Integrative Neuroscience Initiative on Alcoholism Stress

"INIA: Stress, Anxiety of Alcohol Abuse"

Abstracts

Research Society on Alcoholism
June 23-29, 2006
Baltimore, Maryland

Research Society on Alcoholism
29th Annual Meeting
Baltimore, Maryland
June 23-29, 2006

Colorado

IDENTIFICATION OF ETHANOL-RESPONSIVE GENE NETWORKS BY EXPRESSION PROFILING ACROSS LXS RECOMBINANT INBRED LINES

M.F. Miles, P. Vorster, C. Downing, B. Bennett, and T. Johnson Institute for Behavioral Genetics, Univ. of Colorado at Boulder, Boulder, CO 80309; Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298

Maine

EFFECTS OF REPEATED CYCLES OF ETHANOL ACCESS AND DEPRIVATION ON FREE-RUNNING CIRCADIAN ACTIVITY RHYTHMS AND VOLUNTARY ETHANOL INTAKE IN ETHANOL PREFERRING (P, HAD-2) RATS

M.E. Fecteau; Y. Miura; L.M. Turner; A.M. Rosenwasser Departments of Psychology and Biological Sciences, University of Maine, Orono, ME 04469

EFFECTS OF RUNNING WHEEL RESTRICTION ON VOLUNTARY ETHANOL INTAKE IN ETHANOL PREFERRING (P, HAD-2) RATS

M.E. Fecteau; L.M. Turner; Y. Miura; A.M. Rosenwasser Departments of Psychology and Biological Sciences, University of Maine, Orono, ME 04469

CHRONOBIOLOGY OF ALCOHOL AND ALCOHOLISM: TOWARDS ANIMAL MODELS

Alan M. Rosenwasser Departments of Psychology and Biological Sciences, University of Maine, Orono, ME, 04469, USA

EFFECTS OF REPEATED PHOTOPERIOD SHIFTS ON VOLUNTARY ETHANOL INTAKE IN ETHANOL-PREFERRING (HAD-1) RATS

A. M. Rosenwasser; J. W. Clark; M. C. Fixaris Departments of Psychology and Biological Sciences, University of Maine, Orono, ME, 04469

EFFECTS OF CHRONIC ETHANOL INTAKE ON FREE-RUNNING CIRCADIAN ACTIVITY RHYTHMS IN C57BU6J AND DBA/2J MICE

A.M. Rosenwasser; J.D. Reed Departments of Psychology and Biological Sciences, University of Maine, Orono, ME 04469

Maryland

ETHANOL EFFECTS ON ELECTROPHYSIOLOGICAL PROPERTIES OF ASTROCYTES IN STRIATAL BRAIN SLICES

L. Adermark; D.M. Lovinger. Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, MD 20852, USA.

MICE LACKING THE NMDA RECEPTOR NR2A SUBUNIT EXHIBIT IMPAIRED CONDITIONED REWARD AND ENHANCED ATAXIC RESPONSES TO ETHANOL

J.M. Boyce-Rustay; A. Holmes National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

TRANSGENIC MICE EXPRESSING GFP UNDER NEURONAL POPULATIONSPECIFIC PROMOTERS FOR EXAMINING THE EFFECTS OF TERATOGENS ON NEURONAL DEVELOPMENT

Margaret I. Davis and David M. Lovinger Laboratory for Integrative Neuroscience, NIAAA Bethesda, MD 20892

EXAMINATION OF THE ROLE OF THE NR2A SUBUNIT IN ETHANOL EFFECTS ON NMDAR-MEDIATED SYNAPTIC RESPONSES USING KNOCKOUT MICE

Y. Honse; D.M. Lovinger LIN, NIAAA, NIH, Rockville, MD 20892-9411

North Carolina

ACETALDEHYDE ADMINISTRATION INCREASES GABAergic NEUROSTEROID LEVELS IN THE RAT BRAIN

Boyd, K.N., Morrow, A.L. Curriculum in Toxicology, Bowles Center for Alcohol Studies, Depts of Psychiatry and Pharmacology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC, USA

COCAINE AND ETHANOL EFFECTS ON NUCLEUS ACCUMBENS DOPAMINE IN 22-TNJ MUTANT MICE

B. R. Brookshire, A. Lapa, T. A. Mathews and S. R. Jones Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC 27127

ACCUMBAL DOPAMINE RELEASE, BUT NOT UPTAKE OR SYNTHESIS, IS ALTERED BY LOW AND MODERATE DOSES OF ACUTE ETHANOL

E.A. Budygin and S.R. Jones. Wake Forest University Health Sciences, Winston-Salem, NC 27157

DOPAMINERGIC MODULATION OF GABAERGIC SYNAPTIC TRANSMISSION IN THE RAT LATERAL/BASOLATERAL AMYGDALA

M.R. Diaz; B.A. McCool Wake Forest University School of Medicine, Winston-Salem, NC 27157

INCREASED ETHANOL SELF-ADMINISTRATION IN NURSERY-REARED MONKEYS

D.P. Friedman[1], K.T. Szeligal, V.M. Maxey[1], and K.A. Grant[2] [1]Wake Forest University Health Sciences, Department of Physiology and Pharmacology, Witnston-Salem, North Carolina 27157 [2]Oregon Health Sciences University, Department of Behavioral Neuroscience, Portland, Oregon 97239

CHRONIC ETHANOL AND WITHDRAWAL INDUCES GLUTAMATERGIC SYNAPTIC ADAPTATIONS IN THE LATERAL/BASOLATERAL AMGYDALA

A.K. Lack; D.W. DuBois; M.R. Diaz; N.J. Anderson; B.A. McCool. Wake Forest University School of Medicine, Winston Salem, NC 27157

BINGE ALCOHOL EXPOSURE DURING ADOLESCENCE PRODUCES BEHAVIORAL, ELECTROPHYSIOLOGICAL AND BIOCHEMICAL ADAPTATIONS IN HIPPOCAMPUS.

D.B. Matthews, S. Tokunaga, J.M. Silvers, R.B, Berry, K. Tinstey & A.L Morrow Department of Psychology, University of Memphis and Bowles Center for Alcohol Studies, University of North Carolina

NALOXONE AND ACTH STIMULATION OF DEOXYCORTICOSTERONE LEVELS ARE ENHANCED FOLLOWING THE INDUCTION OF DRINKING IN CYNOMOLOGUS MONKEYS

P. Porcu; K.A. Grant; A.L. Morrow Univesity of North Carolina School of Medicine, Chapel Hill, NC 27599 (PP and ALM) Oregon Health Sciences Univ. & National Primate Research Center, Portland, OR 97239 (KAG)

NURSERY-REARED RHESUS MONKEYS HAVE BLUNTED NEUROENDOCRINE RESPONSES COMPARED TO MOTHER-REARED MONKEYS

K.T. Szeliga, V.M. Maxey, A. Davenport, A. Bennett, K.A. Grant and D.P. Friedman Wake Forest University Health Sciences, Department of Physiology and Pharmacology, Winston Salem, North Carolina 27157 10regon Health Sciences University, Department of Behavioral Neuroscience, Portland, Oregon 97239

EFFECTS OF ETHANOL SELF-ADMINISTRATION ON PERIPHERAL BLOOD GENE EXPRESSION IN MONKEYS

S.J. Walker; T.R. Sutter; K.A. Grant Center for the Neurobehavioral Study of Alcohol and Integrative Neuroscience Initiative on Alcoholism Department of Physiology and Pharmacology Wake Forest University, School of Medicine, Winston-Salem NC 27101

Oklahoma

ETHANOL WITHDRAWAL ALTERS IN VIVO AND IN VITRO EVOKED HIPPOCAMPAL CHOLINERGIC RESPONSES

L.P. Gonzalez; D.M. Henthom University of Oklahoma Health Sciences Center, Department of Psychiatry & Behavioral Sciences, POB 26901, OKC, OK 73190.

Oregon

ALPHA-SYNUCLEIN POLYMORPHISM AND mRNA LEVELS ASSOCIATED WITH ETHANOL SELF-ADMINSITRATION IN MONKEYS

B. Ferguson; K.A. Grant; S.J. Walker Oregon National Primate Research Center, Beaverton, Oregon, 97006 and Dept of Physiology & Pharmacology Wake Forest University School of Medicine, Winston- Salem NC 27101

South Carolina

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

HC Becker; KG Femandes; MF Lopez; K Hamre; D Goldowitz Charleston Alcohol Research Center, Medical University of South Carolina & VAMC, Charleston, SC 29425 and the Tennessee Mouse Genome Consortium, Memphis, TN 38163

NEUROSTEROID ALLOPREGNANOLONE INVOLVEMENT IN ETHANOL DEPENDENCE AND RELAPSE BEHAVIOR IN C57BL/6J MICE

H.C. Becker; M.F. Lopez; A.L. Morrow Charleston Alcohol Research Center; Medical University of South Carolina & VAMC; Charleston, SC, 29425

PROBING THE MECHANISM OF ACTION OF ACAMPROSATE: A WHOLE GENOME MICROARRAY STUDY IN THE MOUSE FRONTAL CORTEX

M.G. Blanton; M.F. Olive Center for Drug and Alcohol Programs, Medical University of South Carolina, Charleston, SC 29425.

VOLUNTARY CONSUMPTION OF ETHANOL IN MICE: RELATIONSHIP BETWEEN LICKING BEHAVIOR AND ETHANOL LEVELS IN THE NUCLEUS ACCUMBENS

WC Griffin III; AB Yanke; MF Olive; LD Middaugh; HC Becker Charleston Alcohol Research Center, Medical University of South Carolina & VAMC, Charleston, SC 29425.

REPEATED CHRONIC ETHANOL EXPOSURE AND WITHDRAWAL INCREASES ETHANOL SELF-ADMINISTRATION IN C57BL/6J MICE

MF Lopez; MP Overstreet; HC Becker Charleston Alcohol Research Center and VA Medical Center, Medical University of South Carolina, Charleston, SC 29425

ETHANOL SEEKING AND DRINKING BEHAVIORS: COMPARISON OF MALE AND FEMALE C57BL/6J MICE

MF Lopez; LA Ralston; HC Becker. Charleston Alcohol Research Center, Medical University of South Carolina & VAMC, Charleston, SC 29425

ROLE OF THE GABA-A RECEPTOR IN TOLERANCE TO ETHANOL DISCRIMINABILITY IN C57BL/6J MICE

KJ Smith; AM Crissman; HC Becker. Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425

ETHANOL DISCRIMINATION IN ADOLESCENT AND ADULT C57BU6J MICE USING A WATER T-MAZE TASK

KA Willet; LD Middaugh; HC Becker. Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425.

Tennessee

PRENATAL ALCOHOL EXPOSURE REDUCES THE SIZE OF THE FOREPAW REPRESENTATION IN PRIMARY SOMATOSENSORY CORTEX (S1) IN JUVENILE RATS

T.D. Chappell; C.P. Margret; C.X. Li; A.J. Elberger; S.G. Matta; A. Oladehin, R.S. Waters Departments of Anatomy and Neurobiology, Pharmacology, and Physical Therapy, University of Tennessee Health Science Center, Memphis, TN 38163.

EFFECT OF PRENATAL ETHANOL EXPOSURE ON NMDA-NR1 RECEPTORS: NEW MECHANISMS FOR TOXIC EFFECTS OF ETHANOL

A.J. Elberger The University of Tennessee Health Science Center, Memphis TN

TIMING OF NEUROCIRCUITRY ABNORMALITIES UNDERLYING A BEHAVIORAL ETHANOL PHENOTYPE IN 22TNJ MICE: AN INIA-STRESS NEUROHISTOLOGY CORE PROJECT

[1]A.J. Elberger, [1]L. Cardenas, [1]T. Clark, [1]Y. Xue, [1]T. Hobson, [1]K.M. Hamre, [2]D. Matthews, [1]D. Goldowitz [1]Dept. Anatomy and Neurobiology, Univ. Tennessee HSC; [2]Dept. Psychology, Univ. Memphis; Memphis, TN

MORE BRAIN ABNORMALITIES UNDERLYING A BEHAVIORAL ETHANOL PHENOTYPE IN 22TNJ MICE: AN INIA-STRESS NEUROHISTOLOGY CORE PROJECT

[1]A.J. Elberger, [1]Y. Xue, [1]L. Cardenas, [1]T. Clark, [1]T. Hobson, [1]K.M. Hamre, [2]D. Matthews, [1]D. Goldowitz [1]Dept. Anatomy and Neurobiology, Univ. Tennessee Health Sci. Ctr., Memphis, TN; [2]Dept. Psychology, Univ. Memphis, Memphis, TN

MAPPING 22TNJ, AN ENU-INUDUCED MUTATION THAT EXHIBITS INCREASED LOCOMOTOR ACTIVATION FOLLOWING ACUTE ETHANOL EXPOSURE

K.M. Hamre, S. Wilkinson, D. Matthews, J. Cockcroft, K. Manly, M. Pletcher, D. Goldowitz Univ. of Tenn. Health Sci. Center and Univ. of Memphis, Memphis, TN 38163. Scripps Res. Inst. of FL., Jupiter, FL

MAPPING THE ETHANOL-RELATED PHENOME SPACE USING GENECENTRIC COMBINATORIAL METHODS

R. Kirova; A. Perkins; S. M. Pitts; Z. Li; E. Baker; Michael A. Langston; E. J. Chesler Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge TN, 37831 Department of Computer Science, University of Tennessee, Knoxville TN, 37996 Department of Computer Science, Baylor, TX, 76798

ENHANCED ACQUISITION OF ALCOHOL CONSUMPTION IN ADULT RAT OFFSPRING WITH GESTATIONAL EXPOSURE TO NICOTINE1ALCOHOL

[1]S.G. Matta, [2]A.J. Elberger. [1]E.E. Roguski [1]Dept. Pharmacology, [2]Dept. Anatomy and Neurobiology; Univ. Tennessee Health Science Center, Memphis, TN

GENETIC ASSOCIATION OF GENE EXPRESSION WITH ALCOHOL PHENOTYPES

J.L. Peirce, A.H. Putman, M.F. Miles, C.C. Parker, B. Bennett, H. Li, J. Wang, K.F. Manly, R.J. Hitzemann, J.K. Belknap, G.D. Rosen, E.J. Chesler, R.W. Williams, L. Lu Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163

MAKING BIOLOGICAL SENSE OF MICROARRAY GENE EXPRESSION DATA

Bing Zhang Biomedical Informatics Department Vanderbilt University Nashville, TN 37232

Texas

CRF STIMULATION OF PLASMA DEOXYCORTICOSTERONE LEVELS IN ABSTINENT ALCOHOL-DEPENDENT SUBJECTS

B. Adinoff, A.L Morrow, M.J. Williams, P.A. Chandler University of Texas Southwestern Medical Center, Dallas, TX; VA North Texas Health Care System; University of North Carolina

ADRENOCORTICAL RESPONSIVENESS TO COSYNTROPIN IN ABSTINENT FEMALE ALCOHOL-DEPENDENT WOMEN

Williams M.J.; Chandler P.A.; Best S.E.; Adinoff B. Department of Psychiatry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75216, USA.

Virginia

PHARMACOGENOMICS OF NALTREXONE AND ETHANOL IN MOUSE BRAIN

R.T. Kerns and M.F. Miles Departments of Pharmacology/Toxicology and Neurology, Virginia Commonwealth University, Richmond, VA 23298

GENE EXPRESSION PROFILING ANALYSIS OF MECHANISMS UNDERLYING ETHANOL DEPRIVATION EFFECT IN C57BL/6 MICE

R.T. Khisti, M.F. Miles Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia 23298.

STRATEGIES AND TOOLS FOR DEFINING GENE NETWORKS AND FORMING MECHANISTIC HYPOTHESES RELEVANT TO ETHANOL BEHAVIORS

M.F. Miles, R. Kems and A. McMullen Departments of Pharmacology/Toxicology and Neurology and the Center for Study of Biological Complexity Virginia Commonwealth University, Richmond, VA

QUANTITATIVE TRAIT LOCI AFFECTING SUSCEPTABILITY TO ETHANOLINDUCED ANXIOLYSIS IN BXD RECOMBINANT INBRED MICE

A.H. Putman and M.F. Miles Virginia Commonwealth University, Departments of Pharmacology/Toxicology and Neurology, Richmond, VA 23298

IDENTIFICATION OF CANDIDATE GENES RELATED TO INDIVIDUAL VARIATION IN ETHANOL CONSUMPTION

JT Wolstenholme, MF Miles Virginia Commonwealth University, Department of Pharmacology and Toxicology, Richmond, VA 23298

Italy

NEUROSTEROID RESPONSES TO ETHANOL ARE ELEVATED FOLLOWING SOCIAL ISOLATION STRESS - RELATIONSHIP TO ENHANCED DRINKING IN RATS?

G. Biggio and M. Serra Department of Experimental Biology, Center of Excellence for Neurobioiogy of Drug Dependence, University of Cagliari, Cagliari, Italy.

IDENTIFICATION OF ETHANOL-RESPONSIVE GENE NETWORKS BY EXPRESSION PROFILING ACROSS LXS RECOMBINANT INBRED LINES

M.F. Miles, P. Vorster, C. Downing, B. Bennett, and T. Johnson Institute for Behavioral Genetics, Univ. of Colorado at Boulder, Boulder, CO 80309; Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298

Acute responses to ethanol have been shown to have predictive ability for ethanol drinking behavior in both animal models and humans. We have previously identified limited networks of genes responding to acute ethanol by expression profiling in the B6 and D2 inbred lines (Kerns et al., J. Neurosci. 2005). However, trying to relate gene networks to behaviors by using only two strains or conditions is prone to extremely high type 1 errors. We therefore combined expression profiling of ethanol responses with powerful genetic models by performing microarray studies across an extended panel of recombinant inbred (RI) mice. The goal of these studies was to identify robust ethanol-responsive gene networks, map potential common chromosomal regions (expression QTLs) controlling such networks and correlate such expression networks with acute behavioral responses to ethanol across the same inbred lines. We performed behavioral assays (locomotor activity) across 42 LXS RI lines. Four hours after an injection of saline or ethanol (1.8 g/kg), brain regions from these strains were rapidly dissected and prefrontal cortex was processed for microarray analysis with 420A type 2 arrays (Affymetrix) containing _26,000 genes. Initial analysis of data from 480 microarrays focused on ethanol expression responses conserved across the 42 strains. This identified extended networks containing many genes previously identified in studies with B6 and D2 mice. Correlation analysis of expression and behavioral data in the LXS strains identified several genes having high correlation with ethanol loss-of-righting in both LXS and BXD recombinant inbred lines. These genes are very strong candidates for contributing to acute behavioral responses to ethanol. Ongoing linkage analysis with R/QTL is being used to identify expression QTLs for basal and ethanol-responsive gene expression. These studies have the potential to identify robust, novel networks of genes contributing to behavioral responses to ethanol and chromosomal loci regulating such networks. Supported by NIH Grants RO1 AA13678 and RO1 AA08940.

EFFECTS OF REPEATED CYCLES OF ETHANOL ACCESS AND DEPRIVATION ON FREE-RUNNING CIRCADIAN ACTIVITY RHYTHMS AND VOLUNTARY ETHANOL INTAKE IN ETHANOL PREFERRING (P, HAD-2) RATS

M.E. Fecteau; Y. Miura; L.M. Turner; A.M. Rosenwasser Departments of Psychology and Biological Sciences, University of Maine, Orono, ME 04469

Chronic ethanol intake results in persisting disruptions of sleep and circadian biological rhythms in both humans and experimental animals. While clinical alcoholism is frequently characterized by repeated episodes of abstinence and relapse, the chronobiological effects of alternating bouts of ethanol access and ethanol deprivation have not been explored. In the present study, we examined the effects of repeated ethanol access cycles on free-running circadian activity rhythms in two lines of selectively bred ethanol preferring rats. Male and female ethanolpreferring (P) and high alcohol-drinking (HAD-2) rats were housed individually in running-wheel cages under constant darkness with water and food freely available. Following a 2-week baseline condition, 10% (v/v) ethanol solution was offered in freechoice with water for repeated 15-day epochs alternating with 15-day epochs of ethanol deprivation. P and HAD-2 rats exhibited very similar changes in free-running circadian period, ethanol intake, and ethanol preference across repeated ethanol access cycles. Thus, male and female rats of both lines showed shortening of freerunning circadian period during the initial ethanol access period, but while male rats appeared to develop tolerance to this effect across ethanol access cycles, females showed more persistent effects on circadian period. In addition, ethanol intake and ethanol preference increased gradually across repeated ethanol access cycles in males, but not in females, of both lines. Following our previous findings in unselected male (Long-Evans) rats, the present results confirm that chronic ethanol intake alters a fundamental property of the circadian pacemaker - specifically, its free-running period, and extends these observations to include both male and female rats of two independently bred ethanol-preferring lines. Further, the present results suggest that tolerance to the chronobiological effects of ethanol may develop in a genderdependent manner. Supported by NIAAA R21 AA013893.

EFFECTS OF RUNNING WHEEL RESTRICTION ON VOLUNTARY ETHANOL INTAKE IN ETHANOL PREFERRING (P, HAD-2) RATS

M.E. Fecteau; L.M. Turner; Y. Miura; A.M. Rosenwasser Departments of Psychology and Biological Sciences, University of Maine, Orono, ME 04469

Voluntary ethanol intake may be modulated by the availability of other pharmacological or non-pharmacological rewards in the environment. While considerable evidence indicates that access to running wheels may serve as a source of reward in rodents, previous studies have reported both decreases (McMillan et al., 1995) and increases (Werme et al., 2002) in voluntary ethanol intake during running wheel availability in rats. These inconsistent results are likely due to several factors, including the experimental scheduling of access to both ethanol and running wheels, and the genetic background of the animals. In our ongoing work on the chronobiological effects of ethanol, rats are routinely housed in running wheel cages for monitoring of circadian activity rhythms (cf. Fecteau et al., this meeting). Thus, the present study was conducted to examine the effects of wheel restriction on ethanol intake following long-term access to running wheels and extensive prior history with both ethanol access and ethanol withdrawal. Male and female ethanolpreferring (P) and high alcohol-drinking (HAD-2) rats were housed individually in running-wheel cages under constant darkness with water and food freely available, and exposed to five cycles of ethanol access (10% v/v, 15 days) alternating with ethanol deprivation (15 days). At the end of the fifth ethanol access period, running wheels were locked in place for one ethanol access cycle, and were then unlocked for one additional cycle. While P rats exhibited generally increased ethanol intake during wheel restriction, HAD-2 rats displayed decreased ethanol intake. However, water intake was also modulated by wheel restriction, such that changes in ethanol intake were associated with alterations in ethanol preference only in females. These results indicate that both genetic background and gender influence interactions between running wheel access and ethanol drinking in rats.
Supported by NIAAA R21 AA013893.

CHRONOBIOLOGY OF ALCOHOL AND ALCOHOLISM: TOWARDS ANIMAL MODELS

Alan M. Rosenwasser Departments of Psychology and Biological Sciences, University of Maine, Orono, ME, 04469, USA

Chronic ethanol intake results in dramatic disruptions of sleep and other circadian rhythms in both human alcoholics and experimental animals. In alcoholics, these disruptions persist during extended periods of abstinence, and may promote relapse to drinking. Similarly, disruptions in sleep and circadian timing are associated with increased susceptibility to alcohol abuse in non-alcoholic populations, including adolescents and shift-workers. Thus, excessive ethanol intake results in chronobiological disturbances that may in turn promote and/or sustain excessive drinking. Recent research in my laboratory has focused on behavioral analysis of the chronobiological effects of ethanol and ethanol preference in various animal models. These studies reveal that chronic ethanol intake alters fundamental properties of the circadian pacemaker, including free-running circadian period, as well as responsiveness to perturbation by both photic and non-photic stimuli. In addition, genetic predisposition to excessive ethanol intake is associated with alterations in circadian phenotype, even in ethanol-naı¨ve animals. Taken together, these results indicate that both genetic and physiological linkages underlie reciprocal interactions between the neural systems regulating ethanol intake and the expression of circadian rhythmicity. While the precise nature of such mechanisms remains to be elucidated, several plausible candidates may be identified. At the physiological level, a number of ethanol-sensitive neurotransmitter receptors (e.g., GABAA, NMDA) are known to play critical roles in circadian pacemaker regulation, while at the genetic level, mutations and/or natural polymorphisms of specific circadian ‘‘clock genes’’ alter biological responses to ethanol (and other drugs of abuse) in flies, rodents and humans. In turn, chronic ethanol exposure modifies the expression of these same clock genes within neural components of the circadian system, including the suprachiasmatic nucleus (SCN), site of the circadian pacemaker. An improved understanding of these mechanisms based on studies in animal models is likely to lead to novel strategies for promoting abstinence and avoiding relapse in clinical alcohol abuse disorders.

EFFECTS OF REPEATED PHOTOPERIOD SHIFTS ON VOLUNTARY ETHANOL INTAKE IN ETHANOL-PREFERRING (HAD-1) RATS

A. M. Rosenwasser; J. W. Clark; M. C. Fixaris Departments of Psychology and Biological Sciences, University of Maine, Orono, ME, 04469

Sleep disturbance is associated with an increased risk of subsequent alcohol abuse, both in normal populations and in abstinent alcoholics. Similarly, the increased incidence of alcohol and drug abuse seen in shift workers may be mediated, in part, by the chronobiological disruptions associated with non-traditional work schedules. In turn, alcohol intake exacerbates sleep disturbances and produces widespread disruption of circadian rhythms, which could promote further problem drinking. While several studies have confirmed that ethanol exposure alters both sleep and circadian rhythms in experimental animals, the converse effects of chronobiological disruption on voluntary ethanol intake have been largely ignored in animal work. A notable exception is the study by Gauvin et al. (1997), who showed that ethanol intake could be increased by complex photoperiod manipulations designed to mimic rotating shiftwork schedules. In the present study, male and female rats of the selectively bred ethanol-preferring HAD-1 line were maintained with free access to both 10% (v/v) ethanol solution and water, while licking at the ethanol drinking tube was monitored continuously by a contact-sensing drinkometer, and ethanol and water intakes were assessed weekly. At repeated 3- to 4-week intervals, the LD cycle was phaseadvanced by 6 hours, thus simulating a rapid eastward translocation across 6 time zones, and providing a potential experimental model of jet-lag. Surprisingly, ethanol intake appeared to show transient decreases, rather than the expected increases, over the course of circadian re-entrainment to the new LD cycle. This result could be related to reports that ethanol-preferring, ethanol-nonpreferring, and unselected rats may display very different intake modulation when exposed to a variety of other environmental stressors. Thus, future work should examine the possible genetic dependence of photoperiod-related effects on ethanol intake in this model. Supported by NIAAA R21 AA013893.

EFFECTS OF CHRONIC ETHANOL INTAKE ON FREE-RUNNING CIRCADIAN ACTIVITY RHYTHMS IN C57BU6J AND DBA/2J MICE

A.M. Rosenwasser; J.D. Reed Departments of Psychology and Biological Sciences, University of Maine, Orono, ME 04469

Several lines of evidence from both clinical and experimental research indicate that chronic ethanol intake results in disruptions in sleep and other circadian biological rhythms. Nevertheless, the extent to which these chronobiological disruptions are mediated by ethanol-induced alterations in circadian pacemaker function has not been extensively studied. Recent research from our laboratory reveals that chronic ethanol exposure alters free-running circadian period, a fundamental property of the underlying circadian pacemaker, in rats. While forced intake of 10–20% ethanol solution results in relatively small and inconsistent alterations in free-running circadian period in unselected Long-Evans rats (Rosenwasser et al., 2005a), voluntary intake of 10% ethanol solution results in consistent period shortening in selectively bred ethanol-preferring P and HAD2 rats (Rosenwasser et al., 2005b). In the present report, we describe similar experiments using the ethanol-preferring C57BL/6J (B6) and the ethanol-avoiding DBA/2J (D2) strains of inbred mice. Male mice of both strains were maintained in running wheel cages under constant darkness, and free-running circadian activity rhythms were monitored for three weeks before, during, and after forced exposure to 10% (v/v) ethanol as the sole drinking fluid. While B6 mice consumed similar volumes of water and ethanol solution, fluid intake was reduced by about 50% during ethanol drinking in D2 mice. Mice of both strains showed shortening of circadian period during ethanol treatment, and while this effect was reversed following the termination of ethanol treatment in D2 mice, it generally persisted in B6 mice, possibly as a result of the relatively greater ethanol intake in this strain. These results provide further evidence that the chronobiological effects of chronic ethanol intake are mediated in part by pharmacological modulation of the circadian pacemaker. While the mechanisms mediating these effects have not been identified, they are likely to include ethanolinduced alterations in gene expression and neurotransmission within the circadian system. Supported by NIAAA R21 AA013893.

ETHANOL EFFECTS ON ELECTROPHYSIOLOGICAL PROPERTIES OF ASTROCYTES IN STRIATAL BRAIN SLICES

L. Adermark; D.M. Lovinger. Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, MD 20852, USA.

Astrocytes are a complex group of glial cells that is considered to be pivotal for neuronal development and function. Astrocytes are influenced by neuronal activity, and can in turn modulate neuronal function. Thus, to gain a more complete understanding of ethanol (EtOH) effects on brain function it is necessary to understand the impact of this drug on astrocytes. We evaluated acute effects of EtOH on resting and voltage-activated membrane currents using whole-cell patch clamp recordings from striatal astrocytes in rat brain slices. Cells were categorized as passive or complex astrocytes, based on the absence (passive) or presence (complex) of any evidence of voltage-gated channels. Ten min exposure to 50 mM EtOH increased input resistance and decreased capacitance but did not affect resting membrane potential in passive astrocytes. The slope conductance of these cells was reduced by 20% with no change in the linear shape of the I/V relationship. Ten, 25, 50 or 100 mM EtOH inhibited current generated by a hyperpolarizing pulse by 10, 15, 20 and respectively 25% in passive astrocytes, while no significant EtOH effect was observed in complex astrocytes or neurons. This EtOH effect was blocked when KCI was replaced with CsCI in the internal solution, but not during chelation of intracellular calcium with BAPTA, suggesting that ethanol affects calcium insensitive potassium channels. Astrocytic cell-to-cell coupling through gap junction channels was blocked with 1 mM octanol or intracellular loading of 2 mM CaCI2. The EtOH inhibition of responses to hyperpolarization was reduced but persisted when gap junctions were closed. Interestingly, EtOH effects were largely irreversible when gap junctions were open, but were fully reversible within 10 min after ethanol withdrawal in cells in which gap junctions were closed. These data suggest that EtOH inhibits a calcium-insensitive potassium channel, most likely a passive potassium channel, but also affects gap junction coupling in a way that is sustained after ethanol withdrawal. Since astrocytes are important for spatial buffering of potassium and activation of astrocytes is required for some forms of synaptic plasticity, EtOH effects on astrocytic function could influence neuronal activity.

MICE LACKING THE NMDA RECEPTOR NR2A SUBUNIT EXHIBIT IMPAIRED CONDITIONED REWARD AND ENHANCED ATAXIC RESPONSES TO ETHANOL

J.M. Boyce-Rustay; A. Holmes National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

The ionotropic NMDA glutamate receptor is a tetramer composed of the NR1 subunit and two NR2 subunits (NR2A-D). While there is compelling evidence that NMDA receptors mediate behavioral effects of ethanol, there is little understanding of how the subunit composition of the NMDA receptor determines these effects. In the current study, we sought to assess the relative roles of NMDA subunits via phenotypic assessment of ethanol-related behaviors in NR2A knockout (KO) mice. Results demonstrated that NR2A KO mice failed to show ethanol-induced conditioned place preference (CPP). NR2A KO mice showed a modest baseline deficit in motor coordination as compared to WT controls, and significantly increased sensitivity to ethanol-induced ataxia on the accelerating rotarod and elevated plus maze, and to a lesser extent, the balance beam and wire-hang, but not grip strength, tests. In contrast, locomotor-stimulant, sedative/hypnotic and hypothermic responses to ethanol were no different between genotypes. Voluntary ethanol consumption and preference in a 2-bottle choice paradigm was also normal in NR2A KO mice, as was ethanol metabolism. Data demonstrate that NR2A subunitcontaining NMDA receptors may be necessary for learned reward-related responses to ethanol, and that loss of NR2A causes hypersensitivity to ethanol-induced ataxia. possibly via alterations in NMDA receptor sensitivity to ethanol in brain regions mediating motor coordination. However, NR2A inactivation does not alter other measures of acute ethanol intoxication or ethanol consumption, implicating other NMDA subunits in these effects. These data provide novel insight into the role of NMDA receptors in modulating the behavioral effects of ethanol.

TRANSGENIC MICE EXPRESSING GFP UNDER NEURONAL POPULATIONSPECIFIC PROMOTERS FOR EXAMINING THE EFFECTS OF TERATOGENS ON NEURONAL DEVELOPMENT

Margaret I. Davis and David M. Lovinger Laboratory for Integrative Neuroscience, NIAAA Bethesda, MD 20892

Transgenic mice have emerged as powerful tools for understanding the pharmacological effects of drugs of abuse. The recent development of the Gene Expression Nervous System ATIas (GENSAT) has produced multiple lines of mice expressing GFP under neuronal subtype-specific promoters using bacterial artificial chromosome technology (Gong et al., 2003). These animals represent a powerful tool for examining the effects of ethanol and other teratogens on neuronal development. We have examined the development of cerebellar GABAergic neurons and granule cells using the GAD65 and GAP-43 lines, respectively. We compared the expression pattern of GFP with classical descriptions of interneuron and granule cell development. GAD65-driven GFP is expressed from birth and continues to increase until the third postnatal week. GFP is primarily expressed in the while matter during the first 2 postnatal (PN) weeks in the GAD-GFP pups. GAD65-GFP has a patchy distribution in the white matter at PN7. At PN14, basket cells in the inner third of the molecular layer begin to express parvalbumin but the white matter continues to be populated with GFP1 cells. By PN21, the interneurons have reached the molecular layer and express makers of mature GABAergic neurons. Interestingly, these mice did not show significant expression of the transgene in Purkinje cells, which are known to produce GAD65 protein and mRNA. The GAP43-GFP construct is expressed primarily in granule cells during all phases of development. Granule cells in the external granule layer (EGL) show GFP expression at PN7 with a shift toward expression in the internal granule layer (IGL) by PN 14. Very few GFP positive cells are present in the EGL at PN21. The development of the GFP expression in these 2 GENSAT lines recapitulates classical descriptions of cerebellar ontogeny. These animals will be useful in examining the effect of ethanol on cerebellar granule cells and interneurons, 2 populations that have not been extensively examined with respect to temporal windows of vulnerability to ethanol exposure.

EXAMINATION OF THE ROLE OF THE NR2A SUBUNIT IN ETHANOL EFFECTS ON NMDAR-MEDIATED SYNAPTIC RESPONSES USING KNOCKOUT MICE

Y. Honse; D.M. Lovinger LIN, NIAAA, NIH, Rockville, MD 20892-9411

Ethanol (EtOH) inhibits NMDAR-mediated synaptic transmission and long-term potentiation (LTP) in rat hippocampus. NMDARs exist as heteromers, and differential assembly of NR2 and NR1 subunits is thought to provide the basis for NMDAR heterogeneity. In the hippocampus, the predominant NR2 subunits are NR2A and NR2B. Past studies indicate that NR2A and NR2B subunit-containing receptors do not show substantial differences in sensitivity to EtOH. However, emerging evidence indicates differential sensitivity to some behavioral effects of EtOH in NR2A_/ _mice (see Boyce-Rustay and Holmes' abstract). To determine if differences in EtOH sensitivity of synaptic NMDARs in the NR2A_/_mice could contribute to these behavioral results, we examined LTP or synaptic NMDAR-mediated responses in these mice and C57BL/6N wild-type controls. Ethanol inhibited NMDAR-mediated fEPSPs in the CA1 region of hippocampal slices from both NR2A1/1and_/_mice, and no difference in inhibition of NMDAR-fEPSP slope was observed at EtOH concentrations ranging from 1 to 100 mM. The slope was not influenced by the application of 1 mM EtOH (99 _ 3% of baseline in NR2A_/_, 99 _ 4% in1/1), but was attenuated by 100 mM EtOH (64 _ 12% in_/_, 71 _ 4% in1/1). We next examined the effect using autaptic neurons grown for>6 days in vitro. Ethanol reduced NMDAR-mediated EPSCs in a similar manner in both NR2A1/1and_/_mice. NMDAR-mediated EPSCs in the control mice were inhibited by ifenprodil to a greater extent than in NR2A_/_mice. Thus, it is unlikely that deletion of the NR2A subunit directly alters EtOH sensitivity of synaptic NMDARs. We next examined the effect of EtOH on LTP (induced by 2 100 Hz, 1 sec trains) in field potential recordings from the CA1 hippocampal region in the two mouse groups. In the absence of EtOH the magnitude of LTP was slightly decreased in NR2A_/_mice compared to1/1. In the presence of EtOH, LTP was reduced in control mice and more greatly decreased in NR2A_/_mice (preliminary results). Stronger EtOH inhibition due in part to the diminished magnitude of LTP could contribute to altered alcohol sensitivity in tasks involving cognition (e.g. conditioned place preference) in the NR2A_/_mice. This study was supported by the DICBR of the NIAAA, NIH.

ACETALDEHYDE ADMINISTRATION INCREASES GABAergic NEUROSTEROID LEVELS IN THE RAT BRAIN

Boyd, K.N., Morrow, A.L. Curriculum in Toxicology, Bowles Center for Alcohol Studies, Depts of Psychiatry and Pharmacology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC, USA

Acetaldehyde is formed through the metabolism of ethanol by alcohol dehydrogenase. Acetaldehyde is a biologically active compound and is more toxic than ethanol. Acetaldehyde that has not been metabolized or bound by endogenous molecules can pass the blood brain barrier and is toxic to neurons in the central nervous system. Previous research suggests that acetaldehyde may play a significant role in many of the behavioral and rewarding effects of ethanol. These effects involve many neurotransmitter pathways in the brain including the GABAergic system. Since systemic ethanol administration is known to elevate GABAergic neuroactive steroids, it is important to determine if acetaldehyde plays a role in these changes in neurosteroid levels. In the present study, 250 mg/kg of acetaldehyde (10% v/v in saline) was administered i.p. to naı¨ve rats. The neuroactive steroid 3a - hyroxy-5a-pregnan-20-one (3a,5a-THP) was measured by radioimmunoassay. Acetaldehyde administration increased 3a,5a -THP levels by 144% (po.05) in the cerebral cortex of naı¨ve rats compared to saline controls. These findings suggest that acetaldehyde may contribute to ethanol's effects on the GABAergic neurotransmission through the stimulation of neuroactive steroid formation.

COCAINE AND ETHANOL EFFECTS ON NUCLEUS ACCUMBENS DOPAMINE IN 22-TNJ MUTANT MICE

B. R. Brookshire, A. Lapa, T. A. Mathews and S. R. Jones Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC 27127

The 22-TNJ mouse strain, generated by the Integrative Neuroscience initiative on Alcoholism and the Tennessee Mouse Genome Consortium, is a result of chemically-induced mutagenesis (N-ethyl nitrosourea, ENU) on a mix C3H:C57BI strain (1-TNH). In tests of locomotor activity, 22-TNJ mutant mice showed an enhanced locomotor response to 1.0 and 2.25 g/kg ethanol and 10 mg/kg cocaine compared to controls (1-TNH). There was no difference in activity between the two strains following saline injection. Therefore, we utilized in vivo microdialysis to evaluate changes in DA levels in the nucleus accumbens of 22-TNJ and 1-TNH mice in response to ethanol and cocaine. In awake, freely-moving animals, no diffierences were observed in baseline extracellular levels of DA. Additional studies showed differences between the 22-TNJ strain and the 1-TNH strain in response to 10 mg/kg cocaine (_170% vs. _250% increase, respectively) but no difference in response to 2.25 g/kg ethanol. Slice voltammetry experiments in the NAc core showed that the 22-TNJ strain exhibited faster DA uptake compared to wildtype controls, with no difference in DA release. Additionally, the 22-TNJ strain exhibited supersensitive release-regulating D2 autoreceptors. Extracellular levels of DA cannot account for the locomotor phenotype of the 22-TNJ mice, and it is possible that supersensitive DA receptors may be involved.

ACCUMBAL DOPAMINE RELEASE, BUT NOT UPTAKE OR SYNTHESIS, IS ALTERED BY LOW AND MODERATE DOSES OF ACUTE ETHANOL

E.A. Budygin and S.R. Jones. Wake Forest University Health Sciences, Winston-Salem, NC 27157

The present study was designed to evaluate the effects of low (0.5 g/kg) and moderate (1 g/kg) doses of ethanol on dopamine (DA) dynamics in rat nucleus accumbens (NAc). Fast-scan cyclic voltammetry was used to achieve a detailed examination of the kinetics of DA release and uptake in freely moving rats. A bipolar stimulating electrode was lowered to the ventral tegmental area and its depth was optimized to evoke DA release in the NAc (24 rectangular pulses, 60 Hz, 120 mA, 2 ms/phase, biphasic). Voltammetric recordings were made every 100 ms at a carbon fiber electrode using a triangular waveform (_0.4 to 1 1.2 V, 300 V/s). Recordings were collected at 5 min intervals for 60 min following ethanol or saline injection. We found that acute ethanol did not alter DA uptake parameters. Averaged Vmax and Km values were not significantly different after ethanol injections (0.5 and 1 g/kg). Therefore, the present data are in good agreement with our previous results obtained in NAc slice preparations, where DA release was locally evoked by single electrical pulses. However, the maximal amplitudes of DA signals were dose-dependently decreased after the drug. No alterations in DA biosynthesis rates, measured by LDOPA accumulation after NSD 1015 (50 mg/kg) administration, were observed after low and moderate doses of ethanol. Therefore, changes in DA synthesis are not involved in release effects. Pretreatment with GBL (500 mg/kg), an agent which inhibits the firing rate of DA neurons and decreases DA release in terminal fields, decreased the effect of ethanol on electrically evoked DA release. Taken together, these data suggest that the decrease in the amplitude of the DA signal following low and moderate doses of ethanol is due to autoreceptor feedback inhibition induced by increased cell firing rates and accumulating DA concentrations in the NAc. These findings are important for clarifying the actions of acute ethanol administration on DA neurotransmission. Supported by: AA014686

DOPAMINERGIC MODULATION OF GABAERGIC SYNAPTIC TRANSMISSION IN THE RAT LATERAL/BASOLATERAL AMYGDALA

M.R. Diaz; B.A. McCool Wake Forest University School of Medicine, Winston-Salem, NC 27157

It is established that withdrawal from chronic alcohol leads to anxiety in both humans and rodents. Given its intimate association with the behavioral and physiological aspects of these behaviors, the lateral/basolateral amygdala (BLA) is likely to be a central component of the brain circuitry governing withdrawal-related anxiety. The BLA is innervated by dopaminergic afferents from the ventral tegmental area. These afferents help mediate dopamine-dependent dis-inhibition of the BLA related to the GABAergic system (Bissiere et al., 2003). BLA dopamine therefore causes an overall increase in excitation (Rosenkranz and Grace, 1999) and may be one of the modulatory systems that help increase anxiety-like behavior during withdrawal. It is therefore possible that exposure to chronic ethanol and subsequent withdrawal can alter the effect of DA on the BLA GABAergic system. To test this, we initially performed whole-cell patch clamp recordings in BLA slices prepared from adult, ethanol-naı¨ve Sprague-Dawley rats. We specifically measured the effects of DA (50 mM) on electrically-evoked, pharmacologically isolated GABAergic postsynaptic currents (IPSCs). At more hyperpolarized membrane potentials, DA potentiated the GABAA-mediated IPSC by 25% (po0.05). In contrast, DA inhibited the GABAAmediated IPSC by 25% (po0.01) compared to baseline at more depolarized membrane potentials. To measure presynaptic contributions, paired-pulse electrical stimuli were delivered at increasing interpulse intervals. DA had no effect on the ratio of the second synaptic response relative to the first at either membrane potential. Together, these findings suggest that, in ethanol naı¨ve animals, DA may act on BLA GABAergic transmission in opposing manners that are dependent on the postsynaptic membrane potential. Preliminary data using chronic ethanol inhalation and a 24 hour withdrawal period suggests that the inhibitory effects of DA at depolarized postsynaptic membrane potentials may be down-regulated during withdrawal. With these studies we hope to elucidate the neurophysiological contribution of dopamine in the BLA as it pertains to withdrawal anxiety. This work supported by AA014445 and T32NS007422.

INCREASED ETHANOL SELF-ADMINISTRATION IN NURSERY-REARED MONKEYS

D.P. Friedman[1], K.T. Szeligal, V.M. Maxey[1], and K.A. Grant[2] [1]Wake Forest University Health Sciences, Department of Physiology and Pharmacology, Witnston-Salem, North Carolina 27157 [2]Oregon Health Sciences University, Department of Behavioral Neuroscience, Portland, Oregon 97239

In people, adverse childhood experiences increases the risk for the later development of substance-abuse disorders. The disruption of the maternal-infant relationship by rearing monkeys in a nursery forms the basis of a robust experimental model for studying the outcome of early adverse experience in non-human primates. This early stress may fundamentally alter brain and neuroendocrine development, and some evidence suggests these alterations may be related to an increase in the risk for excessive ethanol self-administration. Eight male rhesus macaques [motherreared controls (n54) or nursery-reared (n54)] were induced under scheduled pellet deliveries (i.e., schedule-induced polydypsia) to self-administer first water, and then ethanol in increasing doses (0.5, 10, 1.5 g/kg) over 30 day epochs. The subjects were then allowed free-access to the drinking panels for 22 hour/day for 12 months where ethanol and water were continuously available and the schedule of pellet delivery was discontinued. Thus, following the induction procedure the animals could choose to drink unlimited amounts of ethanol or water each day. During the induction procedure, the NR animals drank the required dose of fluid significantly faster than the MR animals whether including water in the analysis (F 1,24513.82; po0.001) or including just the ethanol doses (F 1,1859.265; po0.01. The mean ethanol consumed over the 12 month period of free-access revealed that NR animals (2.9 _ 0.8 g/kg) drank more than the MR animals (2.2 _ 0.2 g/kg). The NR animals drank significantly more ethanol than the MR animals during the first 6 months (po0.02), but not over the entire 12 month period (po0.08). The individual and group consumption of ethanol between the two rearing conditions shows that these groups do appear different in both the quantity and pattern of drinking. (Supported by INIA: AA 14106)

CHRONIC ETHANOL AND WITHDRAWAL INDUCES GLUTAMATERGIC SYNAPTIC ADAPTATIONS IN THE LATERAL/BASOLATERAL AMGYDALA

A.K. Lack; D.W. DuBois; M.R. Diaz; N.J. Anderson; B.A. McCool. Wake Forest University School of Medicine, Winston Salem, NC 27157

It has long been proposed that the anxiogenic effects of withdrawal following chronic ethanol exposure represent a disruption of the balance between excitatory and inhibitory influences within discrete brain regions. As a central hub in the brain that processes anxiety cues into behavioral and physiological responses, the lateral/ basolateral amygdala (BLA) is one such region. Therefore, examining the effects of chronic ethanol and withdrawal in the amygdala may lead us to the cellular mechanisms related to the anxiogenic effects of withdrawal. In this study, we examined the effects of chronic intermittent ethanol exposure (CIE) and subsequent withdrawal (WD) on glutamate mediated synaptic transmission in the BLA. Rats were exposed to ethanol vapor 12 hours per day for 10 days and were studied immediately or following withdrawal for 24 hours. Blood ethanol concentrations during the exposure were 200–250 mg/dl. Using blind whole cell patch clamp electrophysiology in amygdala slices, we examined NMDA/AMPA ratios and paired pulse facilitation at glutamatergic synapses. CIE treatment and WD decreased paired pulse ratios relative to control tissue. For example, the ratio for paired pulse 50 (50msec interval) was decreased by 25% (po.01) in CIE animals (1.71 _ 0.109, n524 cells) and 27% (po.001) in WD animals (1.65 _ 0.092, n519) compared to control (2.27 _ 0.16, n516). This would suggest an increased probability of release at BLA glutamate synapses in CIE and WD animals. Studies measuring spontaneous and miniature EPSC's are currently being performed to confirm this. Finally, preliminary results indicate that CIE and WD may also alter AMPA/NMDA ratios. These findings could suggest CIE- and WD-related adaptations in the relative contributions by AMPA and NMDA receptors at BLA synapses. AMPA and NMDA input/output measurements are being made to distinguish CIE- and WD- effects on these different receptor populations. Together, our results suggest that chronic ethanol and subsequent withdrawal induce significant adaptations to BLA glutamatergic synaptic transmission and may increase the overall excitatory tone in this brain region. This may play a role in the heightened anxiety state seen in withdrawing alcoholics. Supported by AA014445 and T32 AA007565.

BINGE ALCOHOL EXPOSURE DURING ADOLESCENCE PRODUCES BEHAVIORAL, ELECTROPHYSIOLOGICAL AND BIOCHEMICAL ADAPTATIONS IN HIPPOCAMPUS.

D.B. Matthews, S. Tokunaga, J.M. Silvers, R.B, Berry, K. Tinstey & A.L Morrow
Department of Psychology, University of Memphis and Bowles Center for Alcohol Studies, University of North Carolina

Alcohol use by adolescents is a profound, yet understudied, problem in the United States. Estimates suggest that over 2 million adolescent engage in binge alcohol use, yet the underlying neurobiological consequences of this use is not clear. Male rats were injected with high dose alcohol, 5.0 g/kg, every other day from postnatal day 30 to 50 and were then alcohol free from postnatal day 50 to 62. This exposure pattern produces tolerance to ethanol-induced hypnosis, metabolism, suppression of hippocampal pyramidal neural activity and hippocampal allopregnanolone levels. In addition, most of these effects last following the ethanol free period while ethanolinduced allopregnanolone levels are actually increased. Finally, adaptation in hippocampal GABAA receptor peptide levels mirror the behavioral and electrophysiological changes. These data suggest that binge alcohol exposure during adolescents produces profound changes in hippocampal neurobiology. Supported by NIAAA.

NALOXONE AND ACTH STIMULATION OF DEOXYCORTICOSTERONE LEVELS ARE ENHANCED FOLLOWING THE INDUCTION OF DRINKING IN CYNOMOLOGUS MONKEYS

P. Porcu; K.A. Grant; A.L. Morrow Univesity of North Carolina School of Medicine, Chapel Hill, NC 27599 (PP and ALM) Oregon Health Sciences Univ. & National Primate Research Center, Portland, OR 97239 (KAG)

Deoxycorticosterone (DOC) is the precursor of the potent endogenous neuroactive steroids 3a, 5a and 3a, 5b-tetrahydrodeoxycorticosterone (THDOC) that exhibit GAB A agonist-like properties including anticonvulsant, anxiolytic and sedative/hypnotic actions. Studies in rodents suggest that plasma DOC levels are highly predictive of THDOC levels. Eleven cynomologus monkeys were tested for plasma DOC levels following activation of the HPA axis by naloxone (375 mg/kg, i m.), adrenocorticotrophic hormone (ACTH, 10 ng/kg, i.v.) challenge, 4– 6 hours after 0.5 mg/kg dexamethasone as well as suppression by dexamethasone alone (130 mg/kg, i.m.). Monkeys were induced to drink alcohol with scheduled pellet delivery that induced drinking up to 1.5 g/kg/day over the course of 3 months and were tested in the pharmacological challenges during scheduled drinking. DOC levels were measured by radioimmunoassay in plasma samples. Prior to induction of alcohol drinking, naloxone increased plasma DOC levels in a time dependent manner with a maximal increase of 97% at 60 minutes (po0.001). Following the induction protocol, naloxone administration increased DOC levels by 218% at 60 minutes. ACTH administration had no effect on DOC levels prior to induction of ethanol drinking, but increased DOC levels by 45.8% following the induction process. The administration of dexamethasone alone resulted in a 45% decrease in DOC levels (po0.001) prior to induction and decreased DOC levels by 72% (po0.001) following induction, consistent with greater suppression of HPA axis function. These results show that DOC levels in non-human primates are regulated by the HPA axis and these effects are modulated by the induction of moderate levels of drinking. These results are consistent with previous data suggesting that alterations in HPA axis function may be related to alcohol drinking in monkeys. Supported by AA10564, AA13515 and AA13510.

NURSERY-REARED RHESUS MONKEYS HAVE BLUNTED NEUROENDOCRINE RESPONSES COMPARED TO MOTHER-REARED MONKEYS

K.T. Szeliga, V.M. Maxey, A. Davenport, A. Bennett, K.A. Grant and D.P. Friedman Wake Forest University Health Sciences, Department of Physiology and Pharmacology, Winston Salem, North Carolina 27157 10regon Health Sciences University, Department of Behavioral Neuroscience, Portland, Oregon 97239

Stress is believed to be a factor in the abuse of ethanol, and chronic stress is known to alter the behavioral effects of ethanol. A model of chronic stress is produced when infant rhesus monkeys are separated from their mothers at birth and raised in a nursery. We examined the effects of this early stress on the status of the HPA (hypothalamus-pituitary-adrenal) axis in both mother-reared (n54) and nurseryreared (n54) male rhesus macaques (Macaca mulatta). A battery of neuroendocrine challenges including ACTH, dexamethasone, ethanol (1.0 and 1.5 g/ kg), saline, naloxone (125 and 375 ug/kg) and CRF were administered prior to ethanol exposure, after limited ethanol self-administration and following chronic ethanol self-administration with resulting plasma samples analyzed for cortisol and ACTH levels. All the tests were conducted with the animals awake, either in their home cages or sitting comfortably in a primate chair to which they had been acclimated over several months. While the animals were ethanol-naı¨ve, the NR animals had significantly smaller ACTH responses when challenged with naloxone, ethanol and CRF; and smaller cortisol responses when challenged with ACTH and ethanol. The cortisol response to naloxone was smaller in the NR group as well, but this difference was not significant. The NR group showed lower levels of both ACTH and cortisol to every challenge. Chronic drinking did not induce an enhanced stress response, as measured by cortisol release, in either group despite increases in ACTH release to a variety of challenges. Thus, in both the NR and MR animals ACTH responses seemed to be more sensitive to drinking than cortisol responses. In general it appears as if one year of drinking has decreased the sensitivity of the adrenals to ACTH stimulation. (Supported by INIA: AA 14106)

EFFECTS OF ETHANOL SELF-ADMINISTRATION ON PERIPHERAL BLOOD GENE EXPRESSION IN MONKEYS

S.J. Walker; T.R. Sutter; K.A. Grant Center for the Neurobehavioral Study of Alcohol and Integrative Neuroscience Initiative on Alcoholism Department of Physiology and Pharmacology Wake Forest University, School of Medicine, Winston-Salem NC 27101

Biological markers that may be associated with risk for alcoholism, or used for assessing chronic ethanol consumption, are limited both by their nature (e.g., variable sensitivity and predictive value) and due to the heterogeneous nature of the human alcoholic population. in an effort to identify new potential alcohol biomarkers, this study examines peripheral gene expression in whole blood taken from macaque monkeys whose drinking behavior models human alcoholism. Four adult male cynomolgus monkeys (Macaca fascicularis) were exposed to oral ethanol selfadministration sessions for 22 hr/day, 7 day/week for 18 consecutive months. Ethanol intake (g/kg/day) as well as blood ethanol concentrations (BEC; mg%) every 5th session was recorded for each animal for the duration of the study. Following 6 consecutive months of drinking, a blood sample for microarray analysis was taken from each ethanol drinking monkey, as well as from four alcohol-naı¨ve adult male control cynomolgus monkeys. Total RNA isolated from these samples was used to query high density oligonucleotide arrays containing probe sets representing 417,000 unique genes. Cynomolgus monkeys in this study self-administered ethanol at average rates of between 1.2 and 3.9 g/kg/day. At intakes _ 2 g/kg the animals routinely had BECs _ 100 mg%. Gene expression analysis from whole blood revealed nearly 1200 genes that were differentially regulated in the alcoholconsuming animals h@fold change _ 1.2 and p _ 0.05i. Ontologic classes of upregulated genes that are overrepresented in this dataset include cellular processes, especially cell proliferation and programmed cell death; cellular components, especially plasmamembrane and; molecular function, especially signal transduction and receptor activity. Overrepresented groups of downregulated genes include: regulation of apoptosis, outer membrane components and protein binding activity. We have a robust nonhuman primate model of chronic alcohol selfadministration that may yield valuable clues to relevant biomarkers for alcoholism in humans. (Supported by grants: AA014984-01, AA 13520, AA 13641, AA13515)

ETHANOL WITHDRAWAL ALTERS IN VIVO AND IN VITRO EVOKED HIPPOCAMPAL CHOLINERGIC RESPONSES

L.P. Gonzalez; D.M. Henthom University of Oklahoma Health Sciences Center, Department of Psychiatry & Behavioral Sciences, POB 26901, OKC, OK 73190.

Following chronic ethanol treatment and withdrawal, neuronal hyperexcitability is observed at numerous sites within the central nervous system. Studies from our laboratory have suggested that hippocampal cholinergic neurotransmission is particularly sensitive to chronic ethanol treatment. The studies reported here examined hippocampal responses to electrical stimulation of the cholinergic septohippocampal pathway in an in vivo preparation and also responses to activation of cholinergic afferents in the stratum oriens in hippocampal tissue slices. In vivo studies were conducted in halothane anesthetized animals, and in vitro hippocampal slices were studied using a substrate-imbedded multi-electrode array (Panasonic Med64) to simultaneously record electrical activity from 64 electrodes across each slice. As we have reported previously, stimulation of the septo-hippocampal pathway with brief trains of stimulation produced long-lasting increases in spontaneous hippocampal single-unit activity in vivo. Similar trains of stimulation applied to the stratum oriens enhanced responses to stimulation in stratum radiatum for up to one minute following oriens stimulation. Oriens stimulation also increased the development of long-term potentiation following brief high-frequency stimulation in stratum radiatum when stimulation in stratum radiatum followed stratum oriens stimulation within 60 seconds. These effects were blocked by pre-treatment with the muscarinic cholinergic antagonists atropine and scopolamine suggesting that these effects are the result of acetylcholine release in response to afferent stimulation. The effects of stimulation in both the in vivo and in the in vitro preparations were significantly greater 6 to 8 hrs following withdrawal from a two-week period of chronic ethanol vapor inhalation when compare to the responses of ethanol-naı¨ve controls. These findings provide further support for the conclusion that hippocampal cholinergic function is altered during acute withdrawal from chronic ethanol treatment and suggest that this effect may contribute to the neuronal hyperexcitability observed during the ethanol withdrawal syndrome.
[Supported in part by NIAAA grants AA09959 & AA12283 to LPG]

ALPHA-SYNUCLEIN POLYMORPHISM AND mRNA LEVELS ASSOCIATED WITH ETHANOL SELF-ADMINSITRATION IN MONKEYS

B. Ferguson; K.A. Grant; S.J. Walker Oregon National Primate Research Center, Beaverton, Oregon, 97006 and Dept of Physiology & Pharmacology Wake Forest University School of Medicine, Winston- Salem NC 27101

The gene SNCA (or NACP), which codes for alpha-synuclein, contains a 50 repeat polymorphism (NACP-REP1) that is associated with the amount of alpha -synuclein expression in peripheral blood and craving for alcohol in patients with alcoholism. The aim of this study was to (1) determine if a-synuclein expression in peripheral blood of monkeys (n58) was elevated following 12 months of 22 hr/day access to ethanol (4% w/v) or water compared to alcohol naı¨ve controls (n510) and (2) determine if the 50 repeat polymorphism exists in cynomolgus monkeys and correlates with gene expression, alpha -Synuclein mRNA levels were measured by microarray. Monkeys in this study self-administered between 1.2 and 4.2 g ethanol/ kg body weight/day. This group of ethanol drinking monkeys had a highly significant 3.21 -fold higher level of alpha -synuclein mRNA in peripheral blood than alcohol naı¨ve controls. Ten different REP1 allele sizes were identified in this population. Among the drinking animals, those with the highest levels of SNCA expression were also those with the largest REP1 alleles. These data agree with recent reports of elevated alpha -synuclein mRNA and protein in the blood of human alcoholics, and support the concept of an association between alpha -synuclein and alcoholism. (Supported by grants: AA014984-01, AA 13520, AA 13641, RR0163-46)

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

HC Becker; KG Femandes; MF Lopez; K Hamre; D Goldowitz Charleston Alcohol Research Center, Medical University of South Carolina & VAMC, Charleston, SC 29425 and the Tennessee Mouse Genome Consortium, Memphis, TN 38163

The 7-TNJ ENU-induced mutant mouse pedigree has been reported to exhibit an exaggerated locomotor stimulant response to acute ethanol (EtOH) challenge. This study was designed to examine whether repeated EtOH administration results in locomotor sensitization in these mutant mice, and whether such treatment influences EtOH drinking. Adult male 7-TNJ mutants and their respective controls (1-TNH mice) were divided into 2 groups (n56–8/group). One group (SE) received saline injections (ip) for nine consecutive days and then 2.0 g/kg EtOH on day 10. Mice in the EE group, received 2.0 g/kg EtOH on day 1 followed by eight daily injections of 2.25 g/kg EtOH, and then 2.0 g/kg EtOH on day 10. All mice were tested for locomotor activity in an open field (15 min test session) 5 min after injection on day 1 and day 10. Two weeks following the final activity test, all mice were evaluated for 24- hr voluntary (2-bottle choice) EtOH intake (15% v/v vs. water) over a 3-week test period. Results indicated that basal (saline) activity was similar for both genotypes. Acute EtOH (2.0 g/kg) did not alter locomotor activity in the 7-TNJ mutants compared to saline baseline levels, but reduced activity in the 1-TNH controls. Following repeated injections of EtOH (EE groups), sensitization to the locomotor stimulant effects of EtOH was evident in both 7-TNJ mutants and 1-TNH controls, with the magnitude of effect greater in 7-TNJ mice. Analysis of EtOH intake indicated that prior treatment with 1 or 10 EtOH injections did not significantly influence average daily intake (1.4 _ 0.2 g/kg EtOH) in 1-TNH mice. In contrast, 7-TNJ mice that received a single prior EtOH injection (SE group) evidenced a transient increase in EtOH consumption compared to mutants that had a history of repeated EtOH injections (EE group) (5.2 _ 0.7 and 1.8 _ 0.2 g/kg EtOH for the SE and EE groups, respectively). These results indicate that both 7-TNJ and 1-TNH genotypes develop sensitization to the locomotor stimulant effects of EtOH, but voluntary EtOH intake in 7-TNJ mutant mice is differentially affected by prior EtOH treatment.
Supported by NIAAA grant AA014095 and NIMH grant MH061971.

NEUROSTEROID ALLOPREGNANOLONE INVOLVEMENT IN ETHANOL DEPENDENCE AND RELAPSE BEHAVIOR IN C57BL/6J MICE

H.C. Becker; M.F. Lopez; A.L. Morrow Charleston Alcohol Research Center; Medical University of South Carolina & VAMC; Charleston, SC, 29425

The neurosteroid allopregnanolone has been shown to play a role in modulating a variety of pharmacological effects produced by acute and chronic ethanol exposure. We have previously demonstrated that repeated cycles of chronic ethanol exposure and withdrawal experience results in enhanced voluntary ethanol drinking in C57BL/ 6J mice. To examine the potential role for changes in brain allopregnanolone levels in mediating such an effect, adult male C57BL/6J mice were separated into two groups and exposed to chronic intermittent ethanol vapor (EtOH) or air (CTL) in inhalation chambers (16 hr/day) for four days. EtOH and CTL mice were sacrificed either immediately upon final withdrawal (HR-0), or at later times following withdrawal (HR- 8 and HR-72) (n55/group/timepoint). Brains were removed, and cortex was dissected and frozen until later assay for allopregnanolone levels by RIA. Results indicated a significant increase in cortical allopregnanolone levels in EtOH mice compared to the CTL group at HR-72, but not HR-0 or HR-8. Interestingly, elevated ethanol intake is typically observed starting at 72 hr post-withdrawal. In a separate study, C57BL/6J mice were trained to lever respond (FR-8) to gain access to 15% (v/ v) ethanol for a 60 min drinking session. Once daily ethanol responding/intake stabilized, mice were tested under extinction conditions (responses did not provide access to ethanol). After extinction responding during daily 15 min sessions stabilized, mice (n58/group) were injected (IP) with allopregnanolone (0, 10, or 20 mg/kg) and then tested under extinction conditions. Results indicated that allopregnanolone produced a dose-related increase in responding on the active lever; responses on the inactive lever remained low. Studies are currently examining whether a history of ethanol dependence influences the ability of allopregnanotone to reinstate ethanol-seeking behavior. Collectively, these results suggest that chronic ethanol exposure increases allopregnanolone levels in cortex at 72 hr postwithdrawal, and this effect may impact the propensity to engage in excessive drinking in this mouse model of dependence and relapse. Supported by NIAAA and VA Medical Research

PROBING THE MECHANISM OF ACTION OF ACAMPROSATE: A WHOLE GENOME MICROARRAY STUDY IN THE MOUSE FRONTAL CORTEX

M.G. Blanton; M.F. Olive Center for Drug and Alcohol Programs, Medical University of South Carolina, Charleston, SC 29425.

Acamprosate has proven to be effective in prolonging abstinence and reducing alcohol craving and relapse in alcoholics. However, its neurobiological mechanisms of action remain poorly understood. To gain a further understanding of the mechanisms of action of this drug, we used high-density oligonucleotide microarrays to determine the effects of acamprosate on gene expression in the frontal cortex, a region known to be involved in alcohol craving and relapse. Male C57Bl/6J were group housed on a reverse light-dark cycle (lights on at 1000 h) and weighed, handled, and injected with saline for 2 days prior to the commencement of treatment. Next, mice were treated i.p. with acamprosate (200 mg/kg) or saline twice daily for 5 days. Approximately 1 hr following the last injection, mice were sacrificed and the frontal cortices were harvested and pooled within treatment groups of n53 mice each. Three biological replicates were obtained for each treatment group. Brain tissue was homogenized and RNA was extracted prior to microarray analysis by GenUs Biosystems (Northbrook, IL). Total RNA was reverse transcribed to cDNA, biotinylated, and hybridized to GE CodeLink whole genome microarrays containing 30-mer probes representing approximately 26,000 mouse genes. Preliminary data analysis revealed that acamprosate treatment upregulated 47 genes by 1.5-fold or greater, whereas 37 genes were downregulated by 1.5-fold or greater. Genes with altered expression levels were identified as having biological functions including protein kinase and phosphatase activity, cell growth, division and differentiation, DNA binding and transcription regulation, G-protein coupled receptor signaling, posttranslational protein modification, and cellular metabolism. Changes observed in microarray experiments are currently being confirmed by quantitative PCR and are being subject to more refined data analysis. The present results suggest that acamprosate has wide-ranging effects on biological processes in the brain, and these data will hopefully lead to a better understanding of the neurobiological mechanisms by which acamprosate reduces alcohol craving and relapse.
This research was supported by grants AA013276 and AA013852 from NIAAA, and by the MUSC Center for Drug and Alcohol Programs.

VOLUNTARY CONSUMPTION OF ETHANOL IN MICE: RELATIONSHIP BETWEEN LICKING BEHAVIOR AND ETHANOL LEVELS IN THE NUCLEUS ACCUMBENS

WC Griffin III; AB Yanke; MF Olive; LD Middaugh; HC Becker Charleston Alcohol Research Center, Medical University of South Carolina & VAMC, Charleston, SC 29425.

This study examined the relationship between voluntary EtOH consumption and brain levels of EtOH in a mouse model of EtOH dependence and drinking, EtOH levels in the Nucleus Accumbens (NAcc) were assessed in C57BL/6J mice (n59– 10/group) using conventional in vivo microdialysis and gas chromatography. Mice were trained to drink 15% EtOH using a limited access (2 hr/d) 2-bottle choice paradigm and consummatory behavior was measured throughout the experiment using standard lickometer circuits. Guide cannulae were implanted over the NAcc and EtOH access continued for 4 weeks. Mice were then either exposed to chronic EtOH vapor in inhalation chambers 16 hr/d for 4d (EtOH group) or similarly treated in air chambers (CTL group). Chamber exposure alternated with 5d of limited access drinking for 3 cycles and EtOH intake reached 3.74 and 3.10 g/kg for EtOH and CTL groups, respectively, at the end of the 3rd cycle. Microdialysis was conducted after the 3rd cycle. The microdialysis procedure disrupted drinking behavior in both groups (EtOH consumption was 2.35 g/kg and 3.25 g/kg for the EtOH and CTL groups, respectively). Nevertheless, the total number of licks was positively correlated with the total amount of EtOH consumed (r250.7653). Licking rates during microdialysis were high initially, but declined as EtOH levels in the NAcc increased. This was supported by a negative correlation between EtOH levels in the NAcc and the number of licks in each 20 min bin (r250.7883 for the EtOH group and r250.6904 for the CTL group). Finally, assays are currently being conducted to determine dopamine and glutamate levels in the NAcc dialysate samples of these same mice. Supported by NIAAA grant AA10761.

REPEATED CHRONIC ETHANOL EXPOSURE AND WITHDRAWAL INCREASES ETHANOL SELF-ADMINISTRATION IN C57BL/6J MICE

MF Lopez; MP Overstreet; HC Becker Charleston Alcohol Research Center and VA Medical Center, Medical University of South Carolina, Charleston, SC 29425

This study was conducted to examine the effect of a history of repeated ethanol (EtOH) exposure and withdrawal on operant EtOH self-administration in C57BL/6J mice. Adult male mice were trained to respond (FR-4) for EtOH (12% v/v EtOH and Sucrose 1% w/v) in 15 min sessions. Mice were not food or water deprived at any time. After stable baseline responding/intake (_1.0 g/kg EtOH) was established, mice received either chronic intermittent EtOH exposure (16 hr/d for 4 days) or air exposure in inhalation chambers, with self-administration sessions resuming 72 hr after final EtOH (or air) exposure for 5 consecutive days. This pattern of chronic intermittent EtOH exposure followed by 5 daily EtOH self-administration sessions was repeated 4 times (Test Cycles 1–4). EtOH and CTL groups were further divided based on having free access (or no-access) to drink EtOH (15% v/v) in their home cage during the first 48 hr after each inhalation treatment cycle. Results indicated that EtOH self-administration remained stable for CTL mice (both access and no-access groups) and did not differ from baseline levels (1.0–1.2 g/kg). However, the EtOH/noaccess group evidenced a significant increase in EtOH self-administration compared to CTL groups (and their own baseline) during Test Cydes 2–4 (1.7–2.0 g/kg). Mice in the EtOH/access group also showed an increase in EtOH responding/intake, but only during Test Cycle 4 (1.3–1.5 g/kg). BECs measured immediately after the final test session revealed higher levels for EtOH compared to CTL mice. These results indicate that experience with repeated cycles of chronic EtOH exposure and withdrawal can induce higher levels of EtOH self-administration behavior. Additionally, it appears that providing access to EtOH during acute withdrawal may attenuate this enhanced EtOH self-administration behavior in dependent subjects. Supported by VA Medical Research and NIAAA grant AA10761.

ETHANOL SEEKING AND DRINKING BEHAVIORS: COMPARISON OF MALE AND FEMALE C57BL/6J MICE

MF Lopez; LA Ralston; HC Becker. Charleston Alcohol Research Center, Medical University of South Carolina & VAMC, Charleston, SC 29425

This study was designed to assess possible sex-related differences in ethanol (EtOH) self-administration using an operant procedure that enabled separate analysis of EtOH seeking and drinking behaviors in adult C57BL/6J mice. Male and female C57BL/6J mice (n515/sex) were trained to respond on an active lever (FR- 8) to gain access to EtOH (15% v/v) for 60 min. Access to the EtOH tube was preceded by presentation of a tone/light stimulus. Once EtOH responding/intake stabilized over daily baseline sessions, mice were tested under extinction (15 min daily sessions with both active and inactive levers presented, but responses had no consequences). Number of responses and latency to reach 8 responses was recorded. Once stable extinction behavior was achieved (o10 responses on the active lever), mice were tested for cue-induced reinstatement (response-contingent cue presentation under extinction conditions). Results indicate that EtOH was equally reinforcing for male and female mice during the baseline self-administration phase (latency to fulfill FR-8: 139.6 _ 36.8 and 162.3 _ 49.1 sec, intake: 1.67 _ 0.37 and 1.87 _ 0.22 g/kg EtOH for mates and females, respectively). Blood EtOH levels (BEC) registered after a baseline session were positively correlated with intake (g/kg) and similar in both males (61.0 _ 18 mg/dl) and females (68.0 _ 12 mg/dl). During the extinction phase, female mice exhibited greater EtOH seeking behavior than males (i.e., slower rate of extinction). Additionally, female mice showed higher responses on the active lever than males when tested under cuereinstatement conditions (19.5 _ 6.3 and 34.8 _ 10.9 responses for males and females, respectively). Reponses on the inactive lever were minimal in both sexes during baseline, extinction, and reinstatement testing sessions. These results indicate that male and female C57BL/6J mice exhibit similar operant EtOH selfadministration profile in terms of EtOH seeking and drinking behaviors. However, when tested in the absence of the reinforcer (extinction), female mice exhibited greater EtOH responding and were more sensitive to cue-induced reinstatement of EtOH seeking behavior.
Supported by NIDA and ORWH grant P50 DA16511.

ROLE OF THE GABA-A RECEPTOR IN TOLERANCE TO ETHANOL DISCRIMINABILITY IN C57BL/6J MICE

KJ Smith; AM Crissman; HC Becker. Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425

Previous research has shown that chronic ethanol (EtOH) exposure subsequently decreases EtOH discriminability in C57BL/6J mice. The present study was conducted to determine the role of the GABA-A receptor-mediated component of the EtOH cue in this tolerance effect using the GABA-A positive modulators midazolam (MDZ) and pregnanolone (PREG). Adult male C57BU6J mice were trained to discriminate EtOH (1.5 g/kg; ip.) from saline using a food reinforced two-lever operant task. Once criterion discrimination performance was achieved, generalization testing was conducted with EtOH, MDZ, and PREG using a cumulative dosing procedure. Baseline EtOH (0.5–2.5 g/kg) generalization testing demonstrated dose-related increases in EtOH-appropriate responding (ED5050.62 g/kg). Separate groups of mice were used to generate baseline dose-effect curves for MDZ (n518) and PREG (n512). MDZ (0.25–4 mg/kg) was found to completely substitute for the EtOH cue at 4 mg/kg (ED5050.65 mg/kg) while PREG (5–25 mg/ kg) partially substituted for EtOH at 25 mg/kg (ED5055.1 mg/kg). Mice were then divided into two groups, receiving either chronic (64 hr) EtOH vapor (EtOH group) or air (CTL group) in inhalation chambers. EtOH, MDZ, and PREG dose-effect curves were re-determined 24 hr after inhalation treatment. Chronic EtOH exposure resulted in tolerance to EtOH discriminability as evidenced by a significant shift to the right in the dose-effect curve (ED5051.7 g/kg EtOH). Results indicated a significant shift to the right in the MDZ (ED5051.34 mg/kg) and PREG (ED50510.5 mg/kg) doseeffect curves as well. Re-determination of EtOH, MDZ, and PREG dose-effect curves indicated no difference from baseline in the CTL condition. These results suggest that changes in the GABA-A receptor-mediated component of the EtOH cue participate in tolerance to EtOH discriminability following chronic EtOH exposure. Supported by VA Medical Research and NIAAA grant AA10761.

ETHANOL DISCRIMINATION IN ADOLESCENT AND ADULT C57BU6J MICE USING A WATER T-MAZE TASK

KA Willet; LD Middaugh; HC Becker. Charleston Alcohol Research Center, Medical University of South Carolina and VAMC, Charleston, SC 29425.

Traditionally, the discriminative stimulus properties of ethanol (EtOH) have been studied using food/water-reinforced lever responding in operant chambers. Previously, we showed that adult C57BU6J mice were able to learn an EtOH discrimination using a water T-maze task. Since training the animals to learn the discrimination task took a relatively short period of time (12 sessions), it was possible to extend the experiment to determine if the discriminative stimulus (subjective) properties of EtOH differed in adolescent and adult mice. Adult (10 weeks) and adolescent (4 weeks) C57BL/6J mice were trained to discriminate between EtOH (1.5 g/kg; ip) and saline by swimming to a hidden platform in one of two distinct (black vs. white) arms of a water T-maze. Dependent measures included percent correct choices and latency to reach the platform. Results reconfirmed successful acquisition of the discrimination task by adult mice within 12 sessions. Adolescent mice, however, did not learn the discrimination task, perhaps due to increased vulnerability to stress related to the brightness of the testing room and/or the swimming task. To examine the contribution of the light variable, a separate group of adolescent mice were trained under dimmer lighting conditions. Under reduced lighting, adolescent mice learned the discrimination task, although performance was not as accurate as for the adult group. These results indicate that both adolescent and adult C57BL/6J mice can discriminate EtOH from saline using this water T-maze task. However, adolescent mice appeared to be less sensitive to the interoceptive cues associated with 1 .5 g/kg EtOH. Studies are currently under way to examine the basis for this apparent age-dependent difference in EtOH discriminability.

Supported by NIAAA grants AA10761 and T32 AA007474, and VA Medical Research.

PRENATAL ALCOHOL EXPOSURE REDUCES THE SIZE OF THE FOREPAW REPRESENTATION IN PRIMARY SOMATOSENSORY CORTEX (S1) IN JUVENILE RATS

T.D. Chappell; C.P. Margret; C.X. Li; A.J. Elberger; S.G. Matta1; A. Oladehin2, R.S. Waters 1Departments of Anatomy and Neurobiology, Pharmacology, and 2Physical Therapy, University of Tennessee Health Science Center, Memphis, TN 38163.

Introduction: We hypothesized that sensorimotor deficits and delayed reaction times in FASD children may reflect impaired sensory cortical processing. To test this hypothesis, we mapped the forepaw representation and measured response latencies in SI evoked by forepaw stimulation in prenatal alcohol exposed (PAE) juvenile rats. Methods: Pregnant Sprague-Dawley dams were given alcohol (6 g/kg, 25% w/v) or a 25% solution of maltose-dextrin by intragastric intubation on gestational days 1 to 20. The sizes of the forepaw representations from alcohol (EtOH), pairfed (PF), and chowfed (CF) offspring were compared at six weeks of age. The forepaw cortex in S1 was exposed and covered with silicone fluid. The receptive fields of neurons responsive to mechanical stimulation of the forepaw were recorded from a depth of 700 microns, using carbon fiber electrodes. Receptive fields were projected to the cortical surface and used to derive a map of the forepaw representation. The maps were digitized, reconstructed using Photoshop (7.0), and measured using ImageJ. Following forepaw mapping, evoked responses were recorded in SI following electrical stimulation of the forepaw digits; latencies were averaged (50 stimulus presentations) and compared between the three groups. Results: EtOH rats had significantly smaller (po0.05) representations of the total forepaw (16% reduction), total ventral digits (9% reduction), total dorsal digits (31% reduction) and individual digits 2, 3, and 5 (20–23% reduction) compared to CF rats. Furthermore, averaged evoked responses from digits 2 to 4 resulted in a 13% increase in latency in EtOH rats compared to CF rats. Conclusion: PAE reduced the forepaw representation in SI and delayed forepaw input to SI. These results support the hypothesis that PAE effects central somatosensory cortical processing in juvenile rats. (NIAAA, R01 AA013437 to R.S.W.)

EFFECT OF PRENATAL ETHANOL EXPOSURE ON NMDA-NR1 RECEPTORS: NEW MECHANISMS FOR TOXIC EFFECTS OF ETHANOL

A.J. Elberger The University of Tennessee Health Science Center, Memphis TN

Projection neurons in cortex use glutamate as a neurotransmitter. Prenatal exposure to ethanol affects the structure and function of glutamatergic cortical projection neurons. Therefore, the effects of prenatal exposure to ethanol on mice with deletion of the NMDA-NRl (NR1) receptor gene were examined. Cortical development was altered in knockout mice with and without prenatal ethanol exposure. Analyses were based on the adult offspring of NR1 heterozygous breeding pairs, i.e., comparing littermates with total deletion (NR1_/_), 50% deletion (NR11/_), and no deletion (NR11/1). A range of prenatal ethanol-induced effects wee blocked in NR1_/ _mice and attenuated in NR11/_mice. Furthermore, the NR 11/1mice show similar effects in neocortex including in the development of corpus callosum projection neurons as do Sprague-Dawley rats with the same amount of prenatal ethanol exposure. Studies have explored possible mechanisms by which ethanol toxicity is modified by NR1 receptors, and one promising approach is examining the relationship of the endocannabinoid pathway and its receptors (CB1 and CB2) with NRl receptors. CBl, CB2, and NRl receptors regionally co-localize in NR11/1and NR11/_, although the distribution of all three receptors is reduced in NR 11/_. Following even a single dose of ethanol, NR 11/_mice fail to show the increased brain and blood levels of endocannabinoids that NR11/1show. In NR11/1the blood levels of lipids, which form the precursor pool for endocannabinoids, are gradually reduced with increasing levels of ethanol. Overall, these results support the possibility that the change in endocannabinoid levels is a physiologically significant consequence of ethanol exposure and are potentially responsible for toxicity at all ages. Supported by NIAAA grants AA12163 and AA13516.

TIMING OF NEUROCIRCUITRY ABNORMALITIES UNDERLYING A BEHAVIORAL ETHANOL PHENOTYPE IN 22TNJ MICE: AN INIA-STRESS NEUROHISTOLOGY CORE PROJECT

[1]A.J. Elberger, [1]L. Cardenas, [1]T. Clark, [1]Y. Xue, [1]T. Hobson, [1]K.M. Hamre, [2]D. Matthews, [1]D. Goldowitz [1]Dept. Anatomy and Neurobiology, Univ. Tennessee HSC; [2]Dept. Psychology, Univ. Memphis; Memphis, TN

The 22TNJ mouse was created by ENU mutagenesis through the Tennessee Mouse Genome Consortium (TMGC). In the TMGC Ethanol Phenotyping Domain it displayed a hyperactive locomotor response to an acute EtOH injection. The Neurohistology Core of INIA-STRESS explored possible neurocircuitry abnormalities as a basis for this phenodeviance. Male and female 22TNJ were compared to control parental strains C57BL/6J and C3H for differential neurochemical labeling. A range of primary antibodies were studied, with differences found for choline acetyltransferase (ChAT), dopamine transporter (DAT), enkephalin (ENK), and serotonin transporter (SET). We reported on adult 22TNJ abnormalities in ChAT and ENK last year, and in DAT and SET this year. To further analyze the 22TNJmutation, the present study examines ChAT, DAT, ENK and SET staining during postnatal development to determine whether phenodeviant neurocircuitry is always present, or whether normal neurocircuitry precedes it. Male and female 22TNJ mice were studied at 1,2, 4 postnatal weeks (PW), and compared with adult (43 months) 22TNJ, C57BL/6J and C3H. Brains were analyzed in alternating 50mm coronal sections stained with one antibody. Labeled regions were identified for areal distribution. In adults, the 22TNJ areal distribution of ChAT, DAT, ENK and SET partially overlaps that of C57BL/6 and/or C3H, but there are differences in labeling compared to both parental strains. In general, the 22TNJ and C57BL/6J have more intense labeling of all antibodies than the C3H. Results show that adult 22TNJ areal distribution appears after 4 PNW for all 4 antibodies. ENK and SET staining show that some areas are not labeled until later ages, but phenodeviant neurocircuitry is always present. However, ChAT and DAT staining show that some regions are labeled at 1–2 PNW and are also labeled in normal adults, but are no longer labeled in older 22TNJ mice. The results reveal 2 patterns, and that ChAT and DAT phenodeviant neurocircuitry occurs after a period of normal neurocircuitry. These patterns may underlie the phenodeviant EtOH behavior. Support: AA13516 (AJE), MH61971 (DG)

MORE BRAIN ABNORMALITIES UNDERLYING A BEHAVIORAL ETHANOL PHENOTYPE IN 22TNJ MICE: AN INIA-STRESS NEUROHISTOLOGY CORE PROJECT

[1]A.J. Elberger, [1]Y. Xue, [1]L. Cardenas, [1]T. Clark, [1]T. Hobson, [1]K.M. Hamre, [2]D. Matthews, [1]D. Goldowitz [1]Dept. Anatomy and Neurobiology, Univ. Tennessee Health Sci. Ctr., Memphis, TN; [2]Dept. Psychology, Univ. Memphis, Memphis, TN

The 22TNJ mouse, created through the Tennessee Mouse Genome Consortium (TMGC) by ENU mutagenesis, was identified as a phenodeviant in the TMGC Ethanol Phenotyping Domain by displaying a hyperactive locomotor response to an acute EtOH injection. The Neurohistology Core of INIA-STRESS explored possible neurocircuitry abnormalities of mice with phenodeviant traits. Male and female 22TNJ and control parental strains C57BL/6J and C3H were compared for differential neurochemical labeling. Last year we reported on choline acetyltransferase (ChAT) and enkephalin (ENK); the present study shows additional findings of phenodeviant neurocircuitry. Entire adult (43 months) brains were coronally sectioned at 50 mm. Separate sections were stained with primary antibodies to BDNF, CRH, dopamine, dopamine transporter (DAT), serotonin, serotonin transporter (SET), and tryptophan hydroxylase. Comparisons were made for the areal extent of labeling. Significant differences were found for dopamine, DAT, serotonin, and SET. Since dopamine and DAT results, and serotonin and SET results matched, results were characterized in depth for DAT and SET. DAT had similar results between genders. Only the 22TNJ had staining in neocortex, lateral preoptic area, Purkinje cells, lateral hypothalamic area, locus ceruleus, and nigrostriatal bundle. Regions labeled in one or both parental strains but not 22TNJ are globus pallidus, amygdala, and limbic areas. SET labeled neocortex and hippocampus in bom genders of 22TNJ but not parentals. SET labeled the dorsal raphe nucleus in 22TNJ males but not females, but results were opposite in C57BL/ 6J. When DAT or SET labeling was present, it was less intense in C3H than in C57BL/6J or 22TNJ. The phenodeviant neurocircuitry of adult 22TNJ versus normal parental controls represents neurochemicals and structures related to substance abuse and locomotion. Combined with our previous findings, results suggest that changes in the distribution of selective neurochemical pathways may underlie the altered EtOH behavioral phenotype.
Supported by AA13516 (AJE), MH61971 (DG).

MAPPING 22TNJ, AN ENU-INUDUCED MUTATION THAT EXHIBITS INCREASED LOCOMOTOR ACTIVATION FOLLOWING ACUTE ETHANOL EXPOSURE

K.M. Hamre, S. Wilkinson, D. Matthews, J. Cockcroft, K. Manly, M. Pletcher, D. Goldowitz Univ. of Tenn. Health Sci. Center and Univ. of Memphis, Memphis, TN 38163. Scripps Res. Inst. of FL., Jupiter, FL

Responses to ethanol are controlled, at least in part, by the genetics of the organism. However, the genes that mediate these responses remain unknown. To identify genetic loci that mediate various ethanol-induced responses, we are using the mutagen N-ethyl nitrosourea (ENU) that makes random, single base-pair mutations within the genome. However, we are using a refined targeted mutagenesis approach in which the presence of a particular chromosomal region from the mutagenized parent is tracked through successive generations. The use of this approach can significantly reduce the portion of the genome that needs to be searched to find the mutated gene. Mice generated in this manner, and homozygous for the relevant chromosome, were then screened for their responses to a bolus of 2.25 g/kg of ethanol. From this screen, we identified one pedigree, 22TNJ that exhibits increased locomotor activation following ethanol. The tracked chromosome in this pedigree of mice is 30 cm on distal chromosome 15.22TNJmice were backcrossed with balancer mice to assess whether the mutation was indeed on chromosome 15. The results failed to find an association between the mutagenized chromosome and the altered phenotype demonstrating that the mutation was off-target and thus, not localized on this region of chromosome 15. Therefore, a whole-genome scan was undertaken to localize the mutation. 22TNJ mice were outcrossed with Balb/c and DBA/2J mice in separate crosses. Phenotypic assessment of the F2 offspring showed that these mice exhibited the expected range of phenotypes with mice showing approximately 25% of the mice exhibiting high locomotor activity in the activity chamber following 2.25 g/kg of ethanol. Current efforts are focused on genotyping these mice to determine the chromosomal localization of the mutation and ultimately to identify the gene that underlies this mutant behavior. Supported by UO1-AA-13503 and MH61971.

MAPPING THE ETHANOL-RELATED PHENOME SPACE USING GENECENTRIC COMBINATORIAL METHODS

R. Kirova; A. Perkins; S. M. Pitts; Z. Li; E. Baker; Michael A. Langston; E. J. Chesler Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge TN, 37831 Department of Computer Science, University of Tennessee, Knoxville TN, 37996 Department of Computer Science, Baylor, TX, 76798

Global analysis of gene-gene, gene-phenotype and phenotype-phenotype relations in genetic reference populations allows the amplification of knowledge around alcohol-related traits. In our present work, we apply a graph theoretical approach to discover relations among sets of genes and phenotypes, construct a map of the behavioral phenome, and associate this map with known and novel sets of genes and gene products. Amajor goal of this work is to determine empirically the functional categories of genes and traits that comprise meaningful biological units. In taking such an approach, we have been able to define sets of alcohol-related phenotypes and the biological networks that they have in common. Moving forward, we will be able to integrate analytically the findings from diverse explorations of the central nervous system structure and function with specific research on alcohol related phenotypes. Our prototypical tools have been developed for genetic reference populations, largely including the BXD recombinant inbred strains. One such tool, GeneNetwork.org's ''COMPARE CORRELATES'' feature, uses graph-based approaches to analyze the connectivity of genes and phenotypes. Users select an input trait set, an association threshold, and a comparison database. All sets of comparison traits associated with each member of any combination of the input traits are then reported. We present a sample result from the use of this tool. It suggests (1) that alcohol preference phenotypes, hippocampal morphology, and seizure related phenotypes are highly connected with an inverse relation, and (2) that hippocampal mRNA abundance for many genes involved in synaptic translation, splicing and potassium influx are associated with these related phenotypes, allowing us to hypothesize that genetic variation affecting potassium efflux may result in altered excitation and elevated synaptic translation. The converse mechanistic hypothesis may also be proposed, namely, that genetic variation in translation increases the synthesis of potassium channels and their functionality. These hypotheses are amenable to biological validation. Additional combinatorial approaches to global analysis of gene-phenotype and phenome mapping are described.

ENHANCED ACQUISITION OF ALCOHOL CONSUMPTION IN ADULT RAT OFFSPRING WITH GESTATIONAL EXPOSURE TO NICOTINE1ALCOHOL

[1]S.G. Matta, [2]A.J. Elberger. [1]E.E. Roguski [1]Dept. Pharmacology, [2]Dept. Anatomy and Neurobiology; Univ. Tennessee Health Science Center, Memphis, TN

Exposure of the human fetus to drugs of abuse results in significant deleterious effects; however, the outcome of co-morbid exposure has received little attention. Nicotine (Nic) is a psychoactive drug commonly abused by young women of childbearing age, who frequently combine smoking with significant alcohol consumption. The present study investigates whether concurrent exposure to Nic and EtOH during gestation is a factor in the acquisition of EtOH consumption as a young adult. Nic1EtOH Sprague-Dawley dams received 4g/kg EtOH gavage daily on gestational days (G)1-21 along with a Nic osmopump that delivered 2g/kg/d subQ from G4-21. Since the rodent postnatal period is the human third trimester equivalent and a period of rapid brain growth and synaptogenesis, treatments were continued postnatally (P) by implanting nursing dams with an 8 mg/kg/d Nic osmopump on P2 and EtOH gavaging pups in 2 separate 2 g/kg dosings 30 min apart from P2-12. Control dams were pair-fed (PF) to the Nic dams, gavaged with an isocaloric solution, and implanted with saline pumps; PF pups received isocaloric gavage. At P60, offspring were allowed to acquire Nic self-administration (SA) in our open access paradigm [30 mg/kg/inj Nic or saline controls (Sal); 23 h/d access]. After stable Nic SA was achieved, 2 bottle-choice EtOH (Hovrous and Peters 1985) was initiated with increasing EtOH concentrations every 4 d from 2% through 10%; Nic SA was ongoing. PF control females with stable Nic SA showed some EtOH preference, whereas Nic1EtOH females exhibited enhanced (2–3 fold) EtOH acquisition, even at 2% and increasing with dose. Also, even without Nic SA, these co-morbid offspring acquired EtOH consumption starting at 4% EtOH, although at a lower level. These results indicate that unlimited Nic SA contributes to acquisition of EtOH consumption. Notably, the combination of gestational co-morbid drug exposure (Nic1EtOH) with unlimited Nic SA elicits rapid and elevated EtOH consumption in female offspring; male offspring are currently being tested. Our results implicate these environmental factors in the continuing cycle of substance abuse. Supported by DA015525 (SGM).

GENETIC ASSOCIATION OF GENE EXPRESSION WITH ALCOHOL PHENOTYPES

J.L. Peirce, A.H. Putman, M.F. Miles, C.C. Parker, B. Bennett, H. Li, J. Wang, K.F. Manly, R.J. Hitzemann, J.K. Belknap, G.D. Rosen, E.J. Chesler, R.W. Williams, L. Lu
Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163

The use of genetic reference populations for relating gene expression and complex alcohol-related phenotypes is a powerful tool. Analyzing genetic correlations between brain gene expression in naive animals and alcohol-related phenotypes is an approach that is becoming more commonly used as gene expression data sets in reference populations become available. There are advantages, drawbacks, and caveats to such approaches that are worth considering, including the interpretation of genetic correlations between gene expression results and polygenic alcohol-related phenotypes. Once a set of interesting genetic relationships is identified, confirming the relationships is a valuable first step towards identifying which should be targeted for further study. In this context, relatively inexpensive F2 data sets can provide a valuable complement to deep RI data sets as can the availability of independent expression data in similar tissues, with caveats that will be discussed. A complementary approach to genetic correlation between naive gene expression and the behaviors of alcohol-treated animals is being attempted in several labs - gene expression analysis of alcohol-treated brains and genetic correlations between expression under treatment conditions and alcohol-related phenotypes. This approach also has advantages, including powerful comparisons of betweencondition effects, but can be challenging to implement. We will discuss and compare the utility of using gene expresssion in both naive and alcohol treated brains to understand alcohol-related phenotypes. Challenges of this approach will also be discussed, including the effects of treatment timing on gene expression and comparison across different treatment conditions. Examples will be drawn from ongoing work in an LXS RI-based comparison of gene expression using naive, alcohol-treated, stressed, and alcohol1stress treated animals.

MAKING BIOLOGICAL SENSE OF MICROARRAY GENE EXPRESSION DATA

Bing Zhang Biomedical Informatics Department Vanderbilt University Nashville, TN 37232

Microarray technology is becoming a routine tool in alcoholism research for the identification of candidate genes, as well as the elucidation of the underlying genetic networks. While in the past biologists studied single genes at a time, now researchers can use this high-throughput technology to analyze tens of thousands of genes simultaneously and generate a massive amount of experimental data at an exponential rate. Computational methodologies have been well developed to identify differential and correlated gene expression from microarray data. However, extracting biological insight from these results remains a big challenge for today's biologists. Rapid and easy access to the existing functional information for large sets of genes identified from microarray experiment is the first step to successful data mining and biological discovery. Traditional resources that are available for retrieving functional information about genes and gene products, such as the Entrez Gene from NCBI, are typically displayed and manipulated in a one-gene-at-a-time format. I will first introduce several new resources that could facilitate batch information retrieval for large number of genes. The next step in biological discovery involves the application of appropriate statistical analysis on the retrieved functional information. One of the most widely used approaches is to organize the genes identified from a microarray experiment based on common functional features, such as the Gene Ontology (GO) categories or the KEGG metabolic pathways, apply statistical tests to identify enriched functional categories, and highlight the significant biological areas. Methods and existing software packages for performing such kind of biological discovery will be introduced. Applications of these approaches to several INIA microarray datasets will be presented. The power, limitations and problems of these approaches will be discussed.

CRF STIMULATION OF PLASMA DEOXYCORTICOSTERONE LEVELS IN ABSTINENT ALCOHOL-DEPENDENT SUBJECTS

B. Adinoff, A.L Morrow, M.J. Williams, P.A. Chandler University of Texas Southwestern Medical Center, Dallas, TX; VA North Texas Health Care System; University of North Carolina

Neuroactive steroids dampen the anxiogenic response to stress by modulating GABAergic transmission. The most potent neurosteroids are the metabolites of deoxycorticosterone (DOC) and progesterone, and these compounds can be synthesized de novo in the brain, adrenal glands, and other endocrine organs. As ethanol and the neurosteroids have interactive neuropharmacological effects and neurosteroids can alter drinking patterns in animal models, we assessed the DOC response to pharmacological activation in abstinence alcohol-dependent subjects and healthy controls. Eleven one-month abstinent, alcohol-only dependent men and ten age- and sex-matched healthy controls were studied. DOC concentrations were obtained at baseline and over the next 120 minutes after the intravenous infusion of ovine corticotropin releasing hormone (oCRH) (0.4mg/kg) at 2000 hr. As previously reported, ACTH concentrations were not significantly different between groups, whereas the cortisol response was blunted in the alcohol-dependent group. DOC concentrations peaked one hour after oCRH infusion at levels appropriately threefold higher than basal concentrations (baseline vs. peak response: controls po0.0001, patients po0.0005). Preliminary findings reveal that baseline DOC concentrations and peak and net integrated DOC responses were not significantly different between groups. However, oCRH-induced elevations in DOC concentrations appeared to persist in a subgroup of alcohol-dependent subjects relative to controls and the other alcohol-dependent subjects. These preliminary studies demonstrate that oCRH can induce a marked increase in DOC concentrations in humans, presumably mediated through adrenal release, revealing the utility of stress-induced provocation in assessing the peripheral responsiveness of neurosteroid precursors. Persistent elevations in DOC concentrations following oCRH stimulation in some alcohol-dependent subjects may suggest intragroup variability, potentially effecting their central response to stress and/or alcohol.

ADRENOCORTICAL RESPONSIVENESS TO COSYNTROPIN IN ABSTINENT FEMALE ALCOHOL-DEPENDENT WOMEN

Williams M.J.; Chandler P.A.; Best S.E.; Adinoff B. Department of Psychiatry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75216, USA.

Abstinent alcohol-dependent males exhibit a suppressed pituitary-adrenal response to both pharmacologic and psychosocial stressors. Blunted glucocorticoid responsiveness may affect the central nervous system's ability to mount an appropriate response to environmental stressors, possibly heightening the probability of relapse. Since gender differences have been noted in the hypothalamic-pituitary-adrenal (HPA) axis response to stress, it is not known whether female alcohol-dependent women also exhibit adrenocortical hyposensitivity. The aim of the present study was to examine HPA axis functioning in alcohol dependent females. Pursuant to this goal, the authors compared the adrenocortical responsiveness in female alcohol-dependent women to that of sexmatched controls. Adrenocortical sensitivity was assessed in both the presence and absence of endogenous pituitary activation. METHODS: Seven female, 4 to 8 weeks abstinent, alcohol-only-dependent subjects and ten age-matched female healthy controls were studied during the pre-follicular phase of the menstrual cycle. A submaximal dose of cosyntropin (.01 ug/kg), a corticotrophin analogue, was administered to assess adrenocortical sensitivity. In a separate session, cosyntropin was administered following high dose dexamethasone (8 mg iv) to assess adrenocortical sensitivity in the relative absence of endogenous corticotrophin. RESULTS: In contrast to males, female alcohol-dependent and control females did not differ in their net AUC cortisol response to cosyntropin, either in the absence (p5.67) or presence (p5.78) of dexamethasone-induced pituitary suppression. CONCLUSION: These findings suggest that female alcohol-dependent women do not share the disruption of HPA responsiveness observed in alcohol-dependent men. These differences may have relevance to vulnerability to stress-induced relapsed in alcohol-dependent women relative to men.

PHARMACOGENOMICS OF NALTREXONE AND ETHANOL IN MOUSE BRAIN

R.T. Kerns and M.F. Miles Departments of Pharmacology/Toxicology and Neurology, Virginia Commonwealth University, Richmond, VA 23298

The non-selective opioid antagonist naltrexone (NTX) has been used in human alcoholics to prevent relapse. NTX also decreases ethanol drinking in rodent models and modifies ethanol-related behaviors in the C57BL/6J (B6) and DBA/2J (D2) inbred-mouse strains that exhibit contrasting acute responses to ethanol and predisposition to drinking. NTX reduces ethanol consumption in B6 and blocks ethanol locomotor activation in D2 mice. The characterization of the effects of NTX on ethanol responsive gene expression in D2 and B6 mice will provide important clues to understanding the molecular basis responsible for observed phenotypic differences in these mouse strains, and may lead to new insight into the neurobiology of ethanol sensitivity and addiction. Total RNA was extracted from microdissected prefrontal cortex (PFC), ventral tegmental area (VTA), and nucleus accumbens (NAC) of mice harvested 4 hours after injection with either 0.5 mg/kg NTX or 0.9% saline and 30 minutes later either 2 g/kg ethanol or saline. Relative levels of RNA were determined by hybridization of triplicate pooled samples to Affymetrix Mouse Genome 430A GeneChipTM oligonucleotide arrays. Expression levels were first analyzed by the Sscore method developed in this laboratory, followed by SAM and multivariant analysis. Expression profiling showed strain-, brain region- and treatment-specific patterns of gene expression, including the blockade or enhancement of ethanolregulated gene expression by NTX. Bioinformatics analysis detected significantly changed functional classes and gene networks specific to strain and brain region. Of special interest was the observed potentiation by NTX and ethanol of ribosomal protein gene expression in B6 but not D2 PFC. In summary, the inbred mouse strains DBA/2J and C57BL/6J exhibited differential changes in ethanol-responsive gene expression with NTX treatment. These changes may help elucidate the underlying mechanisms by which NTX alters ethanol-related behaviors. Supported by NIAAA RO1 AA014717to MFM and F32 AA014726 to RTK.

GENE EXPRESSION PROFILING ANALYSIS OF MECHANISMS UNDERLYING ETHANOL DEPRIVATION EFFECT IN C57BL/6 MICE

R.T. Khisti, M.F. Miles Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia 23298.

The ethanol deprivation effect (EDE) is one of the most robust and widely used animal behavioral models of ethanol-craving known to affect voluntary ethanol intake. Renewed access to ethanol solutions after a period of deprivation for several days/weeks leads to a pronounced, although temporary, increase in voluntary ethanol intake. Although several studies have investigated EDE in rodents, molecular components underlying this behavior are not clearly understood. In the present study we investigated genomic changes underlying EDE in mice. Adult male C57BL/6NCrl mice (n542) from Charles Rivers Laboratory were presented at the beginning of dark phase with 2-tubes containing either 10% w/v ethanol or tap water for 18 hrs/day, as well as food ad libitum. Following establishment of stable baseline drinking for 13 days, mice were deprived of ethanol for 4 days. To study genomic changes following EDE, mice (n518) were sacrificed at 6- or 96-hrs after ethanol deprivation and brain regions dissected. Ethanol deprivation-induced change in ethanol drinking was studied in the remaining mice (n518) by reinstating availability of ethanol (10% w/v). Mice established stable ethanol drinking of 2.5–2.8 g/kg/18hr (19.8–18.8% ethanol preference/18-hr) after 10 days. A robust EDE was observed after reinstatement of ethanol, as evidenced by 1.48 fold increases in ethanol consumption (po0.004 vs. prior to EDE) and 1.73 fold increases in % ethanol preference (po0.005). Oligonucleotide microarrays were used to characterize patterns of gene expression in three brain regions of the mesolimbic reward pathway. Using a rigorous stepwise method for microarray analysis, we identified genes differentially expressed in control versus ethanol deprived mice in the nucleus accumbens, prefrontal cortex, and ventral tegmental area. In conclusion, this study of behavioral and genomic components responsible for the ethanol deprivation effect provides important targets for therapeutic intervention of alcohol craving.
Supported by NIAAA Grant RO1 AA014717 to MFM.

STRATEGIES AND TOOLS FOR DEFINING GENE NETWORKS AND FORMING MECHANISTIC HYPOTHESES RELEVANT TO ETHANOL BEHAVIORS

M.F. Miles, R. Kems and A. McMullen Departments of Pharmacology/Toxicology and Neurology and the Center for Study of Biological Complexity Virginia Commonwealth University, Richmond, VA

Microarray data analysis can present a bewildering array of options for even the experienced genomics investigator. A particularly important stage of microarray studies concerns the quaternary level of analysis where gene correlation networks are combined with other biological information to define functional networks useful for hypothesis generation. This process can encompass correlations across microarray data, genetic variation in behavioral or gene expression data, proteinprotein interaction databases, natural language processing mining of literature associations, membership in known biochemical pathways, and many other such large databases. This lecture will discuss practical approaches to experimental design, useful tools and formulation of testable hypotheses. For example, use of the Cytoscape modular software package for integrating diverse biological correlation networks will be discussed. Biological examples will include studies on acute ethanol action. The combined use of various genetic models (e.g. recombinant inbred strains and null animals from genetic targeting) will be discussed as amechanism, combined with bioinformatic tools, for focusing on the most relevant gene patters for future study. Problems such as linkage dysequilibrium effects on gene expression networks and limitations of behavioral phenotypes will be discussed. An effort will be made to demonstrate how investigators can use the approaches and tools discussed to form novel, testable hypotheses regarding ethanol actions.

QUANTITATIVE TRAIT LOCI AFFECTING SUSCEPTABILITY TO ETHANOLINDUCED ANXIOLYSIS IN BXD RECOMBINANT INBRED MICE

A.H. Putman and M.F. Miles Virginia Commonwealth University, Departments of Pharmacology/Toxicology and Neurology, Richmond, VA 23298

Due to the high comorbidity between anxiety and ethanol abuse and human reports of consuming ethanol to relieve anxiety, there has been a long-standing interest in understanding the relationship between anxiety and ethanol abuse. Although anxiety is hypothesized as a factor in the initiation of ethanol abuse and risk for relapse, the molecular mechanisms underlying anxiety, ethanol addiction, and their correlation are poorly understood. The identification of ethanol-induced anxiolysis-like behavioral quantitative trait loci (QTL) will offer an opportunity to elucidate the underlying molecular mechanisms involved in the variance of this trait. Therefore, anxiety-related responses to ethanol were measured across the BXD recombinant inbred panel and C57BL6J and DBA/2J progenitors using the light-dark transition model of anxiety. Animals were restrained for 15 minutes, immediately injected (I.P.) with either 0.9% saline or 1.8 g/kg ethanol and tested in the light-dark box for 10 minutes. Preliminary QTL analysis has identified potential genetic loci associated with various anxiety-related behaviors. A suggestive basal anxiety QTL mapped to chromosome 2 while two suggestive QTLs on chromosome 1 and chromosome 12 may influence ethanol-induced anxiolysis. These QTLs are all consistent across multiple anxiety-related behaviors including percent time spent in the light and percent distance traveled in the light. Further analyses will include the identification of brain expression patterns across BXD recombinant inbred lines using Affymetrix oligonucleotide microarrays and identify expression QTL. Combining genetic and genomic analyses will allow us to correlate ethanol-responsive expression patterns with behavioral QTL data and ultimately identify genetic variation in expression networks relevant to ethanol-induced anxiolysis. Since differences in the anxiolyiclike response of ethanol may alter the liability of ethanol abuse, any insight into this mechanism may have novel implications in the study of ethanol abuse and potentially identify targets for the treatment of anxiety and ethanol disorders. Supported by NIAAA Grants RO1 AA014717 to MFM and F31 AA016052 to AHP.

IDENTIFICATION OF CANDIDATE GENES RELATED TO INDIVIDUAL VARIATION IN ETHANOL CONSUMPTION

JT Wolstenholme, MF Miles Virginia Commonwealth University, Department of Pharmacology and Toxicology, Richmond, VA 23298

Genetic differences have been described as accounting for only 40–60% of the variability in individual ethanol consumption. Several laboratories have documented persistent individual variability in ethanol consumption and preference patterns within an inbred strain of mice. However, neurobiological factors underlying ethanol consumption and preference in these animals are not completely understood. Therefore, the present studies were designed to identify genes differentially expressed between mice with high and low ethanol consumption and preference. C57BL/6Crl mice were given access to 10% ethanol and water in a two-bottle choice paradigm for at least two weeks. In three independent experiments, we observed persistent individual differences in ethanol drinking patterns. Overall, each animal's intake level was consistent over the drinking sessions. However, ethanol consumption in each experiment ranged from less than 1 g/kg to over 15 g/kg ethanol intake. Ethanol consumption did not appear to be a result of taste sensitivity since consumption did not correlate to the amount of an aversive solution (0.1 mM quinine) consumed in a two-bottle choice test. Ethanol consumption also did not correlate to basal anxiety as measured in the light-dark model. Interestingly, ethanol consumption and preference could be altered by changing the social status of individual mice. High density oligonucleotide microarrays were used to perform differential expression profiling in the ventral tegmental area (VTA) and nucleus accumbens (NAc) between individual mice. Gene expression was correlated to each animal's average ethanol intake level. Out of more than 22,000 genes probed, 200 transcripts in the VTA were significantly correlated to ethanol intake levels of individual mice, while 266 transcripts in the NAc were differentially expressed. Of these, two genes within the a-synuclein/NSF network correlated to ethanol consumption levels. This network of genes includes Stxbp1, a candidate for an ethanol preference drinking locus on mouse Chromosome 2. Thus, these studies may identify candidate genes associated with ethanol consumption and preference. Supported by NIAAA Grant R01 AA14717 to MFM.

NEUROSTEROID RESPONSES TO ETHANOL ARE ELEVATED FOLLOWING SOCIAL ISOLATION STRESS - RELATIONSHIP TO ENHANCED DRINKING IN RATS?

G. Biggio and M. Serra Department of Experimental Biology, Center of Excellence for Neurobioiogy of Drug Dependence, University of Cagliari, Cagliari, Italy.

We have previously demonstrated that social isolation of rats is associated with both a reduction in the cerebrocortical and plasma concentrations of 3a, 5a-TH PROG and 3a, 5a-THDOC and an increase of the positive effects of acute ethanol on the concentrations of these neuroactive steroids. The ethanol-induced increase in the abundance of 3a, 5a-TH PROG is also more pronounced in the brain than in the plasma of isolated rats. Behavioral studies have also indicated that the ability of ethanol to inhibit isoniazid-induoed convulsions is greater in isolated rats than in group-housed animals and this effect of isolation is prevented by treatment with the 5a-reductase inhibitor finasteride. Social isolation modified the effects of ethanol on the amounts of StAR mRNA and protein in the brain suggesting an alteration in the mechanism of cholesterol transport in mitochondria. Moreover, the amounts of the a4 and d subunits of the GABAA receptor in the hippocampus were increased in isolated rats, and these effects were accompanied by an increase in GABAA receptormediated tonic inhibitory currents in granule cells of the dentate gyrus. Ethanol also increased the amplitude of GABAA receptor-mediated miniature inhibitory postsynaptic currents (mIPSC) recorded from CA1 pyramidal neurons with a greater potency in hippocampal slices prepared from socially isolated rats than in those from group-housed animals, an effect inhibited by finasteride. Voluntary consumption of ethanol during social isolation abolished both the reduction of the cerebrocortical and plasma concentrations of 3a, 5a-TH PROG and 3a, 5a-THDOC and the enhanced potency of ethanol on mIPSC recorded from CA1 pyramidal neurons. The increased rate of ethanol intake in isolated rats (Wolffgramm et al., 1990) may thus result from a rewarding effect of this drug that is attributable to the marked increase in the concentrations of endogenous anxiolytic steroids induced by its consumption. Thus, the natural preference for ethanol may be related to the effect of this drug on the levels of neuroactive steroids, an effect that is enhanced by social isolation. Wolffgramm J. Psychopharmacol. 101. 233-39,1990. Supported by INIA (U01AA13641).