S28.4 ETHANOL DISRUPTS IMMATURE NETWORK NEURAL ACTIVITY DURING ETOL醉 SENSITIZED WITHDRAWAL IN ALZHEIMER'S DISEASE-MODELED MICE.
C.F. Valenzuela, R. Galindo. Dept of Neurosciences, U of New Mexico HSC, Albuquerque, NM 87131, USA.

The hippocampus is a brain region that is important for learning and memory processes and studies have demonstrated that it is particularly sensitive to the neurotoxic effects of ethanol. In this brain region, there is a developmentally-regulated pattern of network-driven electrical activity known as the mature dentate granule cell network (MDGCN). In immature neural networks, ethanol receptors are excitatory due to a shift in Ca^2+ equilibrium potential towards more depolarized potentials. The excitatory effects of ethanol are mediated by GABA receptors, which are associated with a shift in the equilibrium potential for chloride (Cl^-) in intracellular calcium. These oscillations contribute to activity-dependent modulation of growth neural system(s) controlling ADE in sP rats. Also the GABAB receptor agonist baclofen has been shown to disrupt the control over alcohol and the episodes of alcohol relapse of human alcoholics. ADE is a relevant feature of alcohol drinking behavior of the selectively bred Svalbard alcohol-prefering (SP) rats. Indeed, after an exposure to alcohol and a subsequent deprivation, SP rats display a pronounced, although short-lasting, ADE when alcohol is represented. When multiple concentrations of alcohol are presented, ADE in SP rats is accompanied by a shift in preference towards the highest concentrated solutions (a likely manifestation of the increased demand for alcohol which results in the search of the alcohol preparations which may give faster central effects). Recent work from this lab demonstrated that the acute administration of the cannabinoid CB1 receptor antagonist, SR 141716, suppressed ADE in SP rats, suggesting the involvement of the cannabinoid CB1 receptor in the neural circuitry mediating ADE. More recently, we found that the combination of SR 141716 and the opioid receptor antagonist, naloxone, synergistically suppressed for SP rats. It is suggested that the GABA receptor is a stress-related protein in the brain and that it could be an important target for the treatment of human alcoholism.

S29.1 PHARMACOLOGICAL REVERSAL OF CYCLED WITHDRAWAL- OR STRESS-SENSITIZED WITHDRAWAL ANXIETY AND ENHANCED ETHANOL DRINKING.
DJ Knapp, DH Overstreet, GR Breese. Ctr. for Alcohol Studies, UNC, Chapel Hill, NC 27599 USA.

These investigations focused on conditions that may link stress, anxiety, and relapse. Previous data demonstrated that ethanol withdrawal dramatically disrupted GABAA receptor-mediated spiny postsynaptic currents in pyramidal cells (33% ± 6%; n=12) and interneurons (45% ± 10%; n=11), and of glutamate receptor-mediated spontaneous postsynaptic currents in interneurons (35% ± 14%; n=7). Ethanol did not affect the intrinsic excitability of either interneurons or pyramidal neurons. We are currently investigating if the effect of ethanol involves changes in the probability of GABA or glutamate release. These novel actions of ethanol on immature neuronal circuits are likely to contribute to the pathophysiology of alcohol-related neurological disorders and fetal alcohol syndrome. Supported by NIH grant AA12684.

S29.2 ALCOHOL CRAVING AND RELAPSE IN RATS GENETICALLY SELECTED FOR HIGH ALCOHOL PREFERENCE.
Zachary Rodd, Indiana University, School of Medicine, Indianapolis, IN, USA.

The alcohol-preferred (P) line of rat has been well characterized both behaviorally and neurobiologically and satisfies criteria proposed as essential for an animal model of alcoholism. Recently, we have examined various animal models of alcohol relapse and craving in the P rat. The alcohol deprivation effect (ADE) phenomenon following single or multiple cycles of ETOL deprivation-access has been examined in P rats. Following a single ETOL deprivation period, 1) an ADE can be observed following a deprivation period of 5 weeks (operant conditions) or 8 weeks (24-hour free-choice conditions). 2) there is typically a two-fold increase in ETOL intake (10 g/kg/day intake; 300 cycle response to 10 g/kg ETOL intake; 5 consecutive cycles of >50% operant ETOL responses). 3) there is a marked shift in preference for higher concentrations of ETOL (from 10% to 30% ETOL). 4) there is a marked shift in preference for higher concentrations of ETOL (from 10% to 30% ETOL). 5) there is a marked shift in preference for higher concentrations of ETOL (from 10% to 30% ETOL). Overall, these results indicate that the rat is a good animal model of both relapse drinking and alcohol craving, that the P line of rats may be unique in its predisposition to expressing pronounced alcohol craving/relapse, and that the PSR procedure may be a potentially valid, important measure for studying alcohol craving behavior. (AA07611, AA11261, AA07291)

S29.3 EXPOSURE TO STRESS INCREASES DOMAPINERGIC BURSTING FACING IN AWAKE RATS. 
DJ Knapp, DH Overstreet, GR Breese. Ctr. for Alcohol Studies, UNC, Chapel Hill, NC 27599 USA.

Exposure to stress may contribute to relapse in alcoholics and addicts. While it is clear that ethanol administration (DA) increases ethanol consumption, the electrophysiological responses of single brain regions to stress are unknown. Distribution of ventral tegmental area (VTA) neurons was recorded under restraint and stressful conditions to determine how neuronal activity contributes to the stress response. Arrays of single- and multi-electrode probes were chronically implanted in the VTA of 22 male, Sprague Dawley rats. After habituation to the recording chamber, neural activity was recorded across a 30-minute session where the animal was allowed to move freely in the recording chamber and was immediately followed by another 30-minute recording session where the rat was restrained. The next day, some rats were subjected to another restraint session and others injected with haloperidol. Putative DA neurons could be identified on the basis of waveform duration, firing rate and response to haloperidol. Mean firing rates were similar to those reported in anesthetized animals with average firing rate of 2.45 Hz and range of 1.07-5.2 Hz. Interspike interval histograms demonstrated that a subpopulation of DA neurons fire uniquely in a pacemaker fashion while others display both burst firing and pacemaker activity. Restraint stress increased mean firing rate of all dopaminergic neurons and increased burst firing only in DA neurons that displayed some burst activity under resting conditions. These data suggest that increases in extracellular DA levels due to stress lead to an increase in population activity and increased burst firing in a subset of DA neurons. Increased burst firing in DA neurons may represent alterations in circuit activity correlated with behavioral states leading to relapse.

S29.4 INVOLVEMENT OF CANNABINOID CB1 AND GABAB RECEPTORS IN THE CONTROL OF RELAPSE-LIKE DRINKING IN ALCOHOL-PREFERING SP RATS.

Alcohol deprivation effect (ADE) is defined as the transient increase in alcohol intake which occurs after a period of total abstinence. The ADE is characterized by a two-fold higher in repeatedly cycled than non-deprived P rats). An animal model of alcohol craving is the Pavlovian Spontaneous Recovery (PSR; reinstatement of responding (goal seeking) or conditioned salivation). We have recently proposed to use alcohol deprivation-induced reinstatement of alcohol drinking as a measure for studying alcohol craving/relapse. (AA07611, AA11261, AA10721) 

S29.5 STRESS-INDUCED ETHANOL DRINKING IN CBI-1, POMC AND PINK KNOCKOUT MICE.
I. Racz, A. Bikes-Girou, K. Michel, A. Zimmer, University of Bonn, Germany, 53105

Stress is known as one of the main causes of relapse in human alcoholics. Although the opioid receptor antagonist naloxone is currently the only drug used for relapse prevention, it is the role of the endogenous opioid system, and other neuromodulator systems, in alcohol drinking behaviour not well understood. We have therefore studied the effects of different types of stress on ethanol drinking in POMC, PINK and CBI1 receptor mutant mice. The two-bottle choice procedure was used for assessment of alcohol preference under base line conditions and in different stress models: foot shock, social stress and forced-swimming. We found a significant sex difference in alcohol drinking and in stress-modulated alcohol preference in all mutant strains and in wild type controls. CBI1 animals and wild type mice had a similar preference for alcohol. On the other hand, PINK- and POMC- mice showed no preference for ethanol. The wild type animals showed changes in alcohol drinking in stress-modulated alcohol preference in all mutant strains and in wild type controls. Stress- induced ethanol drinking in CBI-1, POMC and PINK knockout mice. Stress is known as one of the main causes of relapse in human alcoholics. Although the opioid receptor antagonist naloxone is currently the only drug used for relapse prevention, it is the role of the endogenous opioid system, and other neuromodulator systems, in alcohol drinking behaviour not well understood. We have therefore studied the effects of different types of stress on ethanol drinking in POMC, PINK and CBI1 receptor mutant mice. The two-bottle choice procedure was used for assessment of alcohol preference under base line conditions and in different stress models: foot shock, social stress and forced-swimming. We found a significant sex difference in alcohol drinking and in stress-modulated alcohol preference in all mutant strains and in wild type controls. CBI-1 animals and wild type mice had a similar preference for alcohol. On the other hand, PINK- and POMC- mice showed no preference for ethanol. The wild type animals showed changes in alcohol drinking in stress-modulated alcohol preference in all mutant strains and in wild type controls. Stress- induced ethanol drinking in CBI-1, POMC and PINK knockout mice.
S30.1

EFFECTS OF ALCOHOL AND BENZODIAZEPINES ON BRAIN METABOLISM IN ALCOHOLIC HUMAN SUBJECTS: [11C]RO15 4513 PET STUDIES.

G. Gründler,1,4, M. Schreckenberg,1 T.F. Dietlenstätter,1 M. Lochmann,2 K.mann,1 Ch. Lange-Asschendorf,3 T. Siessmeier,3 H.-G. Buchholz,1 R. Amberg1, P. Bartenstein,1 Departments of Psychiatry,1 Legal Medicine,2 University of Mainz, Mainz, Germany, 4Institute of Psychiatry, University of Heidelberg, Germany.

We have used PET and [11C]Ro15 4513 to study the metabolite of benzodiazepines in abstinent alcoholics. Using [11C]Ro15 4513, we observed a delay in metabolism in the frontal cortex. PET studies on healthy volunteers showed a higher and faster metabolism in the frontal cortex as in alcoholics. This effect can be explained by the higher metabolism in the brain of alcoholics, which might be linked to the higher metabolic rate of the brain. In conclusion, PET can be used to study the metabolism of benzodiazepines in abstinent alcoholics.

S30.2

IMAGING GABA_A RECEPTOR SUBTYPES WITH PET.

A. Lingford-Hughes in collaboration with colleagues at Psychopharmacology Unit, University of Birmingham, Birmingham, UK, and the Nuclear Medicine Unit in the School of Medicine, Du Cane Rd, London, W12 0NN, UK.

We have used PET and SPECT to study the GABA_A benzodiazepine receptor (GABA_A-BDZR) in abstinent alcoholics. Using [11C]flumazenil, we observed a delay in metabolism in the frontal cortex. This effect can be explained by the higher metabolism in the brain of alcoholics, which might be linked to the higher metabolic rate of the brain. In conclusion, PET can be used to study the metabolism of benzodiazepines in abstinent alcoholics.

S30.3

CENTRAL PROCESSING OF ALCOHOL CUES AND CRAVING CORRELATE WITH DOPAMINE D2 RECEPTORS IN VENTRAL STRIATUM.


Dopamine release in the ventral striatum including the nucleus accumbens is stimulated by acute and chronic alcohol intake. Chronic alcohol intake is associated with a down-regulation of central dopamine D2 receptors. Neuroendocrinological challenge studies suggested that delayed recovery of D2 receptor sensitivity after detoxification is correlated with a high risk for relapse. Prolonged D2 receptor dysfunction in the ventral striatum may bias the brain reward system towards excessive allocation of incentive salience to alcohol-associated stimuli. We used a multidimensional imaging approach with the radioligand [11C]flumazenil and positron emission tomography (PET) to study the distribution of dopamine D2 receptors (D2R) to measure the association between D2R-like dopamine receptors in the ventral striatum, alcohol craving, and central processing of alcohol cues. We observed that alcohol-associated versus neutral visual stimuli activated different brain regions associated with the ventral striatum and cortex. We observed a delay in metabolism in the ventral striatum of the alcohol-dependent group compared to the healthy controls. Our findings indicate dopaminergic dysfunction in the ventral striatum of detoxified alcoholics may interfere with incentive salience attribution to alcohol-associated stimuli; as a result, alcohol cues can elicit craving and excessive activation of neural networks associated with attention and behavior control.

S30.4

THE MEDIAL TEMPORAL LOBE IN ALCOHOLISM AND PSYCHOPATHOLOGY: EVALUATION WITH METABRIC MR IMAGING.

Lauko MP, Vaurio A, Salminen A, Tiihonen E, Soininen H, Tiihonen J, Aro A, Kuopio University Hospital, Departments of Neurology and Radiology, University of Kuopio, Nunnamiemi Hospital, Helsinki Brain Research Center, Finland.

Hippocampal structures are involved in learning, memory and conditioning. Hippocampal volume changes have been reported in Alzheimer's disease, frontotemporal dementia, epilepsy and schizophrenia. These changes are also characterized by behavioral problems. Animal and human studies suggest that different functional organization within the hippocampus along its longitudinal axis is disturbed in alcoholics. Identifications of damage that is localized to certain parts of the hippocampus may provide new evidence about the pathophysiological basis of the disease. The volumes of various parts of hippocampus and amygdala in alcoholics and habitually violent offenders were measured by volumetric MRI and the results were correlated with behavioral assessments. MRI was used to measure volumes of the hippocampus in late-onset type 1 alcoholics and early-onset type 2 alcoholics as proposed by Conyne. The type 2 alcohol subjects were also violent offenders with antisocial personality disorder, derived from a forensic psychiatric sample. There was a tendency towards decreased volumes with aging and also with the duration of alcoholism in the type 1 alcoholics. Surprisingly, there was a significant positive correlation between the right hippocampal volume and age in the type 2 alcoholics and a subgroup of habitually violent offenders with antisocial personality disorder and type 2 alcoholics. Regional volumes along the anteroposterior axis of the hippocampus were correlated with the subjects' degree of psychopathy. Strong negative correlations were observed between the psychopathy scores and the posterior half of the hippocampus bilaterally. In our preliminary study MRI was used to examine amygdalar volumes in violent offenders which were divided into two groups according the Psychopathy Checklist Revised. The high psychopathy personality trait offenders had significantly smaller amygdalar volumes on the right compared with the offenders with low psychopathy trait. Our data support the view that lesions of the dorsal hippocampus impair acquisition of conditioned fear. Data from neuroimaging studies support the view that the type 2 characteristics might represent a primarily antisocial personality disorder within the alcoholism.
MOOD, MINOR NEGATIVE EVENTS, AND ALCOHOL CONSUMPTION: DAILY LIFE INVESTIGATIONS USING THE EXPERIENCE SAMPLING METHOD.
J. Swendsen, Department of Psychology, University of Bordeaux, Bordeaux 33076, France.

The vast majority of the published literature concerning the self-medication hypothesis is based on research paradigms that examine correlations between individual difference variables (e.g., diagnoses, average stress or anxiety levels). However, self-medication is a dynamic, within-person and prospective phenomenon whereby state affect or stress increases the risk of alcohol use over periods that are typically limited to minutes to days. In addition, the study of comorbidity in clinical samples renders it difficult to conclude as to causal mechanisms of association, as alcohol use disorders may generate mood and anxiety syndromes. For these reasons, two recent studies using the Experience Sampling Method have examined this brief life cycle of association in non-clinical samples through repeated ambulatory data collection techniques. Participants in both studies were interviewed by palm micro computers at numerous intervals per day and followed for one to four weeks concerning their experience of mood states, stress, and alcohol use. Of the diverse emotions examined, anxiety was the only negative or unpleasant mood state found to be associated with an increase in alcohol consumption over subsequent hours of the same day. However, anxiety was a significant predictor of alcohol use only among older and regular consumers; no evidence for a general self-medication or stress dampening phenomenon was found for younger adults. The findings provide direct and prospective support for the self-medication hypothesis, but underscore the importance of sample characteristics in its investigation and in assessing its generalizability.

ALCOHOL ATTENUATES PSYCHOSOCIAL STRESS RESPONSE BUT NOT STARTLE REFLEX MODULATION IN SONS OF ALCOHOL-DEPENDENT FATHERS.
U. Zimmerman, Central Institute of Mental Health, 68159 Mannheim, Heidelberg University, Germany.

We tested the hypothesis that a family history of alcoholism is associated with more stress-induced hypothalamic-pituitary-adrenal (HPA) activity and more startle reflex potentiation by negative emotional stimuli, and also with a stronger ethanol-induced dampening of those effects compared to family history negative controls. Methods: Subjects with a paternal history of alcoholism (PHA) and family history negative (FHN) control-aged (aged 18-25y) were recruited. Two stress and two startle experiments were performed, each after participants drank either placebo or alcohol (0.6 g/kg) in a randomized double-blind crossover design. Psychosocial stress was induced by a public speaking task, and plasma ACTH and cortisol were measured up to 90 minutes after this test. The acoustic startle reflex was modulated by threat of aversive electric shocks and by the International Affective Picture System (P. Lang). Results: Generally, stress-induced ACTH and cortisol secre- tion was higher in PHA subjects and was dampened by alcohol only in PHA, not in FHN subjects. A strong effect of test repetition and alcohol administration sequence complicates interpretation of these results. The baseline startle response was significantly lower in PHA than FHN subjects. Fear potentiation and emotional modulation of the startle reflex were unaffected by alcohol, family history, and their interaction. Conclusions: In subjects with positive family history, a proxy for genetic risk of alcoholism in the present study, the endocrine response to psychosocial stress is increased, and it is also attenuated by alcohol. The influence of alcohol administration sequence suggests that part of the alcohol effect might be due to disturbed memory encoding during the first test session. Contrary to our hypotheses, the response to experimental induction of fear and negative emotional states does not vary with family genetic risk.

INDIVIDUAL DIFFERENCES IN ALCOHOL OUTCOME EXPECTANCIES ARE PREDICTIVE OF DRINKING TO MANAGE ANXIETY.
M.G. Kuhnen, University of Minnesota, 2450 Riverside Avenue, Minneapolis, MN 55454, USA.

That stress and anxiety promote alcohol consumption aimed at anxiety dampening is a widely held explanation for the co-occurrence (‘comorbidity’) of anxiety disorders and alcohol use disorders (‘self-medication’ model); however, results from laboratory and field studies do not consistently conform to this model. We argue that by considering individual differences in alcohol outcome expectancies (AOEs), the accuracy of predictions stemming from the self-medication model can be increased. In support of this, we present several of our studies showing that: 1) the extent of anxiety dampening obtained in response to an alcohol placebo, particularly in males, is partially dependent upon their pre-experimental AOEs (experimental study), 2) alcohol consumption among college men is positively correlated with anxiety level, but only when tension reduction AOEs are high (field study), 3) tension-reduction AOEs predict drinking to manage anxiety and panic symptoms better than several anxiety-related personality traits and higher-order personality dimensions (field study); and 4) the risk for relapse to drinking following alcoholism treatment associated with comorbid panic disorder is greater in those with higher tension-reduction AOEs (clinical study). We conclude that explicitly incorporating AOEs into the self-medication model increases its practical utility (e.g., predicting who will drink to manage anxiety) and its theoretical development from a cognitive perspective.
Brain-derived neurotrophic factor (BDNF) promotes serotoninergic (5-HT) neurotransmission and the structural plasticity of astrocytes in the adult brain. Hereby, BDNF mRNA and protein levels that are 50% of that of wild-type (WT) mice. These BDNF deficient mice display abnormalities in 5-HT neurotransmission, develop deficits in 5-HT innervation of the mesolimbic dopamine system, and are more sensitive to stress.

5-HT1A receptors have been implicated in the pathogenesis of depression, anxiety, and other neurodevelopmental disorders. The 5-HT1A receptor is also located post-synaptically and is present in high density in the forebrain. Both the basal amount of secreted BDNF and CREB activity were larger in differentiated NPCs than in undifferentiated NPCs. The events in the study using Y2 receptor antisense; in post-dependent animals, this treatment suppressed limited access ethanol self-administration. Doses subthreshold in EtOH-naive rats were ineffective, while hypothalamic injections in fact led to increased EtOH drinking. We postulated that potentiated NPY signalling might act to suppress EtOH self-administration. The content of proenkephalin mRNA is decreased in rats subjected to ethanol deprivation. Thus, increases in DA function may contribute to increased ethanol intake associated with the alcohol-deprivation effect, a model of loss of control and relapse. Lastly, chronic ethanol exposure results in DA hypofunction that has been implicated as a neural basis for dysphoria and negative affect that accompanies early and late stages of withdrawal, and may motivate resumption of drinking. Although these data reveal a significant role for DA in ethanol-seeking and relapse, the literature suggests that direct manipulations of DA in the brain systems may prove effective by eliminating problematic side effects associated with chronic DA agonist or antagonist treatments. Indeed, indirect modification of DA activity via agents that act on other neuronal systems may prove effective by eliminating problematic side effects associated with chronic DA agonist or antagonist treatments. Therefore, the search for agonists that can selectively modify DA function is associated with complications that limit therapeutic promise. DA antagonists are likely to contribute to the established "anti-relapse" profile of this agent.

5-HT1A receptors are located in the caudate-putamen, the ventral tegmental area, and the substantia nigra, all of which are involved in alcohol preference and dependence and also in anxiety-like behaviors. (Supported by NIAAA grants and VA merit grant)
or SR 141716. It may be hypothesized that the CB1 and opioid receptors involved in the stimulating effects of ethanol have now revealed that the nAChR antagonists aiming at defining the nAChR subpopulation(s) involved in mediating the regions. We have now obtained both behavioral (using a two bottle free-choice drinking paradigm) and neurochemical (using microdialysis in awake freely moving rats) data indicating that the DA-activating and reinforcing effects of ethanol may in fact involve direct or indirect activation of central nAChR, especially those located in the ventral tegmental area. Studies using various nAChR antagonists aiming at defining the nAChR subpopulations involved in mediating the effects of ethanol have now revealed that the α7β2 or α5 (α-conotoxin MII), but not the α7β2 (dihydro-β-erythroidine) or α5 (methyllycaconitine), subunits could represent targets for developing new drugs for treatment of alcoholism.

THE ROLE OF CANNABINOID RECEPTORS IN THE CONTROL OF ALCOHOL INTAKE.

Different studies have evidenced that pharmacological blockade of the cannabinoid CB2 receptors receptors in rats tested under multiple procedures. Accordingly, CB2 receptor knockout mice showed a higher preference for alcohol in the two-bottle test than wild-type mice. These results suggest the involvement of the cannabinoid CB2 receptor in the neural circuitry controlling alcohol preference, intake and reinforcing properties. More recently, this lab tested the effect of the combination of myo-Inositol and α7β2 (dihydro-β-erythroidine) or α5 (methyllycaconitine), subunits could represent targets for developing new drugs for treatment of alcoholism.

INTRODUCTION AND REVIEW OF THE NEUROPATHOLOGICAL CHANGES SEEN IN ALCOHOL-RELATED ‘BRAIN SHRINKAGE’.
Clive Harper, Director of Neuropathology, Department of Pathology (DoP), University of Sydney, Sydney, Australia.

Studies during life and after death have also consistently revealed a reduction of the brain white matter volume in the cerebral hemispheres and cerebellum in uncomplicated alcoholic subjects. This has also been demonstrated in animal models. There are a number of ways in which alcohol is thought to impact on the central nervous system. Direct neurotoxicity, the toxicity of metabolic by-products (e.g. acetaldehyde), the effects of secondary nutritional deficiency states and chronic liver disease have all been proposed to cause damage. These toxic, metabolic and nutritional factors interact in a complex fashion. Technologies employed to demonstrate brain shrinkage include CT and MRI in vivo and quantitative neuropathology in autopsy studies. Partial reversibility of this change has also been reported in humans following significant periods of abstinence with concomitant improvement in cognitive function. From a structural point of view, the decrease in the volume of white matter could be due to a change in extracellular space, a change of the nerve fibers within the white matter, a combination of these or a change of the structure of the myelin. The extent of white matter loss and lifetime alcohol consumption, particularly in the cases that also have the Wernicke-Korsakoff syndrome (caused by thiamine deficiency) is not related to changes in hydration or changes in the chemical structure of the myelin. Select factors in the diet may influence white matter changes, and ethanol-preferring rats and indicate substantial brain structural and metabolic variability that may underlie individual differences in alcoholism's outward effects on brain structure.

S34.5

CROSS SECTIONAL AND LONGITUDINAL MR SPECTROSCOPY STUDIES OF CHRONIC ADULT ALCOHOLICS.
Michael Taylor, PhD, Department of Psychiatry, University of California, San Diego, CA, USA.

Long-term alcohol abuse results in neurological and cognitive deficits that are associated with localized neuro-pathological damage, including cerebral and cerebellar shrinkage. Alcohol abuse also results in changes in gene expression, which may underlie the regional selectivity and variability of brain damage as well as the adaptive response to chronic alcohol ingestion. In a previous study, DNA microarrays were used to analyze the expression of thousands of genes in the superior frontal cortex of control and alcoholic cases. The most striking changes in expression were in genes coding for myelin proteins that were decreased in alcoholics with respect to controls. A key question, however, was whether these changes in DNA expression are reflected in altered protein expression and whether changes in protein expression levels can be correlated with white matter volume loss. To address this, the expression of myelin basic protein (MBP), myelin protein zero (MPZ), myelin-associated glycoprotein (MAG), and lipoprotein were analyzed in paired samples of frontal white and gray matter from recently detoxified alcoholics and controls matched on age to determine if dissociation was evident. A significant 14.7% reduction in frontal white matter NAA of alcoholics was observed, while NAA levels in parietal white matter were similar in alcoholics and controls. Reductions in NAA were also associated with a longer drinking history. Alcohol withdrawal seizures have been believed to produce brain damage beyond that caused by alcohol itself. A recent study from our laboratory provided evidence of excitotoxicity mediated brain injury. In a study of alcoholics with a recent history of alcohol withdrawal seizure we found significantly lower NAA in frontal white matter regions of alcoholics with a history of withdrawal seizures compared to controls and their alcoholic counterparts without a history of withdrawal seizures. In a longitudinal study of alcoholics recovering from alcohol withdrawal, we have found evidence for improvement in NAA in alcoholics who maintain abstinence over compared to alcoholics who relapsed during the follow-up period. These data suggest at least partial recovery from alcohol induced damage, which is consistent with neurophysiological studies indicating recovery of brain function with continued abstinence.

S34.1

GENE AND PROTEIN CHANGES IN THE BRAINS OF ALCOHOLIC CASES WITH ‘BRAIN SHRINKAGE’.
Jo Lewloh, School of Molecular and Microbial Sciences., University of Queensland, Brisbane 4072, and Department of Pathology, University of Sydney, Sydney, Australia.

The overall expression pattern of the five proteins was compared and relationships between myelin protein levels and white matter volume, brain weight, age at death and post-mortem interval determined. Overall, the pattern of myelin protein expression differed between the cases. The differences in expression were most marked for PEP which was differentially expressed between brain regions and case groups. CNP was expressed more strongly in superior frontal cortex than in cerebellum. The expression levels of myelin proteins were correlated with white matter volume, brain weight and age at death. The expression of MBP and MPZ was differentially expressed between control and alcoholic cases. In general, the expression of myelin proteins increased in proportion to white matter volume in the control cases whereas the reverse was true for the alcoholics. An alteration in the structure of white matter in alcoholics may affect the propagation of action potentials in these brain areas.
S34.5
COGNITIVE FUNCTION AND ‘BRAIN SHRINKAGE’ IN LONG-TERM ABSENTENT ALCOHOLICS
George Fein and Bennett Landman, Neurobehavior Research, Inc., Corte Madera, CA, USA
Cognitive function and brain structure was studied in 47 abstinent alcoholics and age matched controls. The period of abstinence varied from 6 months to 13 years, with a mean of 6.9 years. As a group, the abstinent alcoholics exhibited normal cognition, except on the Bechira simulated gambling task, which examines individuals’ ability to see beyond short-term rewards to potential long-term consequences. The gambling task impairment existed independent of a positive family history for alcoholism, and was correlated with age, lifetime duration of alcohol use, depression symptom and depressive episode counts, but not any other psychological or cognitive variable or with abstinence duration. The T1-weighted brain images of these subjects, examined using voxel-based morphometry showed reduced gray matter in the general region of the ventromedial prefrontal cortex / anterior mesial temporal cortex. There was also a statistical trend toward reduced white matter volume in the abstinent alcoholics. Within the alcoholics, reduced gray matter was associated with lower socialization scores on the California Psychological Inventory. The results suggest that gambling task performance is negatively affected by chronic alcohol use, and that these effects are associated with reduced gray matter and do not resolve with long-term abstinence. We note that all of the alcoholics studied were able to achieve long-term abstinence in spite of these persistent impairments.

S35.1
INTRODUCTION: RECENT ADVANCES IN THE FORMATION AND MEASUREMENT OF NEUROACTIVE STEROIDS.
Robert H. Pardo and Loren H. Parsons, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, and Department of Veterans Affairs Medical Center, San Diego, CA 92161, USA
Neuroactive steroids are both endogenous and synthetic steroids and their derivatives, which rapidly alter CNS excitability. The endogenous steroids allopregnanolone and allopregna-1,5-dione-3,20-dioxoestradiol are the most potent endogenous positive modulators of GABA A receptors. These steroids have characteristic behavioral effects resembling those of ethanol in many respects, including amnestic, anticonvulsant, and sedative-hypnotic activity. Ethanol itself has similar effects at GABA A receptors, causing a preynaptic potentiation of the inhibitory effects of ethanol. Ethanol and some neuroactive sulfates, such as pregnenolone sulfate and DHEA sulfate, also interact with NMDA receptors. There is accumulating evidence that ethanol and neuroactive steroids have interactive neuropharmacological effects. This symposium begins with a brief review of several recent results which have great bearing on the formation and measurement of neuroactive steroids in the brain, as they relate to alcohol research. It has been accepted for over two decades that pregnenolone sulfate (PREGS) is the major neuroactive steroid present in the adult male rodent brain. Recently, several laboratories have failed to find any evidence of PREGS itself in extracts of these brain tissues. It is now presumed that some other esterified form of pregnenolone sulfate can be quantified by RIA or GC/MS. Subsequent speakers will focus on different approaches to an understanding of the effects of neuroactive steroids in the area of alcohol research.

S35.2
FETAL ETHANOL-INDUCED INCREASE IN BRAIN LEVELS OF PREGNENOLONE SULFATE
Fernando Valenzuela, Manuel Malem and Mario Carta, Department of Neurosciences, University of New Mexico Health Science Center, Albuquerque, NM, USA
Neuroactive steroids are modulators of neuronal function that may play important roles in neurellon development. Neuroteratogens may affect the physiological actions of these agents. Studies suggest that fetal alcohol exposure alters the behavioral effects of neuroactive steroids and that this may be due, in part, to a decrease in the sensitivity of NMDA receptors to the modulatory effects of neuroactive steroids. However, the cause of this reduction in the steroid sensitivity to NMDA receptors remains an open question. We determined whether chronic prenatal ethanol exposure altered some neuroactive steroid levels in the developing brain. Rat dams were exposed to: 1) 3% ethanol-containing liquid diet that produces peak maternal blood alcohol levels near the legal intoxication limit (~0.8 g/dl); 2) an isocaloric liquid diet containing maltose-dextrin instead of ethanol with pair-feeding; 3) rat chow ad isocaloric liquid diet containing maltose-dextrin instead of ethanol with pair-feeding; 3) adult rats ad libitum. Neuroactive steroid levels were assessed in offspring brains at different developmental time points by using radioimmunoassay or gas chromatography-mass spectrometry techniques. We found that prenatal ethanol exposure significantly increased pregnenolone sulfate (PREGS) levels in the fetal and neonatal brain but not in the fetal liver, plasma, and maternal blood, indicating that the effect of ethanol is not secondary to accumulation of peripherally produced steroids. Inductions of PREGS levels on neonate transgenic Drosophila were documented in slices from neonatal rats using patch-clamp electrophysiological techniques. We found that 50 microM PREGS increases the frequency of AMPA receptor-mediated miniature excitatory postsynaptic currents (127±33% vs. 100% in controls, n=7 slices) without affecting their amplitude. Unexpectedly, this effect was also observed in slices from postnatal day 3-5 but not older animals. PREGS did not affect the frequency of GABAergic miniature currents. These findings demonstrate that this neuroactive steroid increases the probability of glutamate release at synapses in an age-dependent manner. We postulated that the fetal ethanol-induced increase in PREGS levels would lead to premature synaptic maturation, leading to abnormalities in the development of neuronal networks. Supported by NIH Grant AA12684.

S35.3
GABAERGIC NEUROACTIVE STEROIDS ALTER ETHANOL SELF-ADMINISTRATION AND RELAPSE.
Patrick H. Janek, Hong Nie and T. Michael Gill, Ernest Gallo Clinic & Research Center, University of California at San Francisco, Emeryville, CA, 94609 USA
Previously, we found that the neuroactive steroid allopregnanolone (3α,5α-THP) enhances ethanol (EtOH) intake by rats. Recent evidence has been obtained to confirm that the effect of 3α,5α-THP is mediated by its effects at the GABA A receptor. In addition, we found that 3α,5α-THP induces relapse in abstinent rats, as indicated by reinstatement of previously extinguished responding for EtOH. This effect of 3α,5α-THP does not depend upon EtOH being made available during the reinstatement test. However, the relapse-inducing effect is specific to previous EtOH availability, as 3α,5α-THP has no effect in rats trained to self-administer sucrose. Pretreatment with a related neuroactive steroid, epipregnanolone (3α,5β-THP), attenuates the reinstatement effects of 3α,5α-THP and reduces the reinstatement produced by either conditioned cues or EtOH delivery. 3α,5α-THP also reinstates EtOH-seeking behavior in C57 mice and this effect is attenuated by 3α,5β-THP. The results suggest that the endogenous steroid 3α,5α-THP alters EtOH self-administration, as well as EtOH-seeking when EtOH is not present. In addition, 3α,5β-THP reduces 3α,5α-THP and attenuates 3α,5α-THP-induced reinstatement in vivo by antagonizing 3α,5β-THP actions at the GABA A receptor. These findings raise the possibility of a potential role for 3α,5β-THP-like compounds in the treatment of relapse for EtOH.

S35.4
NEUROACTIVE STEROID MODULATION OF ETHANOL INTAKE PATTERNS IN C57BL/6 MICE.
Deborah A. Fino, Tamara J. Phillips, Naomi Yoneyama, Stephen T. Hansen and Matthew M. Ford, Portland Alcohol Research Center, Department of Veterans Affairs Medical Research and Development of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR, 97239 USA
Recent findings in the laboratory indicate that the neurosteroid allopregnanolone (3α,5α-THP) significantly increased limited access home-cage ethanol (EtOH) preference drinking in male but not female C57BL/6 (B6) mice. Since 3α,5α-THP is a very potent positive modulator of GABA A receptors, and modulation of GABA A receptors has been shown to alter EtOH intake, the present studies were conducted to assess the impact of several neurosteroids with different pharmacological profiles at GABA A receptors on limited-access ethanol consumption patterns. Two allosteric agonists (3α,5α-THP and pregnenolone, 3α,5β-THP), an allosteric partial agonist (epipregnanolone, 3α,5β-THP) and a non-competitive antagonist (pregnenolone sulfate, PREGS) were tested. Male B6 mice were acclimatized to a reverse light/dark schedule and permitted 2-hr access to lickometer chambers containing either 10% ethanol (EtOH) bottle and one water bottle at the beginning of the dark phase. Drinking patterns were monitored with lickometer circuits attached to each fluid sipper. A saccharin fading procedure was used to establish stable baseline 10E intake. All mice were habituated to vehicle injections (20% w/v 2-hydroxypropyl-γ-cyclodextrin) for 4-5 days, and then received 3-day injection regimens of either vehicle or neuroactive steroid (10 mg/kg 3α,5α-THP, 3α,5β-THP 10 mg/kg or 50 mg/kg PREGS) immediately prior to the drinking session. During the initial 20 min of access, 3α,5α-THP elicited a robust increase (+109%) in 10E lick responses, while 3α,5β-THP transiently elevated 10E licks (+18%), and PREGS elicited only a moderate elevation in this measure (+353%), when compared to vehicle controls. In contrast, 3α,5β-THP decreased 10E licks (-38%) in the first 20min. These initial lick responses were associated with reductions in the latency to first 10E bout in 3α,5α-THP, 3α,5β-THP, and PREGS-treated mice and an increase in the latency to the first 10E bout in the 3α,5β-THP-treated mice. Consistent with earlier findings, neurosteroids with various GABA A receptor pharmacological profiles differentially modulate EtOH intake patterns. Supported by NIH Grants AA09945, AA10760, DA07262, and the Department of Veterans Affairs.
S36.1 KUPFFER CELL-DERIVED MEDIATORS INVOLVED IN ALD: REACTIVE OXYGEN AND NITROGEN SPECIES.

**GE Aruffo**, Department of Pharmacology and Toxicology and the James Graham Brown Cancer Center, University of Louisville Health Sciences Center, Louisville, KY, USA

One mechanism by which alcohol is proposed to mediate its liver damage is through oxidative stress. Reactive oxygen and nitrogen species can be products of normal cellular metabolism, but overproduction of these reactive species can lead to tissue damage. Kupffer cells are a potential major source of oxidant production during ALD. Upon priming, Kupffer cells produce several kinds of proinflammatory mediators, including reactive oxygen and nitrogen species (ROS/RNS) as well as cytokines. Kupffer cells are critical for progression of alcoholic liver disease (ALD). On the other hand, it has been well documented that women are more susceptible to ALD. The mechanisms underlying this phenomenon have not been fully understood. It has been shown that plasma endotoxin levels were significantly higher in females than in males after exposure to ethanol. These phenomena can be explained by the action of estrogens, which increases gut permeability and endotoxin concentrations in the portal vein. Moreover, estrogens increase CYP2B4 expression in Kupffer cells, as well as LPS binding protein (LBP) production in hepatocytes. As a consequence, Kupffer cells in rats treated with estriol exhibited sensitization to LPS, thereby increasing production of toxic mediators, the culprits of alcohol liver damage. It is thus postulated that estrogens induced increase in Kupffer cell sensitization to LPS accounts for, at least in part, the mechanism of the gender difference in ALD.

S36.2 KUPFFER CELL-DERIVED MEDIATORS IN ALD: TNFα

**MR McMullen, LE Nagele**, Case Western Reserve University, Cleveland OH, 44106-4896, USA

Tumor necrosis factor (TNFα) is considered a critical factor in the progression of alcoholic liver injury. TNFα can cause hepatocellular damage, via the generation of superoxide anion by parenchymal cells and increasing expression of interleukin 8, which regulates neutrophil chemotaxis. TNFα can also promote the activation of macrophages by lipopolysaccharide (LPS) during ethanol exposure. TNFα production by KCs in response to LPS. As expected, pioglitazone agonist, has been shown to reduce TNF-α production by KCs through decreasing TNF-α mRNA. Thalidomide thus prevents liver damage caused by chronic ethanol exposure through not only suppression of TNF-α production but also down regulation of LPS sensitization to LPS. However, thalidomide, which is an immunomodulatory agent, has been shown to reduce TNF-α production by KCs in response to LPS. As expected, pioglitazone prevents alcoholic liver injury through abrogation of Kupffer cell sensitization to LPS. Thus, given that thalidomide and pioglitazone have unique mechanisms of action, there appears to be a strong possibility that this type of drugs will prove beneficial to patients with severe alcoholic liver injury.