPH exposure. Since several studies have suggested that the route of MPH administration can have profound effects on the behavioral and neurobiological consequences of chronic drug exposure, in this study we examined the effect of an oral MPH treatment protocol designed to simulate therapeutic drug exposure levels. Two groups of nine male, Long-Evans rats were given oral MPH (5 mg/kg) or vehicle (10% sucrose) twice daily, 5 days/week, from PD 28-91 and EtOH drinking was assessed from PD 50-160 using an intermittent two-bottle choice procedure that engenders relatively high EtOH intake (ACER 32: 1816-23). Subjects rapidly consumed the MPH (< 3 min.) and this treatment resulted in therapeutic MPH blood levels (8.5±0.5 ng/ml, 30 min. post treatment). EtOH consumption was not altered by concurrent MPH exposure however transient increases in EtOH intake were seen during the first two weeks of MPH withdrawal. Notably, anxiety-like behavior, assessed in the elevated plus-maze and light/dark box, was also significantly elevated during this period. In contrast to our findings with i.p. adolescent MPH exposure, oral MPH-treated rats did not show an increased locomotor response to cocaine (10 mg/kg, i.p.) during MPH withdrawal and exhibited significant tolerance to the locomotor stimulant effect of an acute MPH challenge (10 mg/kg, i.p.). Taken together, these data suggest that oral MPH exposure during adolescence does not increase EtOH drinking; however MPH withdrawal may lead to a transient increase in EtOH intake and anxiety like behavior. Moreover, although i.p adolescent MPH treatment results in a long-lasting increase in locomotor responsiveness to cocaine, possibly reflecting a sensitization of the mesolimbic DA system, oral adolescent MPH exposure may not lead to similar changes. Funded by NIH grants AA15568 and AA17056.

EFFECT OF CHRONIC ETHANOL ON 5HT1A DENSITIES AND SEROTONIN TRANSPORTER LEVELS IN THE DORSAL RAPHE OF MOTHER-REARED VS NURSERYREARED MACAQUES.

D.P. Friedman, E.P. Dolson, V.M. Maxey, E.G. Burnett, J.B. Daunais, A.T. Davenport
Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Early childhood stress can fundamentally alter brain and neuroendocrine development, and evidence suggests these alterations may underlie an increase in the risk for excessive ethanol consumption later in life. Because the dorsal raphe nucleus (DRN) is involved in modulation of both the HPA axis and the brain reward circuitry central to the development of excessive drinking behavior, we examined the effect of chronic ethanol self-administration on this structure. Eight male rhesus macaques [mother reared (MR, n=4) or nursery-reared (NR, n=4)] were induced to self-administer ethanol and 10 age matched rhesus males [(MR, n=5) or (NR, n=5)] were induced to self-administer a maltose-dextrin solution isocaloric with ethanol using a schedule-induced polydipsia procedure. After induction, the monkeys self-administered ethanol or maltose-dextrin during daily 22 hr sessions in their home cage for at least 12 months. At necropsy, brains were blocked, flash-frozen, and subsequently processed for in vitro receptor autoradiography using the 5HT1A receptor antagonist [3H] MPPF and the serotonin transporter (SERT) ligand [3H]Citalopram. Non-specific binding was determined usingWAY-10065 or fluoxetine, respectively. Images were analyzed using AIS software and regions of interest were defined using overlaid nissl stained images of the same sections. SERT binding in the DRN was the same in MR and NR controls ($p<0.45$), but was significantly higher ($p<0.01$) in MR ethanol drinkers than in the MR controls. In addition, SERT levels were significantly higher ($p<0.005$) in the MR ethanol drinkers than in the NR ethanol drinkers. There were no significant differences in SERT levels between the NR controls and the NR ethanol drinkers ($p<0.20$). SERT levels in the MRN were unchanged regardless of rearing or treatment group. SERT, there were no significant difference in DRN 5HT1A receptor density between MR and NR controls ($p<0.96$). However, 5HT1A receptor density was significantly higher in the MR ethanol drinkers ($p<0.04$) than their NR counterparts. These data suggest that in the normal s(control) state, early childhood stress (nursery-rearing) does not have a significant effect on SERT and 5HT1A densities in the DRN. The effects of rearing only become apparent after chronic ethanol self-administration. The MR animals show an adaptive response to ethanol whereas their NR counterparts do not. This study was supported by AA014106 and AA015568 (DPF)

DOPAMINE AND SEROTONIN NEUROCHEMISTRY IN LONG-TERM ALCOHOL DRINKING NURSERY AND MOTHER REARED MONKEYS

T.A. Mathews, J. Locke, D.P. Friedman, S.R. Jones
Dept. of Physiology and Pharmacology, Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA

Acute alcohol administration is known to elevate dopamine (DA) and serotonin (5-HT) levels in the brain. The long-term consequences of alcohol exposure on the dopaminergic and serotonergic systems, however, are not fully understood. Alcohol dependence and abuse are generally considered to be the result of both environmental and genetic factors. In the present study, rhesus macaques reared either by their mothers or in a nursery were studied. Nursery rearing is considered a stressful early environmental challenge. The animals were allowed to voluntarily drink alcohol in a long-term (12 months) exposure paradigm in order to examine the effects of rearing condition and chronic alcohol exposure on the tissue content of DA, 5-HT and their metabolites. Several published studies have shown that cerebrospinal fluid levels of the 5-HT metabolite, 5-HIAA, are decreased in nursery-reared (NR) monkeys compared to mother-reared (MR) controls. We examined DA, DOPAC, HVA, 5-HT and 5-HIAA levels in the caudate, putamen, substantia nigra pars compacta, substantia nigra pars reticulata and raphe nuclei. The only statistically significant differences found between alcohol-naïve animals were decreased HVA and 5-HIAA in putamen of NR compared to MR monkeys. These findings provide support for decreased activity in both the DA and 5-HT systems in early-stressed NR monkeys and demonstrates a brain change in 5-HIAA that correlates with the well-documented decreases in CSF 5-HIAA in NR monkeys. With regard to alcohol effects, it was found that DA and HVA tissue levels were significantly increased in the substantia nigra pars compacta of both MR and NR monkeys after 12 months of voluntary alcohol drinking. These changes may be consistent with increased activity in the DA system in alcohol-drinking monkeys. Overall, these data are consistent with decreased 5-HT and DA system function in alcohol-naïve NR monkeys compared to MR controls, and with increased DA system function during chronic exposure to ethanol in monkeys from both rearing conditions. We have consistently shown adaptations in the DA system of chronic alcohol-exposed rodents and monkeys that are consistent with compensatory down-regulation in response to chronically elevated DA activity, and these results are consistent with such adaptations. Funded by NIH grants AA014091 and AA17056

USING MONOSODIUM GLUTAMATE TO INITIATE VOLUNTARY ETHANOL CONSUMPTION IN 'NON-PREFERRING' INBRED MOUSE LINES

B.A. McCool, A.E. Chappell
Wake Forest University School of Medicine and the Translational Center for Neurobehavioral Studies on Alcohol, Winston-Salem NC 27157; Dept. Physiology & Pharmacology, Wake Forest Univ. School of Medicine, Winston-Salem, NC, 27157

Voluntary oral ethanol consumption in rodents is generally limited by strong taste-aversion in these models. Historically, this has been overcome by combining ethanol with a sweetener, typically sucrose or saccharine, and then slowly 'fading' away the sweetener. However, this approach has not proven as useful for some inbred strains of mice (e.g. DBA/2J), despite indications that these same strains have strong conditioned place preference for IP- or IG-administered ethanol (Cunningham et al. 1992 Psychopharmacol 187:382; Cunningham et al. 2002 Pharmacol Biochem Behav 72:659). Importantly, DBA/2J mice carry a polymorphism in a subunit of the 'sweet' taste receptor (Max et al. 2001 Nature Genet 28:58) that reduces the potency of sweet substances in these mice. We hypothesized that the presence of this polymorphism might help explain the contrasting weak voluntary oral ethanol consumption and strong ethanol conditioned place preference in this strain. To test this hypothesis, we compared ethanol consumption initiated by a 'traditional' sucrose-fade with a fade from an alternative tastant, monosodium glutamate (MSG). We used the single-bottle 'drinking in the dark' protocol (Rhodes et al. 2007 Genes Brain Behav 6:1) and measured voluntary consumption of 15% ethanol for six weeks following a sucrose- or MSG-fade. Over the six-week test period, DBA/2J mice subjected to the MSG-fade had a significantly greater daily intake of ethanol (0.47±0.08g/kg, n=8) compared to a separate group of DBA/2J mice subjected to a sucrose-fade (0.2±0.02g/kg, n=7; $P<0.01$, t-test). In the MSG-DBA/2J mice, there was no significant difference in the mean daily intake between the first and sixth week of the test period. In contrast, sucrose-DBA/2J mice drank significantly less on the sixth week (0.11±06g/kg) compared to the first week (0.30±0.05g/kg; $P<0.01$, paired t-test). We then repeated these experiments in the ethanol 'drinking' strain C57BL6/J and again found that MSG-mice (n=8) had a significantly higher mean daily intake (2.73±0.12g/kg) than did sucrose-mice (2.00±0.19g/ kg, n=8; $P<0.01$, 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. Supported by AA014445, AA016671, and AA 017056.

HYPERPOLARIZATION-ACTIVATED CATION CURRENT MEDIATES THE FACILITATORY EFFECTS OF ACUTE ETHANOL ON GABAERGIC TRANSMISSION IN THE BASOLATERAL AMYGDALA

T.E. Spence, D.T. Christian, M.R. Diaz, B.A. McCool
Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

Extensive evidence indicates that ethanol exerts its sedative and anxiolytic effects via modulation of GABAergic transmission. However, acute ethanol is believed to regulate GABAergic transmission through a variety of pre- and postsynaptic mechanisms. Recent findings in other brain regions have shown that acute ethanol can enhance the hyperpolarization-activated, depolarizing cation current, Ih (Okomoto et al. 2006 J Neurophsyiol 95:619). Since Ih is known to play a role in the generation and modulation of spontaneous action potentials, we hypothesized that facilitation of GABAergic transmission by ethanol may be mediated, in part, by ethanol-dependent activation of Ih on inhibitory interneurons. To test this, we used whole-cell (slice) electrophysiological techniques to record GABA-mediated electrically evoked and spontaneous inhibitory post synaptic currents (IPSCs, sIPSCs) in pyramidal neurons in the basolateral amygdala (BLA), a central component of the anxiety neurocircuit.We specifically measured responses of GABAergic transmission to acute application of ethanol and/or ZD7288, a selective Ih antagonist. We found that ZD7288 significantly attenuated the peak amplitude of the evoked GABA IPSCs by 32.16±7.64% and 41.41±5.472% following stimulation of local and lateral paracapsular interneurons, respectively. Importantly, ZD7288 suppressed the frequency, but not the peak amplitude, of sIPSCs. These findings suggest that Ih tonically regulates GABAergic transmission via a presynaptic mechanism, potentially by regulating the spontaneous activity of BLA GABAergic interneurons.We next investigated the potential contribution of Ih to ethanol-induced facilitation of sIPSCs. Preliminary data suggest that Ih can both reverse and occlude ethanol-dependent facilitation of BLA-GABAergic transmission. These observations support our initial hypothesis that Ih may function to positively regulate the effects of ethanol on GABAergic transmission. Because GABAergic interneurons tightly regulate the flow of information through the BLA and BLA synaptic transmission modulates alcohol withdrawal-induced anxiety and drug seeking behavior, we propose that better understanding the mechanisms by which ethanol interacts with Ih to increase BLA GABA function may provide new pharmacotherapeutic targets for alcohol abuse treatment and relapse prevention. This work was supported by grants AA014445, AA016671, and AA016671 S1.

Oregon

BASELINE AND STIMULATED MEASURES OF HPA ACTIVITY IN NONHUMAN PRIMATES RELATED TO SOCIAL HIERARCHY RANK

K.A. Grant, M. Odagiri, A. Rau, J. Basile, K. Nusser
Oregon National Primate Research Center, OHSU, Beaverton, OR 97006

Circulating levels of the hypothalamic-adrenal-pituitary (HPA) axis hormones adrenocorticotropin hormone (ACTH) and cortisol are commonly used as a measures of stress and heightened anxiety in laboratory studies and for across species comparisons. We have extensively characterized pharmacological challenges to the HPA axis in an attempt to assess if dysregulations prior to or following alcohol self-administration can be traced to hypothalamic, pituitary or adrenal function. Monkeys were housed in groups of 3-4 and social rank was determined. Each monkey was given a series of pharmacological challenges (dexamethasone (0.13 mg/kg, i.m.), corticotropin releasing hormone (CRH, 1 microg/kg, i.v.), adrenocorticotropin hormone (ACTH, 10 ng/kg, iv., 4-6 hr after 0.5 mg/kg, i.m. dexamethasone), the opiate antagonist naloxone (2 doses: 0.125 and 0.375 mg/kg, i.m.), ethanol (2 doses: 1.0 and 1.5 g/kg., i.g.) and saline. Circulating levels of cortisol and ACTH were measured over time courses specific to each challenge. All challenges increased HPA response except saline and ethanol, which had no effect or decreased cortisol and ACTH. The ‘‘profile’’ of response to these pharmacological challenges was then compared with social hierarchy rank, with subordinate and intermediate monkeys having higher HPA activity compared to dominant monkeys. These monkeys were subsequently exposed to an ethanol self-administration procedure and allowed to chronically drink ethanol for 12 months. Subordinate and intermediate-ranked monkeys tended to drink more alcohol and have greater ACTH and cortisol responses compared to dominant monkeys. The data suggest that socially dominant monkeys have a lower basal level of stress, self-administer less ethanol and have lower levels of ethanol-induced increases in HPA output.

BRAIN ETHANOL MAGNETIC RESONANCE SPECTROSCOPIC SIGNAL INTENSITY INCREASES FOLLOWING ETHANOL EXPOSURE

C.D. Kroenke, G.S. Flory, K.A. Grant
Oregon National Primate Research Center, Oregon Health and Science University, Portland, OR 97239

Application of in vivo magnetic resonance spectroscopy (MRS) to human subjects has lead to the proposal that the ethanol methyl 1H MRS intensity is larger in heavy drinkers than in controls. This has further been interpreted to indicate the ethanol MRS intensity relates to the degree of tolerance to ethanol's intoxicating effects. To date however, ethanol MRS studies have not compared naïve to ethanol- exposed states within individuals, and they have not accounted for differences in MRS intensity between gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). We have performed MRS measurements in 12 rhesus macaque monkeys following intravenous ethanol administration. Measurements were performed prior to ethanol exposure, and following a 3-month period of daily ethanol exposure (1.0 g/kg mean daily intake). The ethanol MRS intensity is expressed relative to the MRS intensity of N-acetylaspartate (NAA), and separated into GM,WM, and CSF components. In the ethanol- naïve state, the group-averaged ethanol/NAA MRS intensity ratios per mg%blood ethanol concentration are 0.0058, 0.0046, and 0.017 for GM,WM, and CSF, respectively. These respective values increased by 6%, 16%, and 12% following exposure to ethanol. A model is proposed to relate the ethanol MRS intensity to binding interactions with brain macromolecular constituents. We interpret the MRS findings in terms of reductions in macromolecular binding interactions, a potential manifestation of acquired tolerance.

SUBSTITUTION OF ETHANOL AND ALLOPREGNANOLONE FOR THE DISCRIMINATIVE STIMULUS EFFECTS OF ETHANOL IN RATS: EFFECT OF OVARIECTOMY

T. Moschak, C.M. Helms, A. Buckert, K.A. Grant
Oregon Health & Science University, Portland, OR 97239

Allopregnanolone, a GABAA-positive modulatory neuroactive steroid, has been shown to substitute for ethanol in drug discrimination tasks. In females, allopregnanolone is produced in the brain de novo, and derived from progesterone produced by the adrenal glands and ovaries. Past drug discrimination studies suggest that sensitivity to the discriminative stimulus effects of ethanol varies with menstrual cycle phase due to fluctuations in endogenous progesterone and its metabolite allopregnanolone.We hypothesized that loss of ovarian progesterone would alter sensitivity to the discriminative stimulus effects of ethanol and ethanol-like effects of allopregnanolone. Female Long-Evans rats were either ovariectomized or given sham surgery (age = 57-69 days) and subsequently trained to discriminate 1.0 g/kg ethanol from water (i.g.) with a 30-min pre-treatment. Following training, acute doses of ethanol (i.g.) and allopregnanolone (i.p.) were administered to determine the half maximal effective dose (ED50) of both drugs to substitute for 1.0 g/kg ethanol. Preliminary data indicate no difference between the groups in ED50 for ethanol substitution (mean ± SEM: ovariectomized, n=9, 0.63 ± 0.03 g/kg; sham, n=13, 0.60 ± 0.09 g/kg). Allopregnanolone completely substituted (‡ 80% ethanol-appropriate responding) in 4/7 sham and 5/6 ovariectomized rats tested; there was no difference in the percent that fully substituted between groups. Among the rats for whom allopregnanolone completely substituted, the dose of allopregnanolone that substituted for ethanol was similar between ovariectomized (n=5, 4.16 ± 1.02 mg/kg) and sham-ovariectomized rats (n=4, 4.87 ±0.85 mg/kg). Similarly, no difference was found for mean maximal ethanol appropriate responding between sham rats (n=7, 65.47±15.27%) and ovariectomized rats (n=6, 83.60±15.43%). These preliminary results suggest that sensitivity to the discriminative stimulus effects of ethanol is not influenced by endogenous neuroactive steroids when discrimination training occurs in ovariectomized rats. Furthermore, the potency of allopregnanolone to produce ethanol-like discriminative stimulus effects appears to be independent of ovarian-derived progesterone.

REPEATED ABSTINENCE AND HPA RESPONSE IN A MONKEY MODEL OF CHRONIC ETHANOL SELF-ADMINISTRATION

M. Odagiri, A. Rau, J Basile, K.A. Grant
Department of Behavioral Neuroscience, Oregon National Primate Research Center, OHSU, Portland, OR 97239 USA

Background: In human alcoholics, abstinence from alcohol is associated with changes in HPA axis function, particularly in circulating adrenocorticotropin hormone (ACTH) and cortisol levels. We have developed an animal model of chronic alcohol self-administration that results in reliable ethanol consumption under open access (22 hr/day) conditions with food and water concurrently available. In this study, we investigated the effect of repeated abstinence from ethanol self-administration on the resting levels of ACTH and cortisol in monkeys. Methods: Adult male cynomolgus monkeys (Macaca fascicularis, n=12) were induced to drink water and 4% (w/v in water) ethanol by a Fixed-Time 300 sec (FT-300 sec) schedule of banana-flavored pellets. The FT-300 sec schedule was in effect for 120 consecutive sessions, with daily induction doses increasing from 0.0 to 0.5 g/kg to 1.0 g/kg to 1.5 g/kg every 30 days. Following induction, the monkeys were allowed concurrent access to 4% ethanol and water for 22 hr/day for 16 months. Following this chronic exposure to ethanol a 28 day abstinence period was imposed with only water available. At 18 days of withdrawal 5 blood samples were taken at 15, 30, 60, 90 and 120 minutes 4 hours into the light phase (morning) and assayed for ACTH and cortisol. After this first abstinence period, the monkeys were returned to alcohol self-administration for 22hrs/day for 4 months and then again subjected to 28 days of alcohol abstinence. Again, at 18 days of withdrawal resting levels of ACTH and cortisol were determined. Results: Prior to the first abstinence, average daily ethanol for all 12 monkeys was 2.5 g/kg/day and ranged from 1.8-4.8 g/kg/day. Following the abstinence periods, ethanol intakes retuned to previous levels (overall mean = 2.6 g/kg for abstinence 1 and mean = 2.5 g/kg/day for abstinence 2). The first abstinence period had no significant effect on ACTH or cortisol peak or area-under-the-curve (AUC) measures in a mixed-model analysis. However, during the second abstinence ACTH peak and AUC significantly fell whereas cortisol peak and AUC significantly rose. Conclusion: This model of ethanol self-administration suggests that repeated withdrawals from chronic alcohol self-administration have a cumulative toll on functioning of the HPA axis. The model may aid in identifying mechanisms of HPA axis dysfunction seen in abstinent alcoholics. (Supported by AA13510)

SPECIES DIFFERENCES IN A MONKEY MODEL OF CHRONIC ETHANOL SELFADMINISTRATION

A. Rau, B Ferguson, J. Bahk, M. Odagiri, J. Basile, K.A. Grant
Department of Behavioral Neuroscience, Oregon National Primate Research Center, OHSU, Portland, OR 97239 USA

Background: Rhesus (Macaca mulatta) and Cynomolgus (Macaca fascicularis) macaques are the most widely used non-human primates in biomedical research, however they differ in their susceptibility to cardiovascular, infectious and other disease states. To explore if there is also a species difference in susceptibility to alcohol-induced pathologies, we have characterized both of these species for predisposition to chronically self-administer ethanol following the same ethanol induction procedure and given the same self-administration protocol. Methods: Adult male cynomolgus (n=22) and rhesus macaques (n=12) were SNP genotyped to ascertain each monkey's geographical background. Genetically, we can distinguish subspecies differences in rhesus macaques (Indian origin vs. Chinese origin and we can distinguish cynomolgus macaques from Indonesia, Indochina, Mauritius, and the Philippines. All animals were induced to drink water and 4% (w/v in water) ethanol by a Fixed-Time 300 sec (FT-300 sec) schedule of banana-flavored pellets. The FT-300 sec schedule was in effect for 120 consecutive sessions, with daily induction doses increasing from 0.0 to 0.5 g/kg to 1.0 g/kg to 1.5 g/kg every 30 days. Following induction, the monkeys were allowed concurrent access to 4% ethanol and water for 22 hrs/day. Results: Rhesus monkeys took significantly longer to finish each induction dose of ethanol and achieved lower blood ethanol concentration (BEC) in induction compared to cynomolgus monkeys. Under the 22 hr availability of ethanol, the average daily intakes of rhesus monkeys was lower than cynomolgus monkeys during the first 6 months of access. Further, cynomolgus monkeys had a higher proportion of heavy drinking day (>3.0 g/kg/day) and correspondingly higher BECs (>200 mg%) compared to rhesus monkeys. Conclusion: This model of ethanol self-administration suggests that there are species differences within the macaque population in the predisposition to chronically self-administer high doses of ethanol. Whether this difference in intake and BECs is influenced by genetically distinct subspecies differences is under investigation. (Supported by AA13510)

South Carolina

ALTERED ETHANOL DISCRIMINABILITY IN NR2A DEFICIENT MICE

H.C. Becker, M. Cunningham, M. Overstreet, A. Holmes, D. Lovinger
Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425

Genetic deletion of the NR2A subunit of NMDA receptors has been shown to alter some pharmacological effects of ethanol. The present study examined whether NR2A deficient mice exhibit altered sensitivity to EtOH's interoceptive cues. NR2A knockout (KO) mice, along with C57BL/6J mice (used as controls) were trained to discriminate between EtOH (1.5 g/kg) and saline injections (IP) using an operant 2-lever food-reinforced discrimination procedure. Results indicated that NR2A KO mice displayed a slower acquisition rate for EtOH discrimination compared to C57BL/6J mice (i.e., after 150 days of training, 67% of C57BL/6J and 38% of NR2A KO mice met criterion performance indicating successful discrimination between EtOH (1.5 g/kg) and saline). Generalization testing using a cumulative dosing procedure was then performed in a subset of NR2A KO and C57BL/6J mice (n= 6/genotype) that had achieved criterion discrimination performance. A full EtOH dose-response curve was generated within a single session both prior to (baseline) and 24 hr following chronic (64 hr continuous) EtOH vapor exposure (tolerance testing). Analysis of calculated ED50 doses revealed that sensitivity to the EtOH cue during baseline assessment was similar in NR2A KO mice (ED50= 0.92 ± 0.17 g/kg) and C57BL/ 6J mice (ED50= 0.77 ± 0.14 g/kg). As previously demonstrated, chronic EtOH exposure produced a significant shift to the right in the EtOH dose-response function for C57BL/6J mice (ED50= 1.34 ± 0.18 g/kg). This apparent tolerance to EtOH's interoceptive cue was also evident in the NR2A KO mice (ED50= 1.25 ± 0.31 g/kg) and, in contrast to C57BL/6J mice, NR2A KO mice did not reach criterion level of discrimination performance even at the highest (2.5 g/kg) dose tested. Overall, these results indicate (a) NR2A KO mice require more training sessions than C57BL/6J mice to acquire EtOH discrimination; (b) fewerNR2AKOmice than C57BL/6Jmice learn the EtOH discrimination even after extensive training; (c) once EtOH discrimination is mastered (criterion performance is achieved), NR2A KO mice and C57BL/6J mice exhibit similar sensitivity to EtOH's interoceptive cue (as determined by generalization testing); and (d) chronic EtOH exposure produces tolerance to EtOH's cue (shift to the right in the dose-response curve) in both genotypes, although there is a trend for a greater magnitude of effect in the NR2A KO mice. Supported by NIAAA grant U01 AA014095 and VA Medical Research.

ALTERED SENSITIVITY TO ETOH IN B-ENDORPHIN DEFICIENT MICE MAY BE MEDIATED BY GABAA RECEPTOR HETEROGENEITY

J. E. Grisel, J. Crawford, M.E. Locklear, T. E. Spence, N.y J. Anderson, B. A. McCool
Furman University, Greenville, SC; Wake Forest University, Winston Salem, NC

Behavioral effects of EtOH are influenced by the opioid b-endorphin.We have previously shown that transgenic mice deficient in this peptide are more sensitive to the anxiolytic effects of EtOH as measured in plus maze and light-dark box assays. Despite this, they also evidence enhanced basal levels of anxiety in these two tests and enlarged adrenal glands. We therefore hypothesized that the constitutive absence of b-endorphin leads to a general increase in sensitivity to environmental stressors, and that this may predispose subjects to compensatory changes that are especially revealed in the presence of EtOH. Further evidence for this idea comes from the finding that b-endorphin deficient mice show a dramatic (2-3 fold) increase in loss of righting reflex (LORR) to EtOH (3.6 g/kg). Thus we tested the hypothesis that absent b-endorphin induces compensatory genetic modifications in the GABAa receptor. We dissected the BLA, BNST, hippocampus (HIP) and lateral hypothalamus (LH) from adult, naïve, male b-endorphin deficient and control (C57BL/6J) mice. These tissues were pooled by genotype and evaluated using RT-PCR for specific expression of various subunits contributing to the GABAa receptor. B-endorphin deficient mice exhibited a small overall increase in alpha1 expression across all brain regions. They also showed a large increase in alpha2 subunits in all regions (3-4 fold), and moderate increases in alpha3 and alpha4 subunits in specific areas (alpha3 expression was higher in the BLA and LH; alpha4 in the HIP and LH. No differences were seen in alpha5, or gamma1 or 2 subunits. However beta2 expression in the BLA and LH was also upregulated in b-endorphin deficient mice, as was delta expression in the LH. Delta subunit expression in opioid-deficient mice was significantly down-regulated in the BLA (this was the only decrease in subunit expression found in transgenic subjects). These alterations may contribute to an increase in behavioral sensitivity to EtOH and yield insight into the circuitry mediating alcohol effects, particularly as they relate to stress. Supported by: P20 RR-016461 / SC INBRE; AA13259 (INIA Stress consortium) and AA13641.

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

M.F. Lopez, W.C. Griffin III, M.P. Overstreet, H.C. Becker
Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425

Repeated cycles of chronic intermittent ethanol exposure results in enhanced voluntary ethanol drinking in C57BL/6J mice. This study evaluated the hypothesis that stress associated with repeated cycles of chronic ethanol exposure and withdrawal produces region-specific changes in brain corticotrophin release factor (CRF) levels and plasma corticosterone (CORT) levels that relate to enhanced ethanol drinking. Adult male C57BL/6J mice were trained to drink 15% ethanol in a 2-bottle choice (water as alternative), limited access (2 hr/day) procedure. Once stable baseline intake was achieved, mice were exposed to either chronic intermittent ethanol vapor (EtOH) or air (CTL) in inhalation chambers (16 hr/day for 4 days). At 72 hr following the last 16-hr cycle of ethanol or air exposure, mice resumed ethanol drinking for 5 days. This procedure was repeated for a second cycle, and EtOH and CTL mice were sacrificed either immediately upon final withdrawal (HR-0), or at later times following withdrawal (HR-8 and HR-72), or after 4 days access to ethanol (Day-5). Brains were removed and processed to assay CRF content by ELISA. Blood samples were collected for analysis of CORT levels by RIA. A group of ethanol-naïve mice was included to obtain baseline CRF and CORT levels. Results indicated that EtOH mice consumed significantly more ethanol (3.4 g/kg) compared to CTL mice (2.6 g/kg) during testing sessions and at baseline (2.6 g/kg). CORT levels were significantly higher in EtOH mice compared to CTL mice at HR-0 (23.6 vs 14.1 lg/dl) and HR-8 (40.6 vs 14.3 lg/dl), but returned to control levels at 72 hr post-withdrawal. CORT levels for EtOH-naïve mice= 7.9 lg/dl. CRF content in amygdala was significantly elevated at HR-8 in EtOH mice (216.9 pg/mg protein) compared to CTL (168.5 pg/mg) and EtOH-naïve (160.5 pg/mg) groups. Also, CRF content in the bed nucleus of the stria terminalis was significantly lower in EtOH mice 72 hr into withdrawal when compared to CTL mice (266.9 vs 342.9 pg/mg, respectively). Ethanol intake over 4 days during the 2nd test cycle did not alter plasma CORT levels or brain CRF content. These results indicate that repeated cycles of chronic intermittent ethanol exposure that induce increased ethanol intake also produce significant changes in circulating levels of CORT, and differential changes in CRF content in extra-hypothalamic brain structures. Supported by NIAAA grant U01 AA014095 and VA Medical Research.

Tennessee

PHENOTYPIC ANALYSIS OF THE EXPANDED BXD RI LINES OF MICE FOR ALCOHOL- AND OTHER ADDICTION-RELATED TRAITS

K.M. Hamre, G. Mittleman, M. Cook, C.D. Blaha, T. Ansah, V.M. Philip, E.J. Chesler, D. Goldowitz
Dept. Anat. & Neurobiol., UTHSC, Memphis TN 38163; Dept. Psych., Univ. Memphis; Meharry Med. Coll; Sys. Gen. Group, Bio. Div., ORNL & Gen. Sci. & Tech. Prog., Univ. Tenn.; CMMT, UBC.

Genetic reference populations, including the BXD recombinant inbred (RI) lines of mice, have been widely used to identify genetic loci that underlie ethanol-related behaviors. However, traditional studies have typically examined a single phenotype and defined the quantitative trait loci (QTLs) related to that phenotype. This project is part of a multi-site phenotyping consortium to examine a wide range of addiction-related phenotypes including alcohol-related phenotypes. The expanded BXD panel of RI lines of mice consists of approximately 60 different BXD lines and thus has increased statistical power over the 30 strains in the initial panel. In this study, male and female adults from the expanded BXD RI panel and the parental C57BL/6J and DBA/2J mice were examined. For alcohol-related traits, mice were tested after a dose of 2.25 g/kg of alcohol or isovolumetric saline. Alcohol-related traits examined include locomotor activation as measured using an activity chamber, ataxia as measured using an accelerating rotarod and anxiolysis as measured using an elevated plus maze. Separate groups of mice were tested on cocaine-related and morphine-related traits which allowed for the assessment of similarities across modalities. Traits included locomotion in a novel environment, cocaine and morphine induced locomotor activation, cocaine-induced place preference, and naloxone precipitated withdrawal. Additional baseline measures were also examined including those for stress, anxiety and depression to assess whether these measures exhibited genetic correlations with alcohol-related phenotypes. Significant gender related differences were observed on many measures. In most instances, multiple tests were used to assess each phenotypic construct, e.g. anxiety. While the initial analyses show relatively weak genetic correlations among many of the different phenotypes measured, integration of these results within the phenotyping study and with other genomic data sets can reveal genetic pathways or networks that are critical in mediating differences and similarities among these alcohol related phenotypes. Supported by R01DA020677, U01AA016662-02.

EVIDENCE FOR ALCOHOL-INDUCED HOMEOSTATIC METAPLASTICITY IN THE EXTENDED AMYGDALA

T.L. Kash, A.J. Baucum, K.L. Conrad, R.J. Colbran, D.G. Winder
Department of Molecular Biology and Biophysics, Vanderbilt University School of Medicine

Repeated exposure to alcohol causes neuroadaptations that are believed to underlie aspects of the pathological behavior associated with alcoholism. A number of studies have focused on the ability of alcohol to modulate the synaptic strength in key brain regions. Another form of experience-dependent alteration in synaptic function is metaplasticity, or the plasticity of plasticity. Metaplasticity is an alteration in the machinery responsible for plasticity, modulating the ability of synapses to undergo further activity dependent modulation including long-term potentiation (LTP) or long-term depression (LTD). The central extended amygdala, in particular the BNST, has been proposed to be a critical site of action for adaptations associated with alcohol abuse. However, to date there have been no studies that have examined the impact of in vivo alcohol exposure on either synaptic function or metaplasticity in the BNST. In order to better understand how alcohol can alter neuronal function, we examined the ability of in vivo alcohol exposure to alter mechanisms potentially underlying an alteration inmetaplasticity in the BNST using whole-cell voltage clamp recordings and biochemistry in brain slices obtained from C57Bl6 mice. In particular we focused on the vBNST because this region has been shown to regulate activation of both stress and reward pathways. Briefly, we found that chronic intermittent, but not continuous, ethanol vapor exposure increased temporal summation of NMDAR mediated EPSCs, suggesting a metaplastic shift. Multiple electrophysiological approaches suggest that this difference is not due to alterations in glutamate release, but rather an increase in the levels of NR2B in synaptic NMDARs. Our results suggest that NMDA mediated synaptic transmission is sensitized at key synapses in the extended amygdala and thus may be a suitable target for manipulation of the behavioral deficits associated with acute withdrawal from chronic alcohol exposure.

MULTIDIMENSIONAL ANALYSIS OF MOUSE BEHAVIOR FOR GENETIC ANALYSIS

V. M. Philip, M. Cook, K. Hamre, G. Mittleman, T. Ansah, C. Blaha, D. Goldowitz, E. J. Chesler
Oak Ridge Natl Lab. and U. Tenn, Oak Ridge, TN, 37831; U. Memphis, TN, 38152; U. Tenn Hlth Sci Ctr, Memphis, TN, 38163; Meharry Med Coll, Nashville, TN, 37208; U. British Columbia, V6T 1Z4

Intense focus on individual phenotypic assays in mouse models and mapping populations has led to the discovery of genes associated with various alcohol-related phenotypes. However, complex behavioral traits are often inadequately assessed through a single dimension of behavior or a single experimental paradigm. In contrast, multi-dimensional phenotyping approaches allow the detection of genetic loci associated with latent predictors of behavioral variation. These traits, extracted from multivariate phenotype data sets, underlie variation in many individual behavioral measures. This variation is manifest across the lifespan and in different environmental states, including the diversity of laboratory environments and test conditions and the perturbations caused by exposure to alcohol, stress and drugs of abuse. These components of behavioral variation are likely to be important behavioral indicators and predictors of alcohol use and addiction related phenotypes. Deployment of these approaches in mouse genetics has thus far been challenging due to constraints on repeated testing and the limited size of existing reference populations. Newly expanded, and optimized genetic reference populations have the potential to allow comprehensive profiling of behavioral variation across the phenome, under multiple environments, and with multiple apparatus parameters. Our recent large-scale phenotyping project in the newly expanded BXD recombinant inbred strains has generated substantial data toward this end, with an emphasis on stress, anxiety, alcoholism and drug abuse. These data have been subject to multivariate genetic analysis, allowing the identification of several factors underlying trait variation across multiple measures, and amenable to genetic dissection and functional genomic analysis. Assessment of the role of these traits and their regulatory genes in the predisposition to alcohol abuse phenotypes accelerates detection and intervention in alcohol use disorders. The approach also enables the identification of common and unique sources of behavioral variation, leading to a richer, redefined classification of the subtypes of behavioral disorders based on biological substrate, rather than on external manifestations represented by individual phenotypic assays. Supported by R01DA020677, U01AA016662-02, ORNL, managed by UT-Battelle, LLC, for the US DOE under Contract No. DE-AC05-00OR22725.

Texas

SELF-REPORTED ALCOHOL USE EXPECTANCIES AND THE EFFECTS OF STRESS ON EVENT-RELATED POTENTIALS TO ALCOHOL-RELATED SCENES

N. Ceballos, R. Guiliano, N. Y. Y. Wicha, R. Graham
Department of Psychology, Texas State University, San Marcos, TX 78666.; Department of Psychology, Texas State University, 601 University Drive, San Marcos, Texas, 78666

This study examined the relationship between self-reported alcohol use expectancies and Event- Related Potential (ERP) responses to alcohol related scenes before and after stress induction using the Paced Auditory Serial Addition Test (PASAT; Gronwell, 1977). Thirty-one social drinkers (18 male) were randomly assigned to one of two oddball tasks: 1) detect alcohol targets, ignore household object distracters and frequently presented nonsense shapes, or 2) detect household object targets, ignore alcohol distracters and frequently presented nonsense shapes. Self-reported anxiety levels confirmed the aversive nature of the stressor in both groups (ps<.01). Participants were faster to detect targets after stress induction, ($F(1,33) = 15.0$, $p < .001$). In addition, the P300 peaked significantly earlier after stress ($F(1,33) = 19.7$, $p < .001$). P300 latency shifts due to stress for both targets and distracters were examined using difference scores (pre- minus post-stress latency), which were entered into separate stepwise linear regressions with selected items from the Drinking Expectancies Questionnaire (DEQ; Lee et al., 2003) as predictors. Data from oddball paradigms 1 and 2 were examined separately. Results indicated that the negative consequences subscale of the DEQ significantly predicted target P300 latency shifts due to stress for individuals who were required to ignore alcohol distracters ($b = .563$, $t(12) = 2.26$, $p < .05$). Stronger endorsement of the negative consequences of drinking was associated with an enhanced ability to ignore alcohol-related distracters after the stressor, as reflected in a larger change in P300 latency in response to household object targets. Participants who provided a weaker endorsement of the negative consequences of drinking exhibited less cognitive efficiency in ignoring the alcohol-related distracters, as reflected in smaller stress-related changes in the P300 latency in response to household object targets. These results demonstrate an effect of stress on the latency of the P300 waveform, which may be predicted by self-reported expectancies about alcohol use. Although preliminary, the information gained from these experiments could ultimately provide a better understanding of the impact of stress on the relationship between the initial attentional and emotional processes elicited by alcohol related images and more controlled cognitive processes involved in the decision to engage in alcohol consumption.

Virginia

GENE EXPRESSION PATTERN CHANGES IN MONKEY PREFRONTAL CORTEX FOLLOWING CHRONIC ETHANOL SELF-ADMINISTRATION

N.A. Bruce1, J. T. Wolstenholme1, J. B. Daunais3, A. Rau3, C. Keily3, K. A. Grant2, M. F. Miles1
1Depts of Pharmacology/ Toxicology & Neurology, Virginia Commonwealth Univ. 2Dept of Behav Neuroscience, Oregon Health & Sciences Univ. 3Dept of Physiology & Pharmacology, Wake Forest Univ. School of Med; 1217 E. Marshall Street, Medical Sciences Building, Room 630, Richmond, VA, 23298

Many Americans consume alcohol on a regular basis. However, only a small proportion (7-10%) develop excessive drinking tendencies leading to alcohol abuse and alcoholism. Genomic studies in rodent and human autopsy brain material have previously identified gene networks that might influence development of ethanol abuse and alcoholism. Such detailed genomic studies have not been done in primate models of ethanol abuse, despite the powerful comparability of such models to human alcoholism. Here, we report initial results from a microarray analysis of excessive ethanol drinking in a primate model. Female cynomolgus monkeys (n=12) were induced to drink ethanol using a schedule-induced polydipsia paradigm to establish reliable ethanol consumption under open access conditions. Food and water were concurrently available during 12 months of free access to ethanol. Post-induction, animals were allowed to self administer ethanol (4% w/v) for six months in individual housing followed by another six month self-administration period with limited social contact. This model produced a high proportion of heavy drinking monkeys (67%) consuming more than 3 g/kg of ethanol daily. Control (n=4) animals had a similar induction protocol, but with isocaloric diets rather than ethanol, followed by 12 months of housing under identical conditions. Following 12 months of ethanol/water access, animals were euthanized and multiple tissues and brain regions harvested for analysis. Here, we describe whole genome microarray (Affymetrix) studies of medial prefrontal cortex. Multilayered statistical filtering of microarray data identified a robust list of genes (n=1552) with expression correlated with ethanol intake ($p<0.01$). Bioinformatic analysis identified multiple over-represented gene networks, including previously identified ethanol responsive networks such as GABA, dopamine receptor signaling and BDNF signaling. Prominent networks also included neuronal morphogenesis and RNA trafficking. Intriguingly, there was also significant overlap between gene lists from these primate studies and prior genomic studies on ethanol in rodents. These included genes involved in neuronal differentiation and plasticity. Ongoing studies include profiling of additional brain regions. We expect these results to identify gene networks highly relevant to neuronal plasticity occurring in alcoholism. Supported by NIAAA INIA grants AA013641(KAG), AA0116667(MFM) and AA016662(MFM).

ACUTE ETHANOL-RESPONSIVE GENE EXPRESSION IN FYN KINASE KNOCKOUT MOUSE PREFRONTAL CORTEX

S.P. Farris, N.A. Bruce, M.F. Miles
Department of Pharmacology and Toxicology, Virginia Commonwealth University, School of Medicine, Richmond, VA 23298

Alcohol abuse and alcoholism is a devastating disease affecting millions of individuals worldwide. The molecular mechanisms underlying this disease are largely unknown, however, changes in gene expression are proposed as critical molecular adaptations leading to the development of alcohol abuse and alcoholism. Prior gene expression studies from our laboratory and others have demonstrated reduced myelin-associated gene expression in postmortem alcoholic brain tissue. DBA/2J (D2) and C57BL/6J (B6) mice, inbred mouse strains displaying divergent behavioral responses to ethanol, also exhibit differing basal and ethanol-induced myelin gene expression in prefrontal cortex (PFC). Bioinformatic analysis of microarray studies on D2 and B6 mice implicated the non-receptor tyrosine kinase Fyn as a candidate signaling molecule regulating ethanol-responsive myelin gene expression. Fyn kinase knockout mice in addition to having a CNS hypomyelination phenotype are more sensitive to a subset of ethanol related behaviors such as loss of righting reflex. Therefore, we conducted expression profiling using DNA microarrays to identify altered gene expression in Fyn kinase knockout mouse (B6;129S7-Fyntm1Sor/J) prefrontal cortex in response to an acute sedative-hypnotic dose of ethanol (3 g/kg). Characterizing these ethanol-responsive gene networks will test the hypothesis that Fyn kinase is required for ethanol regulation of myelin gene networks, or indentify potential alternative signaling mechanisms regulating myelin gene expression in PFC. Genomic analysis of microarray data from B6;129S7-Fyntm1Sor/J mouse PFC revealed 565 genes altered by genotype, and 746 genes altered by acute ethanol (P-value <0.001). Among the genes differently regulated either by genotype, acute ethanol, or an interaction of genotype and ethanol were a subset of myelin genes, insulin-like growth factor (Igf-1), progesterone receptor (Pgr), and lysophosphatidic acid receptor (Lpar-1/Edg2). Individually these genes have a known relationship to myelination, but together may represent a novel association with acute ethanol signaling in PFC. Ongoing microarray analysis of nucleus accumbens and other brain regions may uncover functional gene networks related to the effects of ethanol on PFC. Further characterizing these ethanol-responsive gene networks may aid in the future development of more effective pharmacotherapies for the treatment of alcohol abuse and alcoholism, as well as associated CNS toxicity.

GENE EXPRESSION NETWORKS IN PREFRONTAL CORTEX CONTRIBUTING TO ETHANOL ANXIOLYTIC EFFECTS

M.F. Miles, A. Wolen, A. Putman, N. Bruce
Dept. of Pharmacology and Toxicology, Virginia Commonwealth University

In addition to other functions, prefrontal cortex (PFC) serves to integrate neural pathways and endocrine signaling affected by stress. Our prior work documented alterations in PFC gene expression with acute ethanol treatment, including a subset of glucocorticoid regulated genes. Acute or chronic ethanol and ethanol withdrawal all modulate anxiety and alter HPA axis activation, actions hypothesized to have an important role in recidivism in alcoholism. We therefore conducted a detailed genetic, behavioral and genomic analysis of ethanol anxiolytic actions and effects on PFC gene expression. Using over 35 BXD recombinant inbred strains, we mapped and verified a prominent ethanol anxiolysis QTL on Chr 12. Genome-wide analysis of PFC gene expression across the BXD strains identified several strong candidate genes for the Chr 12 QTL and PFC expression networks correlated with anxiolytic effects of ethanol. We also identified several large gene networks with PFC ethanol regulation controlled by discrete chromosome regions (‘‘trans-bands’’). This suggests a limited number of central regulators for ethanol effects on PFC gene expression. These results may provide important mechanistic insight into the role of PFC in ethanol anxiolysis.

FINE MAPPING A QUANTITATIVE TRAIT LOCUS FOR THE ANXIOLYTIC-LIKE RESPONSE TO ACUTE ETHANOL IN BXD RECOMBINANT INBRED STRAINS

A.R. Wolen, A.H. Putman, N.A. Bruce, M.F. Miles
Virginia Commonwealth University, School of Medicine, Richmond, VA 23290

Initial responses to acute ethanol are heritable predictors of an individual's proclivity for ethanol consumption and long-term risk for alcohol dependence, and provide a convenient model for investigating genetic correlates of susceptibility to alcoholism. Anxiety attenuation is an acute ethanol response of particular importance because anxiolysis is considered a primary motivator for habitual drinkers and substantial comorbidity exists between anxiety disorders and alcohol dependence. Our lab has used the BXD panel of recombinant inbred mice and the light-dark box model of anxiety to dissect genetic variation of the anxiolytic-like response to acute ethanol. All strains received IP injections of ethanol (1.8g/kg) or saline 10 minutes prior to entering the light-dark box. Global gene expression levels in prefrontal cortex and nucleus accumbens were concurrently measured across BXD lines from both treatment groups using Affymetrix microarrays.We have recently identified a quantitative trait locus (QTL) significantly associated (LOD 4.7) with acute ethanol-induced anxiolysis (Etanq1) on chromosome 12. The initial support interval for Etanq1 covered 26.2 megabases (Mb), including 137 known or predicted genes. Here, we present results of our initial effort to fine-map Etanq1 by studying novel BXD lines from an advanced intercross that carry informative recombinations within the Etanq1 support interval. The additional strains increased the LOD score of Etanq1 to 5.68 and reduced the support interval to approximately 10 Mb. The updated interval now spans 65.9-74.9 Mb on Chr12 and contains 80 genes. We used the BXD microarray data to facilitate the process of screening for candidate genes by conducting expression QTL (eQTL) analysis. Genes showing cis eQTLs within the Etanq1 region are considered strong candidates, since the local sequence polymorphisms driving the allele-specific differences in expression may be modulating the degree of anxiolysis induced by acute ethanol. We have identified 14 cis eQTLs with LOD scores ‡ 3 in the support interval, representing 7 unique genes. Bioinformatic analysis suggests these cis eQTLs are not due to polymorphisms affecting microarray performance and show that several genes have strong correlations with our behavioral phenotype. These results may identify novel mechanisms underlying ethanol anxiolysis and provide new candidates for future interventions in alcoholism. Supported by NIAAA INIA grants AA016667 and AA016662.