S28.4

ETHANOL DISRUPTS IMMATURE NETWORK NEURONAL ACTIVITY DRIVEN BY EXCITATORY ACTIONS OF GABA.

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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 neuroteratogenic effects of ethanol. In this brain region, there is a developmentally-regulated pattern of network-driven electrical activity known as the giant depolarizing potentials (GDPs). In immature neurons, $GABA_A$ receptors are excitatory due to a shift in Cl^- equilibrium potential towards more depolarized potentials. The excitatory actions of GABA generate these GDPs, which are associated with large oscillations in intracellular calcium. These oscillations contribute to activity-dependent modulation of neuronal growth and synaptogenesis by, for example, increasing DNA synthesis and inducing neurotrophic factor expression. Therefore, alterations in this pattern of synaptic activity could have profound effects on the normal maturation of hippocampal circuits. Using acute hippocampal slices from neonatal rats and patch-clamp electrophysiological techniques, it was discovered that ethanol dramatically disrupts GDPs in the CA3 region. Short-term (8 min) application of ethanol (50 mM) increased GDP frequency by 100% ± 30 (n=12) The increment in GDP activity is likely mediated by an increase in action potential-dependent GABA and glutamate release given that we found that ethanol (50mM) significantly increased the frequency of $GABA_A$ receptor-mediated spontaneous 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 neurodevelopmental disorders and fetal alcohol syndrome. Supported by NIH grant AA12684.

S29.1

PHARMACOLOGICAL REVERSAL OF CYCLED WITHDRAWAL- OR STRESS-SENSITIZED WITHRAWAL ANXIETY AND ENHANCED ETHANOL DRINKING.

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These investigations focused on conditions that may link stress, anxiety, and relapse. Previous data demonstrated repeated ethanol withdrawal (EW)-induced sensitization of anxiety-like behavior in Sprague-Dawley (SD) rats tested in the social interaction (SI) and elevated-plus maze tests. Data also showed that alcohol-preferring P-rats had lower thresholds for elicitation of this response and that relatively short-term cycles of voluntary drinking (5-day cycles) and EW could elicit a deprivation-like effect that persisted 24-48 hr without a change in anxiety-like behavior. P rats exposed to restraint stress (60 min) during the 1st and 2nd deprivation/EW periods had comparable early (24-48 hr) deprivation-induced increase in drinking. However, the drinking persisted in these stressed animals. Furthermore, these rats exhibited anxiety-like behavior in the SI test during EW from the voluntary ethanol drinking after the third cycle. Thus, stress increased both drinking and EW-induced anxiety-like behavior in the P rats. Furthermore, when two stress exposures were substituted for EW experiences in SD or P-rats, sensitized anxiety-like behavior occurred following a future EW even though the animals had not experienced multiple EWs. This effect did not appear to be related to circulating levels of corticosterone. IP administration of non-peptide CRF type-1 receptor antagonists to SD or P-rats during early EWs blocked sensitized anxiety-like behavior that was assessed during a future untreated EW. ICV treatment also blocked the sensitization of anxiety-like behavior in SD rats while systemic administration prior to stress episodes in P-rats blocked the sensitizing effect of stress on anxiety-like behavior. When given prior to restraint stress, a benzodiazepine receptor antagonist or a serotonin $5\text{-}HT_{1A}$ receptor agonist were effective in reducing the anxiety-like behavior and the elevated duration of alcohol drinking, but not that due to deprivation alone. In contrast, antagonists for opiate, $5\text{-}HT_{2C}$, or dopamine receptors were ineffective or only partially effective. These data highlight the importance of the pattern of ethanol exposure in the severity of EW-type symptoms and the contribution of stress, receptor, and animal strain in the effects of multiple EW and stress on anxiety-like behavior and voluntary drinking.

S29.2

ALCOHOL CRAVING AND RELAPSE IN RATS GENETICALLY SELECTED FOR HIGH ALCOHOL PREFERENCE.

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The alcohol-preferring (P) line of rat has been well characterized both behaviorally and neurobiologically and satisfies a 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 EtOH deprivation-access has been examined in P rats. Following a single EtOH deprivation period; (1) an ADE can be observed following a deprivation period of 5 weeks (operant conditions) or 8 weeks (24-hr free-choice conditions), (2) there is typically a two-fold increase in EtOH intake (10 g/kg/day intake, 350 operant responses for EtOH), (3) the effects are transient (e.g., lasting less than 3 days). However, following multiple cycles of EtOH deprivation-access in P rats; (1) the magnitude of the ADE is increased (>16 g/kg/day, > 800 operant responses for EtOH), (2) the duration of the ADE is increased (6 consecutive days with > 12 g/kg/day EtOH intake, 5 consecutive sessions of > 550 operant EtOH responses), (3) there is a marked shift in preference for higher concentrations of EtOH (from 10% to 30% EtOH), (4) there is an increase in the reinforcing properties of EtOH (the breakpoint determination for EtOH is 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 conditional response in the absence of the previously trained reward following a period of rest after extinction) model. Recent data has indicated that the expression of an EtOH PSR by P rats can be mediated by numerous factors that influence human relapse and craving; adolescent EtOH drinking, EtOH priming, the presence of an EtOH odor, exposure to repeated cycles of EtOH deprivation-access all enhance the expression of an EtOH PSR by P rats. Novel data has indicated that the opioid antagonist naltrexone can inhibit the expression of an EtOH PSR and ADE by P rats. Overall, the results indicate that there are current valid animal models of both relapse drinking/excessive alcohol intake 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, AA10721)

S29.3

EXPOSURE TO STRESS INCREASES DOPAMINERGIC BURST FIRING IN AWAKE RATS.

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Exposure to stress may contribute to relapse in alcoholics and addicts. While it is clear that extracellular dopamine (DA) levels increase as a result of stress, the electrophysiological responses of single midbrain neurons to stress are unknown. Distributed activity of ventral tegmental area (VTA) neurons was recorded over resting and stressful conditions to determine how neuronal activity contributes to the stress response. Arrays of stainless steel electrodes 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 CB_1 AND $GABA_B$ RECEPTORS IN THE CONTROL OF RELAPSE-LIKE DRINKING IN ALCOHOL-PREFERRING SP RATS.

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Alcohol deprivation effect (ADE) is defined as the transient increase in alcohol intake which occurs in several animal species after a period of alcohol abstinence; ADE has been proposed to model the loss of 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 Sardinian alcohol-preferring (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 CB_1 receptor antagonist, SR 141716, suppressed ADE in sP rats, suggesting the involvement of the cannabinoid CB_1 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 ADE in sP rats exposed to a single alcohol concentration (10%, v/v). These results suggest that SR 141716 and naloxone exert a concomitant and reciprocally potentiating inhibitory action on the neural system(s) controlling ADE in sP rats. Also the $GABA_B$ receptor agonist baclofen has been found to suppress ADE in sP rats, suggesting that the $GABA_B$ receptor is a further player in the neural substrate mediating ADE. More recently, this lab investigated the effect of baclofen on ADE in sP rats exposed to multiple alcohol concentrations (0, 10, 20 and 30%, v/v). Acute baclofen completely suppressed the extra-intake of alcohol as well as of the shift in alcohol preference, suggesting that baclofen blocked both the quantitative (amount of alcohol consumed) and qualitative (preference for the most concentrated solutions) aspects of the increased demand for alcohol associated with alcohol deprivation in sP rats.

S29.5

STRESS-INDUCED ETHANOL DRINKING IN $CB1^{-/-}$, POMC AND PENK KNOCKOUT MICE.

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Stress is known as one of the main causes of relapse in human alcoholics. Although the opioid receptor antagonist naloxon is currently the only drug used for relapse prevention, 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, PENK and CB1 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. $CB1^{-/-}$ animals and wild type mice had a similar preference for alcohol. On the other hand, $PENK^{-/-}$ and $POMC^{-/-}$ mice showed no preference for ethanol. The wild type animals showed changes in alcohol-drinking behaviours in each of the stress models. In contrast, none of the stressors had any effect on ethanol consumption in $CB1^{-/-}$ animals. In POMC mice only swim stress had an effect on alcohol drinking. Surprisingly the effect was different in the two sexes: we found a decrease in alcohol preference in males and an increase in females. $PENK^{-/-}$ mice showed a decrease in alcohol drinking after the foot shock stress. Our results clearly indicate that the CB1 receptor plays an important role in stress-induced relapse-like alcohol drinking. In our animal models, this effect was so striking that a further evaluation of CB1 antagonists for the therapy of alcohol addiction in humans seems warranted. The opioid peptides seem to play more restricted role in the modulation of specific stress responses.

S30.1

EFFECTS OF ALCOHOL AND BENZODIAZEPINES ON BRAIN METABOLISM IN ALCOHOLICS AND HEALTHY VOLUNTEERS: [^{18}F]FDG-PET-STUDIES.

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To further investigate the functional role of GABAergic systems for the neurobiology of alcoholism, we applied functional imaging (PET, EEG) with pharmacological challenges to healthy controls, detoxified alcoholics and subjects at risk for alcoholism. 10 male alcoholics, 10 subjects at risk for alcoholism (sons of alcoholics) and 18 healthy male controls (age 23-57 years) underwent two 18-FDG PET scans in a randomized single-blind study (placebo vs. lorazepam 30μg/kg body weight i.v.). Another sample of 20 healthy volunteers underwent three18-FDG PET scans to test the effects of alcohol on brain metabolism at various stages of alcohol influx (placebo vs. alcohol influx vs. alcohol elimination, 40 g alcohol i.v.). Data analysis was performed on voxel-by-voxel basis using SPM. While lorazepam in controls reduced regional cerebral glucose metabolism (rCGM) in occipital cortex and in thalamus, alcohol stimulates rCGM in anterior parts of the striatum, frontal cortex, and in diencephalic regions. RCGM was increased in the anterior cingulate and right prefrontal cortex during alcohol influx compared to alcohol elimination. RCGM in the baseline condition revealed a significantly higher metabolic activity in the bilateral caudate nucleus in detoxified alcoholics compared to normals; this difference was reduced after administration of lorazepam. Data are currently being analyzed with regard to differential sensitivity to lorazepam in the various populations. At this point our data indicate that the alcohol effects by far exceed those observed under lorazepam. The results obtained in alcoholics demonstrate that even after at least 4 weeks of abstinence there is a persisting elevation of metabolic activity in a region that is highly responsive to alcohol. This hyperactivity can be partially normalized by lorazepam administration. Together with results obtained by Heinz et al. (this symposium), these results point to complex disturbances of multiple transmitter systems involved in human reward.

S30.2

IMAGING GABA$_A$ RECEPTOR SUBTYPES WITH PET.

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We have used PET and SPET to study the GABA-benzodiazepine receptor [GABA-BDZR] in abstinent alcohol dependent patients. Using [^{123}I]iomazenil SPET, alcohol dependence was shown to be associated with reduced levels of the GABA-BDZR, mostly in the frontal cortex[1]. We then studied the function of the GABA-BDZR in alcohol dependence by measuring the pharmacokinetics and pharmacodynamics of the GABA-BDZR using a midazolam challenge with [^{11}C]-flumazenil PET and EEG. We found that alcohol dependence was associated with a trend towards increased midazolam occupancy and no change in EEG beta-power response but a 50% reduction in time spent asleep compared to control subjects. One explanation of these different dynamic responses is that these functions are mediated by specific GABA-BZDR differentially affected by alcohol. Recently particular functions have been ascribed to certain GABA-BDZR subtypes so it is now timely to characterize tracers used for neuroimaging. In order to study GABA-BDZR subtypes, we determined the subtype selectivity of [^{11}C]flumazenil and [^{11}C]Ro15 4513. We have shown that brain uptake of [^{11}C]Ro15 4513 in rat and in man has a primarily limbic distribution consistent with labelling of the GABA-BDZR containing the α5 subunit[2]. Displacement studies in the rat showed that only benzodiazepines with high affinity for the α5-containing subtype reduced [^{11}C]Ro15 4513 uptake to non-specific levels. By contrast, [^{11}C]flumazenil appears to be a more non-selective tracer. The α5-containing subtype of the GABA-BDZR has recently been shown to be involved with mediating alcohol reinforcement. We have acquired [^{11}C]Ro15 4513 images from abstinent alcohol dependent subjects for comparison with controls and analysis is currently underway. [^{11}C]Ro15 4513 will allow us to determine the role of the α5 subtype of the GABA-BDZR in a variety of disorders *in vivo*

. This work was funded by the Wellcome Trust and an MRC Programme Grant. [1]Lingford-Hughes A et al., B J Psych, 173, 116-122 [2]Lingford-Hughes A et al., J Cereb Blood Flow Metab. 22(7):878-89.

S30.3

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

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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 D_2 receptors. Neuroendocrinological challenge studies suggested that delayed recovery of D_2 receptor sensitivity after detoxification is correlated with a high risk for relapse. Prolonged D_2 receptor dysfunction in the ventral striatum may bias the brain reward system towards excessive attribution of incentive salience to alcohol-associated stimuli. We used a multimodal imaging approach with the radioligand [^{18}F]desmethoxyfallypride ([^{18}F]DMFP) and positron emission tomography (PET) as well as functional magnetic resonance imaging (fMRI) to measure the association between D_2-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 the medial prefrontal cortex and striatum in detoxified male alcoholics compared with healthy males. Alcoholics displayed a lower availability of dopamine D_2-like receptors in the ventral striatum, which was associated with increased cue-induced activation of the medial prefrontal cortex and anterior cingulate as assessed with fMRI and with the severity of alcohol craving. These 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 BY VOLUMETRIC MRI.

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Hippocampal structures are involved in learning, memory and conditioning. Hippocampal volume changes have been reported in Alzheimers disease, frontotemporal dementia, epilepsy and schizophrenia. These conditions are also characterized also by behavioural problems. Animal and human studies suggest different functional organization within the hippocampus along its longitudinal axis. Identification of damage that is localized to certain parts of the hippocampus may provide in vivo evidence about the pathological basis from a given 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 Cloninger. The type 2 alcoholic subjects were also violent offenders with antisocial personality disorder, derived from a forensic psychiatric sample. There was tendency towards decreased volumes with aging and also with the duration of alcoholism in the type 1 alcoholic subjects. Surprisingly, there was a significant positive correlation between the right hippocampal volume and age in the type 2 alcoholics. In a subgroup of habitually violent offenders with antisocial personality disorder and type 2 alcoholism 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 hippocampi bilaterally. In our preliminary study MRI was used to examine amygdaloid volumes in violent offenders which were divided into two groups according the Psychopathy Checklist Revised. The high psychopathy personality traits offenders had significantly smaller amygdaloid volumes on the right compared with the offenders with low psychopathy traits. Our data support the view that lesions of the dorsal hippocampus impair acquisition of conditioned fear. Data provide biological evidence that the type 2 characteristics might represent a primarily antisocial personality disorder than alcoholism itself.

S30.5

BRAIN GROWTH AND SHRINKAGE.

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Brain size during adulthood is a function of two processes: brain growth and brain shrinkage. Brain growth determines the maximum size an individual's brain achieves, usually during early adolescence. Brain shrinkage begins late in the second or early in the third decade of life and continues over the remaining lifespan. The intra-cranial volume (ICV) provides an accurate estimate of maximum brain growth while ratio of brain volume to ICV provides a good measure of how much the brain shrinks from its maximum size. Thus, by measuring ICV and brain volume we are able to determine how much pre-morbid differences in brain growth and brain shrinkage during adulthood contribute to the differences in brain volume between alcoholics and healthy non-alcoholic controls. Alcoholics (male and female) have significantly smaller ICV than healthy non-alcoholics. This suggests that alcoholics have less brain growth than non-alcoholics and that this may be a pre-morbid risk factor for alcoholism. Among White male alcoholics and non-alcoholics (the only homogeneous group for which we had a large sample) ICV, independently of age, significantly predicted verbal IQ, but not performance IQ. Verbal IQ did not decrease with age in either group. These results suggest that both verbal IQ and ICV are modestly related and low values of both may be risk factors for the development of alcoholism. Among alcoholics but not controls increasing brain shrinkage, independently of age, predicted performance IQ. Performance IQ decreased and brain shrinkage increased with age among alcoholics, and both were significantly different from non-alcoholics. It appears that brain shrinkage is associated with a decrease in performance IQ as alcoholics age, and that both brain growth and shrinkage independently contribute to differences in brain volume between alcoholics and non-alcoholics. We also compared ICV and brain shrinkage among male alcoholics with differing co-morbid substance abuse. We found no significant differences in ICV or brain shrinkage among alcoholics with alcohol dependence alone, alcohol dependence plus cocaine or cannabis dependence. We also found that years of heavy drinking, independently of age, predicted brain shrinkage among all three groups. Among individuals with alcohol dependence it appears that cumulative alcohol exposure and is more important than other drug use as a cause of brain volume loss during adulthood. Sponsored by the NIAAA Intramural Research Program.

S31.1

DEVELOPMENT OF ADDICTION: THE STRESS DAMPENING HYPOTHESES TESTED WITH BIOLOGICAL FACTORS IN SUBJECTS AT RISK.

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Concerning the question of the development of alcohol dependence ethanol challenge studies are of high interest in subjects at risk (sons of male alcoholics). This FH+ group (family history) is considered to bear a heightened genetic risk for addictive behavior and is much more likely to exploit the autonomic stress response dampening effect of alcohol than FH- subjects. Method: In our alcohol (vs. non-alcohol) challenge study (0.7 ‰ blood ethanol level) adult sons, daughters and siblings of male alcoholics were recruited and matched with controls (104 subjects at risk and 51 controls). A mental arithmetic condition in addition to aversive and rewarding reaction time paradigms was employed. For each subject psychophysiological parameters and the course of neuroendocrine hormones was recorded. Results: Stress dampening effects emerged in female FH+ and FH- participants (heart rate). Interestingly, cortisol dampening phenomena did emerge in male FH+ children only but not in female FH+ and FH- participants. Conclusion: Our findings suggest heart rate and cortisol are separate and distinct indices of alcohol stress-response dampening. Further it might be possible that different thresholds for manifestations of alcohol dampening effects exists. Our results implicate that gender, genetic load, BAC and impact of the stress paradigm have substantial influence on psychophysiological and endocrine results.

S31.2

MOOD, MINOR NEGATIVE EVENTS, AND ALCOHOL CONSUMPTION: DAILY LIFE INVESTIGATIONS USING THE EXPERIENCE SAMPLING METHOD.

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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 or hours. 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.

S31.3

ALCOHOL ATTENUATES PSYCHOSOCIAL STSRESS RESPONSE BUT NOT STARTLE REFLEX MODULATION IN SONS OF ALCOHOL-DEPENDENT FATHERS.

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We tested the hypothesis that a family history of alcoholism is associated with more stress-induced hypothalamic-pituitary-adrenal activation 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) controls (aged 18-26 years) 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 secretion 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.

S31.4

DRINKING TO COPE IN INDIVIDUALS WITH SOCIAL ANXIETY.

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The self-medication hypothesis regarding alcoholism states that because some individuals experience relief from aversive emotional states by using alcohol (ie, stress-response dampening), alcohol is reinforcing for these individuals, and so they are at risk to drink excessively and develop alcohol problems. Social anxiety disorder, an Axis I anxiety disorder characterized by excessive fear of scrutiny and embarrassment, is an especially good model to understand the practice of alcohol self-medication and subsequent risk of alcoholism. Approximately 20% of individuals with social anxiety disorder develop alcoholism; in almost all cases, alcoholism occurs a decade or more after the onset of anxiety symptoms, effectively removing the issue of an alcohol-induced anxiety disorder. In addition, data from the National Comorbidity Survey show that self-medication with alcohol is significantly more prominent in individuals with social anxiety disorder compared to other anxiety disorders. Data will be presented from controlled studies comparing drinking practices of individuals with and without social anxiety regarding (1) the propensity to drink to cope in general, (2) in which situations drinking to cope is more common, and (3) the self-reported degree of anxiolysis from alcohol. While individuals with social anxiety have a greater risk of future alcohol problems, only some use alcohol to cope, and only in some social situations, despite the fact that all have high levels of anxiety to be relieved. This fact will be discussed in terms of how future research studies can be conducted to better study stress-response dampening in this population and better predict who is at greatest risk for future alcohol problems in this at-risk group.

S31.5

INDIVIDUAL DIFFERENCES IN ALCOHOL OUTCOME EXPECTANCIES ARE PREDICTIVE OF DRINKING TO MANAGE ANXIETY.

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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.

S32.1

ETHANOL AND NMDA RECEPTOR COUPLING TO ERK SIGNALING.

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NMDA receptors regulate neuronal development, synaptic plasticity, learning and memory, and neuronal survival and death, and also appear to be important sites of action of ethanol in the brain. The extracellular signal-regulated kinase (ERK) cascade is a prototypical growth factor signaling pathway implicated in neuronal plasticity and survival. We have previously shown that NMDA receptor coupling to ERK activation is bidirectionally regulated. This bidirectionality is mediated by NMDA receptor coupling to opposing stimulatory and inhibitory pathways. Surprisingly, recent evidence suggests that the inhibitory pathway that acts to shut-off the NMDA stimulatory pathway does not inhibit BDNF activation of ERKs. Thus, NMDA receptor-coupled ERK modulatory pathways appear to be compartmentalized with spatially distinct populations of NMDA receptors. While NMDA receptor signaling plays an important role in regulating BDNF expression, NMDA and BDNF coupling to ERK activation appears to function independently. We have also recently observed that prolonged ethanol exposure of cultured neurons leads to a selective increase in synaptic NMDA receptors without altering extrasynaptic receptors. This effect occurs through activity-dependent homeostatic processes that restore stability to the neuronal network in response to the inhibitory effects of ethanol. We propose a molecular model in which ethanol-induced increases in synaptic NMDA receptors enhance BDNF expression (via enhanced NMDA receptor-ERK-CREB signaling) without directly altering BDNF activation of ERKs.

S32.2

ETHANOL MODULATION OF CREB: ROLE IN NEUROGENESIS.

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The transcription factor CREB regulates expression of genes that promote neuron survival, including BDNF and other trophic factors. Both CREB and adult neurogenesis have been implicated in neuronal plasticity and memory that play role in addiction and drug abuse. We examined the effects of 4-day binge ethanol administration on PCREB and NPY immunoreactivity (PCREB-IR and NPY-IR) as a target gene regulated by CREB as well as neurogenesis within rat hippocampus. Binge ethanol treatment decreased PCREB-IR and NPY-IR as well as neurogenesis and caused hippocampal neurodegeneration. In contrast, ethanol withdrawal increased PCREB-IR (10 fold) and NPY-IR ($\approx$ 60 fold) and neurogenesis while no neurodegeneration was found. To further examine the modulation of CREB by acute or chronic ethanol we used hippocampal-entorhinal cortex (HEC) brain slice cultures and EMSA assay to determine CREB or NF-γB binding activity. Ethanol (25-150mM-24hr) dose-dependently reduces CREB, but increases NF-γB binding, which enhances neuronal vulnerability to TNFα-glutamate neurotoxicity. Rolipram, an inhibitor of phosphodiesterase IV, increased CREB and reduced NF-γB binding while reducing chronic ethanol withdrawal neurotoxicity and increasing neurogenesis. Taken together, these results suggest that ethanol modulates nuclear signaling pathways that mediate changes in gene expression and neuronal vitality, which may contribute to ethanol dependence and withdrawal. (Supported by NIAAA).

S32.3

SEROTONIN DYSFUNCTION AND ALCOHOL PREFERENCE IN BDNF DEFICIENT MICE.
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Brain-derived neurotrophic factor (BDNF) promotes serotonergic (5-HT) neuro-transmission and the structural plasticity of 5-HT neurons in the adult brain. Heterozygous BDNF (+/-) mice have forebrain BDNF mRNA and protein levels that are 50% of that of wild-type (WT) mice. These BNDF deficient mice display abnormalities in 5-HT neurotransmission, develop decrements in 5-HT innervation of the forebrain, and exhibit enhanced inter-male aggressiveness. Disturbances of 5-HT neurotransmission in brain have been implicated in the pathogenesis and maintenance of alcoholism, as well as in behavioral patterns relevant to alcoholism, i.e. impulse control disorders, aggression, negative mood and a low response to alcohol intake. The 5-HT1A receptor, located on 5-HT cell bodies in the brainstem, functions as the somatodendritic autoreceptor and plays a key role in regulating 5-HT neurotransmission. The 5-HT1A receptor is also located post-synaptically and is present in high density in cortical and limbic structures. 5-HT1A receptors have been implicated in aggressive behavior and impulse control disorders, as well as alcohol abuse. We have examined in BDNF (+/-) mice alcohol drinking behavior, as well as central 5-HT1A receptor function at the level of 5-HT1A receptor-G protein interaction. BDNF (+/-) mice displayed increased ethanol intake in the two-bottle choice procedure. We measured in the brains of alcohol-naïve mice [^{35}S]GTPγS binding stimulated by the 5-HT1A receptor agonist 8-OH-DPAT. The capacity of 5-HT1A receptors to activate G proteins was attenuated in dorsal and median raphe. A decrease in 5-HT1A autoreceptor function would be expected to result in a decrease in alcohol intake, given the well-established inverse relationship between 5-HT neurotransmission and alcohol consumption. However, the capacity of 5-HT1A receptors in cortical and limbic structures to activate G proteins was also decreased in BDNF (+/-) mice, indicating that postsynaptic 5-HT1A receptor function is diminished in forebrain areas. These mice also show in several forebrain regions a blunted c-Fos induction by the specific 5-HT releaser dexfenfluramaine. Thus, 5-HT neurotransmission appears be attenuated in these animals. In conclusion, attenuated 5–HT1A receptor function may by a factor in alcohol abuse, as well as aggressive behavior and impulse control disorders.

S32.4

BDNF GENE AND RELATED SIGNALING: ROLE IN ANXIETY AND ALCOHOL DEPENDENCE AND PREFERENCE.
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Neurotrophins play diverse roles in regulating neuronal functions, structure and growth during development and adulthood. One of the neurotrophins, brain-derived neurotrophic factor (BDNF) is the cAMP-responsive element binding protein (CREB) target gene, which plays a crucial role in synaptic plasticity in the brain. We investigated the role of amygdaloid BDNF signaling in alcohol dependence and preference and also in anxiety-like behaviors in rats. It was found that chronic ethanol treatment had no effect on protein and mRNA levels of BDNF as well as phosphorylation of extracellular-regulated protein kinases (ERK1/2) in the various structures of amygdala. However, ethanol withdrawal (24 hrs after 15 days of ethanol exposure) produced significant reductions in the protein and mRNA levels of BDNF as well as phosphorylation of ERK1/2 in the central, and medial but not in basolateral amygdala. These results suggest the possibility that the decreased expression and function of BDNF in the central and medial amygdala may be associated with the process of ethanol dependence such as development of anxiety-like behaviors during withdrawal, and this may be promoting the continued consumption of ethanol. We tested these possibilities by altering the expression of BDNF in various amygdaloid structures using anti-sense strategy and then measuring anxiety and alcohol drinking behaviors in normal rats. It was observed that BDNF anti-sense oligodeoxynucleotides (ODNs) infusions into the central or medial but not into basolateral amygdala provoked anxiety-like behaviors and also increased the alcohol preference in rats, which was prevented by BDNF co-infusion. The mRNA and protein levels of BDNF as well as protein levels of phospho-ERK1/2 and phospho-CREB were decreased by BDNF anti-sense but not by sense ODNs infusions, which were restored to normal following BDNF co-infusions. These novel results implicate that decreased BDNF function in the central and medial but not in the basolateral amygdala is involved in alcohol preference and dependence and also in anxiety-like behaviors. (Supported by NIAAA grants and VA merit grant)

S32.5

BDNF AND CREB: ROLE IN ETHANOL INDUCED NEURONAL DAMAGE
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We have previously demonstrated that the cellular cAMP-signaling is impaired in alcoholics: quantitative reduction of both type 1 adenylyl cyclase (AC1) and Gsα protein. Since the cAMP responsive element binding protein (CREB) helps to modulate long-term potentiation and synaptic plasticity, it is plausible that ethanol affects the CREB-induced gene transcriptions, and affect neural differentiation/development. In this study, we focused on the mechanism of the ethanol induced cell damage and the role of the neurotrophic factor signaling on the effect of ethanol using SH-SY5Y cells, neurons and neural progenitor cells (NPCs) from rat brain. The undifferentiated SH-SY5Y cells were more susceptible to ethanol than the retinoic acid (RA)-differentiated cells. Both the basal amount of secreted BDNF and CREB activity were larger in differentiated SH-SY5Y cells than in undifferentiated cells. We tested the activity change of ERK and MSK-1 which are known to be activated by the trophic factors followed by the activation of CREB. We next investigated the ethanol's effects on neuronal differentiation and development in neural progenitor cells. Ethanol inhibited neuronal differentiation of NPCs in the low concentrations that did not affect neuronal survival. Besides promoting effects on neuronal differentiation by BDNF were suppressed by the treatment of ethanol. To investigate the effects of ethanol on the regulation of transcriptional change, we analyze the DNA binding of neuron-restrictive silencer factor (NRSF) of NPCs which is known to be an important factor to promote NPCs to neurons. These results suggest that ethanol-induced downregulation of cAMP system affects CREB activity and BDNF production in the neurons, which then induce to the disruption of neuronal defense mechanism. Furthermore, the ethanol-induced reduction of neurotrophic factor signaling in the NPCs might be related to the inhibition of the differentiation of NPCs to the neurons. The events in the study using our cellular models here must be presented for the brain damage mechanisms induced by ethanol.

S33.1

CENTRAL NPY-Y2 RECEPTORS AS A TREATMENT TARGET FOR ALCOHOLISM.
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NPY, a 36 aa peptide with high brain expression has potent anti-stress actions, indicating that altered NPY function might contribute to long term phenotypes of negative affect. A role for NPY in regulation of EtOH intake was proposed by Ehlers et al on the basis of pharmacological similarities, and by Thiele at al on the basis of inverse correlation between NPY expression and EtOH drinking in mutant mice with deletion of the preproNPY gene, and NPY overexpressors, respectively; while Carr et al. found a major QTL for alcohol preference in P rats, which mapped to the NPY gene. It was postulated that potentiated NPY signalling might act to suppress EtOH drinking. However, icv microinjection studies failed to demonstrate this, while hypothalamic injections in fact led to increased EtOH drinking. We postulated that NPY has a dual role in regulating EtOH intake. Basal EtOH consumption in alcohol naïve, genetically heterogenous rats does not lead to pharmacologically active BAC:s, and is not consumed for pharmacologically reinforcing properties. This consumption is not under major modulation by NPY, and may even be positively regulated through hypothalamic appetite mechanisms. A second component is present in genetically selected EtOH preferring lines, and can also be induced using e.g. prolonged exposure to cycles of intoxication and withdrawal. This component is selectively sensitive to e.g. acamprosate. We therefor evaluated the potential of targeting the NPY system to develop treatments for high drinking states. Blockade of presynaptic Y2 autoreceptors offers an attractive strategy to potentiate release of endogenous NPY. Icv injections of the Y2 antagonist BIIE0246 in rats suppressed operant EtOH self-administration. Doses subthreshold in EtOH-naïve subjects were effective following a history of dependence, indicating a recruitment of the NPY system. This was confirmed using Y2 receptor antisense; in post-dependent animals, this treatment suppressed limited access voluntary drinking by up to 70% in a sequence specific manner. Antagonism at Y2 receptors offers an attractive principle for treatment of alcohol dependence. It is under patent by the NIAAA, and will be developed.

S33.2

IS DOPAMINE NEUROTRANSMISSION A POTENTIAL TREATMENT TARGET FOR ALCOHOL ADDICTION?
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Alcoholism is a chronically relapsing disorder. Mesolimbic dopamine (DA) transmission participates in mediating the reinforcing effects of ethanol. Evidence has accumulated implicating this system also in neurobehavioral processes relevant for susceptibility to relapse. First, ethanol-predictive environmental stimuli increase extracellular DA levels in the nucleus accumbens, and blockade of either D_1 or D_2 receptors reverses ethanol-seeking behavior induced by such stimuli. These findings implicate DA transmission in conditioned drug-seeking and, by inference, craving and relapse associated with alcohol cue exposure. Second, DAergic activation by an ethanol-related context is enhanced in rats subjected to ethanol deprivation. Thus, changes 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 manipulation of this system is associated with complications that limit therapeutic promise. DA antagonists may have potential for ameliorating craving and relapse associated with alcohol cue exposure, but are likely to exacerbate the DA hypoactivity that accompanies chronic ethanol abuse and has been linked to increased relapse risk. DA agonist treatments to reduce susceptibility to relapse by ameliorating DA hypofunction are problematic as well, because these may lead to autoinhibitory reductions in DAergic tone, counteracting the therapeutic effects of these agents. It remains to be determined whether indirect modification of DA activity via agents that act on other neurochemical systems may prove effective by eliminating problematic side effects associated with chronic DA agonist or antagonist treatments. Indeed, indirect modification of DA activity by opiate receptor antagonists is likely to contribute to the established "anti-relapse" profile of this agent.

S33.3

THE ROLE OF OPIOID SYSTEMS IN ETHANOL SELF-ADMINISTRATION.
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The role of central dopaminergic and opioidergic neuronal systems in ethanol self-administration has been examined using the alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rat lines. Data from microdialysis studies suggest that differences in ethanol intake between the lines cannot be explained in terms of differential sensitivity of central dopaminergic neurons to ethanol, and that dopamine probably is not crucially involved in the mediation ethanol reinforcement in AA rats. The strain specific differences in the opioidergic systems between the AA and ANA rats, however, suggest a role for the opioidergic mechanisms in ethanol self-administration behavior in these animals. The content of proenkephalin mRNA is higher in the brain of AA than ANA rats, and there are differences in the densities of mu opioid receptors in numerous brain parts, particularly in the nucleus accumbens and prefrontal cortex, the AA rats showing higher densities than the ANA rats. Such differences are not seen in delta receptor density. When compared to the ANAs, the AAs also consume more etonitazene (an opioid agonist) solution and show higher morphine-induced locomotor activity. Furthermore, AA rats are more liable to the sensitizing effects of repeated morphine injections on both locomotion and accumbal dopamine overflow. Studies on the relationship between morphine-induced behavioural sensitization and voluntary ethanol intake in the AA rats show that activation of opioid receptors by morphine modifies ethanol intake, and that morphine-induced behavioural sensitization is expressed as increased ethanol consumption following a priming dose of morphine. In line with these findings, administration of mu receptor antagonist CTOP or delta receptor antagonist naltrindole into the nucleus accumbens or basolateral amygdala decreases ethanol responding whereas injections into the VTA have little effect on ethanol intake. Since nonselective opioid antagonist naltrexone attenuates ethanol intake dose-dependently also in 6-hydroxydopamine-treated animals, the suppressive effect of opioid antagonists on ethanol self-administration is probably not based on the inhibition of ethanol-induced dopamine release by opioid antagonists in the nucleus accumbens.

S33.4

NEUROCHEMICAL AND BEHAVIORAL STUDIES ON ETHANOL AND NICOTINE INTERACTIONS.

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Ethanol and nicotine are the most abused drugs and it is well-known that co-abuse of ethanol and nicotine is frequent in man. The understanding of the neurochemical basis underlying the addictive properties of ethanol and nicotine is imperative for the development of pharmacological means to reduce the intake of these drugs. Indeed, during the last few years new pharmacological alternatives, most notably acamprosate and naltrexone have been introduced for reducing alcohol consumption and preventing relapse in problem drinkers or alcoholics. The initiation of the clinical trials of these drugs was based on results obtained from research on neuronal mechanisms of importance for ethanol self-administration in experimental animals. Ample evidence has accumulated indicating that dopamine (DA) is implicated in the brain reward systems and that DA is directly or indirectly involved in the acute reinforcing actions of ethanol, although other neurochemical systems including GABA, glutamate, serotonin and opioid peptides also appear to participate in orchestrating the reward profile of ethanol. The molecular events underlying the DA-enhancing properties of ethanol are largely unknown, but recently ethanol was shown to directly interfere with ionic flux through several multi-subunit, ligand-gated ion-channels, including nicotinic acetylcholine receptors (nAChR) and we have previously reported that chronic ethanol administration can produce differential B_{max} changes in ^{3}H-nicotine binding in various brain regions. We have now obtained both behavioral (using a two bottle free-choice drinking paradigm) and neurochemical (using microdialysis in awake freely moving rodents) data indicating that the DA-activating and reinforcing properties 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 subpopulation(s) involved in mediating the effects of ethanol have now revealed that the $\alpha_3\beta_2$ or α_6 (α-conotoxin MII), but not the $\alpha_4\beta_2$ (dihydro-β-erythroidine) or α_7 (methyllycaconitine), subunits could represent targets for developing new drugs for treatment of alcoholism.

S33.5

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

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Different lines of evidence suggest that pharmacological blockade of the cannabinoid CB_1 receptors reduces alcohol intake and alcohol motivational properties in rats tested under multiple procedures. Accordingly, CB_1 receptor knock-out mice displayed lower levels of alcohol intake than wild-type mice. These results suggest the involvement of the cannabinoid CB_1 receptor in the neural circuitry controlling alcohol preference, intake and reinforcing properties. More recently, this lab tested the effect of the combination of the cannabinoid CB_1 receptor antagonist, SR 141716, and the opioid receptor antagonist, naltrexone, on alcohol intake in Sardinian alcohol-preferring (sP) rats. The combination of doses of SR 141716 and naltrexone per se ineffective synergistically suppressed acquisition and maintenance of alcohol drinking behavior in sP rats. These results suggest that SR 141716 and naltrexone exert a concomitant and reciprocally potentiating inhibitory action on the neural systems controlling alcohol drinking behavior. This lab investigated also the effect of cannabinoid CB_1 receptor agonist, CP 55,940, on alcohol intake in sP rats. The acute administration of CP 55,940 markedly stimulated alcohol intake. Pretreatment with SR 141716 or naloxone, given at doses that did not alter alcohol intake when given alone, resulted in the complete suppression of the stimulating effect of CP 55,940. Similarly, the stimulating effect of morphine on alcohol intake was completely blocked by pretreatment with naloxone or SR 141716. It may be hypothesized that the CB_1 and opioid receptors involved in the stimulating effect of CP 55,940 and morphine coexist in the same neuronal circuitry, and that their concomitant activation is needed for the stimulation to occur. The fact that blockade of one or the other receptor prevents the stimulation of alcohol intake suggests that the morphine effect is permitted by the concomitant activation of CB_1 receptors by endogenous cannabinoids and, vice versa, opioid receptor activation by endogenous opioids is needed for the cannabinoid response. These results add further evidence to the hypothesized cross-talk between opioid and cannabinoid brain systems.

S34.1

INTRODUCTION AND REVIEW OF THE NEUROPATHOLOGICAL CHANGES SEEN IN ALCOHOL-RELATED 'BRAIN SHRINKAGE'.

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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 (eg. 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 fibres within the white matter or a combination of these mechanisms. There is a correlation between white matter loss and lifetime alcohol consumption, particularly in the cases that also have the Wernicke-Korsakoff syndrome (caused by thiamine deficiency). The reduced white matter volume is not related to changes in hydration or changes in the chemical structure of the myelin. Select populations of neurones appear to be susceptible to alcohol-related brain damage. There is a 20% reduction in numbers of neurones in the superior frontal cortex. This will contribute to white matter loss due to Wallerian degeneration of myelinated axons but does not explain the partial reversibility of 'brain shrinkage' after significant periods of abstinence. Cortical neuronal dendritic arborisation is also reduced in the alcoholic cases and this has also been shown to be reversible with abstinence (in animal models). Neuropathological studies suggest that poor nutrition and, vitamin B1 deficiency in particular, is an important pathogenetic factor in alcohol-related brain shrinkage and neuronal changes.

S34.2

IN VIVO DETECTION OF MACROSTRUCTURAL AND MICROSTRUCTURAL MARKERS OF BRAIN INTEGRITY IN HUMAN ALCOHOLISM AND A RODENT MODEL OF ALCOHOLISM.

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Severe focal brain white matter pathology is a hallmark of several alcoholism-related clinical syndromes, such as Marchiafava-Bignami disease and central pontine myelinolysis. In vivo and post-mortem studies have also noted widespread white matter pathology as a typical feature in uncomplicated alcoholism. Controlled longitudinal MR structural imaging studies have revealed increase or decrease in gross white matter brain volume depending on alcohol abstinence or consumption by alcoholics. Because tissue volume recovery appears incomplete with abstinence, alcoholic brain pathology may have two components, one reflecting permanent change and one transient change. In addition to bulk changes in white matter volume, the microstructural integrity of white matter can be examined in vivo with MR diffusion tensor imaging (DTI). With DTI, disruption of regional white matter has been noted in alcoholic men, and the extent of the abnormality correlates with performance on tests of working memory and attention. DTI has also detected abnormalities in brain white matter in alcoholic women not detectable with gross measures of structural size. Factors in addition to alcohol itself contribute to observed changes in brain tissue. Thiamine deficiency, for example, has been shown with animal models and by inference from patients with Wernicke-Korsakoff syndrome to account for myelin loss. Translational neuroimaging studies focused on animal models of alcoholism can complement human research and permit control of factors not possible in naturalistic human study. Our initial MRI and MRS investigations have yielded novel data on both chronic and acute alcohol exposure of alcohol-preferring rats and indicate substantial brain structural and metabolic variability that may underlie individual differences in alcoholism's untoward effects on brain structure and function.

S34.3

CROSS SECTIONAL AND LONGITUDINAL MR SPECTROSCOPY STUDIES OF CHRONIC ADULT ALCOHOLICS.

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Several studies have been conducted in our laboratory using proton MR spectroscopy to evaluate the deleterious effects of chronic alcoholism on the brain and subsequent improvement with long-term abstinence. In cross-sectional studies, we have found significantly higher myo-Inositol in frontal gray matter and deep gray matter (thalamus) in recently detoxified alcoholics. Alcoholics who achieve long-term abstinence have similar levels of myo-Inositol to controls. The observed elevation in myo-Inositol may reflect proliferation or activation of glia. The comparable level of myo-Inositol in long-term abstainers compared to controls may reflect osmolar stability in abstinent alcoholics and/or a reduction in glial cell activation. Studies using various methodologies including structural MR, neuropathology, and neuropsychology have suggested a particular vulnerability of the frontal lobes to alcoholism. This issue was addressed using MRS to measure concentrations of N-acetylaspartate in frontal and parietal white matter of 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 provides indirect evidence of excitotoxicity mediated brain injury. In a study of alcoholics with a past history of alcohol-related 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 re-scanned after approximately two years 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 brain damage, and are consistent with neuropsychological studies indicating recovery of brain function with continued abstinence.

S34.4

GENE AND PROTEIN CHANGES IN THE BRAINS OF ALCOHOLIC CASES WITH 'BRAIN SHRINKAGE'.

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Long-term alcohol abuse results in neurological and cognitive deficits that are associated with localized neuropathological 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, is whether the changes in RNA expression so identified are reflected in altered protein expression and whether changes in protein expression levels can be correlated with white matter volume loss. In this study, the expression of myelin basic protein (MBP), myelin proteolipid protein (PLP), myelin-associated glycoprotein (MAG), cyclic nucleotide phosphodiesterase (CNP) and glial fibrillary acidic protein (GFAP) were measured in groups of control and alcoholic cases that were part of the original cohort of cases used in pathological studies, which included assessment of white matter volume changes. Alcoholics were defined on the basis of ethanol intake (>80 g per day); controls were defined by a consumption of less than 20 g/day of ethanol. Cases were matched for age at death, post-mortem delay and gender. Brain weights and white matter volume estimates were available for each case. Myelin protein expression was measured using a combination of Western and slot blotting in two brain regions, the superior frontal cortex and the cerebellum. 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 case groups. The difference in expression was most pronounced for PLP 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 and there was a marked difference 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 ABSTINENT ALCOHOLICS.

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Cognitive function and brain structure was studies 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 Bechara 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 ANALYSIS OF NEUROACTIVE STEROIDS.

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Neuroactive steroids are both endogenous and synthetic steroids and their derivatives, which rapidly alter CNS excitability. The endogenous steroids allopregnanolone and allotetrahydrodeoxycorticosterone 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 anxiolytic, anticonvulsant, and sedative-hypnotic activity. Ethanol itself has similar effects at $GABA_A$ receptors, causing a presynaptic potentiation of the inhibitory effects of GABA . 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 exists in brain, which is easily hydrolyzed by dilute acid in the well-known "solvolysis" procedures, to yield pregnenolone that 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.

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Neuroactive steroids are modulators of neuronal function that may play important roles in neuronal 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) a 5% ethanol-containing liquid diet that produces peak maternal blood alcohol levels near the legal intoxication limit (~0.08 g/dl); 2) an isocaloric liquid diet containing maltose-dextrin instead of ethanol with pair-feeding; 3) rat chow 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 selectively increased pregnenolone (PREG) and pregnenolone sulfate (PREGS) levels in the fetal and neonatal brain but not in the fetal liver, placenta, and maternal blood, indicating that the effect of ethanol is not secondary to accumulation of peripherally produced steroids. The impact of this elevation in PREGS levels on synaptic transmission was evaluated 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±35%; n=8-9) without affecting their amplitude. Unexpectedly, this effect was only 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 presynaptic sites in an age-dependent manner. We postulate that the fetal ethanol-induced increase in PREGS levels could 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.

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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, this 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 (3β,5β-THP), attenuates the reinstating 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 3β,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, 3β,5β-THP reduces 3α,5α-THP and attenuates 3α,5α-THP–induced reinstatement in vivo by antagonizing 3α,5α-THP's actions at the $GABA_A$ receptor. These results raise the possibility of a potential role for 3β,5β-THP-like compounds in the treatment of relapse for EtOH.

S35.4

NEUROACTIVE STEROID MODULATION OF ETHANOL INTAKE PATTERNS IN C57BL/6J MICE.

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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 pregnanolone, 3α,5β-THP), an allosteric partial agonist/ antagonist (epipregnanolone, 3β,5β-THP), and a non-competitive antagonist (pregnenolone sulfate, PREGS) were tested. Male B6 mice were acclimated to a reverse light/dark schedule and permitted 2-hr access to lickometer chambers containing one 10% ethanol (10E) 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-β-cyclodextrin) for 4-5 days, and then received 3-day injection regimens of either vehicle or neuroactive steroid (10 mg/kg 3α,5α-THP , 3β,5β-THP 0.15 mg/kg , 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,5β-THP elicited a robust increase (+109%) in 10E lick responses, while 3α,5α-THP transiently elevated 10E licks (+18%), and PREGS elicited only a moderate elevation in this measure (+33%), when compared to vehicle controls. In contrast, 3β,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 3β,5β-THP -treated mice. Consistent with earlier findings, neuroactive steroids with various $GABA_A$ receptor pharmacological profiles differentially modulate EtOH intake patterns. Supported by NIH Grants AA06945, AA10760. DA07262, and the Department of Veterans Affairs.

S35.5

ROLE OF NEUROSTEROIDS IN ETHANOL DEPENDENCE AND $GABA_A$ RECEPTOR PLASTICITY.

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Prolonged exposure to and subsequent withdrawal of ethanol are associated with marked, specific, and opposite changes in $GABA_A$ receptor subunit gene expression as well as in receptor function and pharmacological sensitivity in cultured rat hippocampal neurons. Down-regulation of $GABA_A$ receptor and a reduction in the efficacy of various benzodiazepine receptor ligands induced by prolonged ethanol treatment are associated with a reduced expression of α1, α 3, γ2L, and γ2S subunits. In contrast, an increase in the abundance of α4-containing receptors induced by ethanol withdrawal may be an important determinant of withdrawal syndrome and is blocked by drugs that are effective in the treatment of ethanol dependence. We now show that, in isolated rat hippocampal tissue, ethanol increases the concentration of 3α,5 α-THP as well as the amplitude of γ-aminobutyric acid type A ($GABA_A$) receptor-mediated inhibitory postsynaptic currents recorded from CA1 pyramidal neurons. This latter action is biphasic, consisting of rapid, finasteride-insensitive and delayed, finasteride-sensitive components. These observations suggest the ethanol may modulate $GABA_A$ receptor function through an increase in de novo neurosteroid synthesis in the brain that is independent from HPA axis. This novel mechanism may have a crucial role in mediating the short and long term effects of ethanol on $GABA_A$ receptor and brain function.

S35.6

ALCOHOL AND NEUROACTIVE STEROID INTERACTIONS IN THE MENSTRUAL CYCLE.

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Alcohol consumption varies during the menstrual cycle in women with premenstrual dysphoric disorder (PMDD), but not in women without cyclical mood changes. Alcohol consumption and number of women abusing alcohol seem to be higher among women with PMDD than controls. Women with PMDD are less sensitive to $GABA_A$ receptor-active substances like benzodiazepines (BZ), pregnanolone (3α,5β-THP), and also alcohol during the luteal phase compared to the follicular phase. Healthy control women do not show any variation depending on the phase of the cycle, and there is no difference between healthy control women and men. Women with severe PMDD are less sensitive to BZ and 3α,5β-THP than women with mild PMDD. Healthy postmenopausal women increase their sensitivity to 3α,5β-THP during progestagen treatment, which is opposite to the pattern in PMDD. Both alcohol and allopregnanolone (3α,5α-THP) will, in low dosage or serum concentration, induce negative mood and aggression, while in high dosages alcohol and 3α,5α-THP both have the opposite effect. Indeed, in this situation alcohol and 3α,5α-THP enhance each other. These findings together indicate that alcohol and GABAergic steroids have some common behavioral effects, which are especially noted in women with PMDD.

S36.1

KUPFFER CELL-DERIVED MEDIATORS INVOLVED IN ALD: REACTIVE OXYGEN AND NITROGEN SPECIES.

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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 prooxidants, among which superoxide (O_2^{-}) has been characterized well for its roles in ALD. One source of O_2^{-} in Kupffer cells is NAD(P)H oxidase. Indeed, injury and free radical production is completely blocked in mice deficient in NADPH oxidase ($p47^{phox}$ knockout), suggesting a key role of O_2^{-} production from this enzyme in alcohol-induced liver injury. However, since O_2^{-} is not itself a potent oxidant, it is likely that O_2^{-} reacts with other biological molecules to create a more reactive oxidizing species. There are multiple pathways involving O_2^{-} that can lead to the formation of potent oxidants. For example, nitric oxide (NO·) and O_2^{-} can combine via a diffusion-limited reaction to form peroxynitrite ($ONOO^-$), a potent oxidizing/nitrating species. In support of the latter pathway, it was recently shown that mice deficient in inducible nitric oxide synthase (iNOS knockout mice) were completely protected against alcohol-induced liver injury and ROS/RNS formation, analogous to studies with NAD(P)H oxidase deficient mice. These results support the hypothesis that the potent oxidant formed during alcohol-induced liver injury is dependent on both O_2^{-} and NO·, such as $ONOO^-$. Interestingly, NO· from eNOS has also been shown to play a protective role in alcoholic liver disease, most likely via maintaining vascular tone. These results therefore suggest that NO· may play pleotropic roles in alcoholic liver disease, depending on the source of the radical. (supported, in part, by NIAAA).

S36.2

KUPFFER CELL DERIVED MEDIATORS IN ALD: TNFA

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Tumor necrosis factor α (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 stimulate expression of intracellular adhesion molecule-1 (ICAM-1), potentially leading to microcirculatory disturbances. This critical role of TNFα in mediating ethanol-induced liver damage is supported by experiments demonstrating that TNFα receptor-knockout mice are resistant to ethanol-induced liver damage. While the essential role for TNFα in mediating ethanol-induced injury is clear, the factors regulating TNFα in response to ethanol are not well understood. Activation of hepatic macrophages by lipopolysaccharide (LPS) during ethanol exposure is thought to be an important mechanism for stimulation of TNFα expression. Chronic exposure of macrophages to ethanol, both in vivo and in culture, impacts on specific elements within the LPS-stimulated signaling cascade, disrupting both the transcriptional and post-transcriptional regulation of TNFα biosynthesis. Stabilization of TNFα mRNA after chronic ethanol exposure is one important mechanism for increased TNFα production by hepatic macrophages. Stabilization of TNFα mRNA after chronic ethanol exposure requires both *cis*-acting elements in the TNFα mRNA and *trans*-acting mRNA binding proteins. The AU-rich element in the 3'-untranslated region of the TNFα mRNA is an important regulator of TNFα mRNA stability. Its activity is required for chronic ethanol-induced stabilization of TNFα mRNA. Moreover, recent studies have demonstrated that at least one mRNA binding protein, HuR, is also involved in stabilization of TNFα mRNA stability after chronic ethanol exposure. Taken together, these studies identify the regulation of TNFα mRNA stability as a novel mechanism by which chronic ethanol exposure increases the expression of TNFα. This work was supported by NIAAA.

S36.3

METFORMIN PREVENTS ACUTE ALCOHOL-INDUCED FAT ACCUMULATION IN MICE.

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Metformin is drug widely used in treatment of Type 2 diabetes whose cellular action seems to be mediated in part through the activation of AMP-activated protein kinase (AMPK), a major regulator of lipid and glucose metabolism. Activation of AMPK by metformin has been shown to reduce activity of acetyl CoA carboxylase (ACC) and suppress expression of SREBP1, a key lipogenic transcription factor as well as induce β-oxidation of fat in the liver. Recently, metformin has been shown to improve steatosis in non-alcoholic steatohepatitis (NASH) in rodents and humans. Whether this compound will also be protective in other forms of fatty liver disease (i.e. alcoholic liver disease; ALD) remains to be determined. Therefore we examined the effect of metformin on acute alcohol induced fat accumulation in a mouse model. Accordingly, 4 groups of female C57 Bl6 mice (6 wks) were treated 4 d with metformin (200 mg/ kg body-weight/ day, i.p.) or saline. On day 4, animals received ethanol (6 g/kg bw i.g.) or isocaloric maltose-dextrin solution by gavage. Hepatic lipids were assessed by triglyceride (TG) determination and by Oil Red O staining. Protein levels of SREBP1 were measured in nuclear extracts by Western blot and mRNA levels of fatty acid synthase (FAS) were determined by real-time RT-PCR. Twelve hours after alcohol treatment, hepatic lipid content was significantly higher compared to control-treated animals; specifically, there was a 20-fold increase in TG content and a similar increase in Oil Red O staining. The effect of ethanol on both parameters was significantly attenuated by metformin treatment. Alcohol also increased nuclear localization of mature SREBP1 and FAS expression; these effects were also blunted by concomitant metformin treatment. No significant differences were found for any of the investigated parameters between control groups. In summary, these data demonstrate that metformin effectively prevents acute alterations in fat metabolism caused by ethanol in mice, presumably though activation of AMPK. These data also support the hypothesis that metformin may be effective in the treatment of ALD as well as in NASH. (supported, in part, by NIAAA)

S36.4

GENDER DIFFERENCE IN ALCOHOLIC LIVER DISEASE.

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Accumulating evidence indicates that sensitization of Kupffer cells to lipopolysaccharide (LPS) and over-production of TNF-α by 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 estriol, which increases gut permeability and endotoxin concentrations in the portal vein. Moreover, estriol increases CD14 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 estriol-induced increase in Kupffer cell sensitization to LPS accounts for, at least in the part, the mechanism of the gender difference in ALD.

S36.5

KUPFFER CELLS AS A THERAPEUTIC TARGET OF ALCOHOLIC LIVER DISEASE.

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Given the roles of Kupffer cell-derived mediators in the pathogenesis of alcoholic liver disease, efforts have been directed to find or develop drugs that have an efficacy to modulate Kupffer cell (KC) functions thereby reducing or correcting overproduction of toxic mediators. Thalidomide has been shown to suppress TNF-α production by macrophages. We determined whether thalidomide could prevent alcohol-induced liver injury. Ethanol for 8 weeks caused marked steatosis, necrosis and inflammation in the liver. These pathological parameters were diminished markedly by thalidomide. In the 4-week ethanol group, KCs were sensitized to LPS. Co-administration of thalidomide with ethanol for 4 weeks prevented the KC sensitization completely. Furthermore, thalidomide abolished the LPS-induced increase in CD14 expression and $[Ca^{2+}]_i$ elevation in KC from rats treated ethanol chronically. Moreover, thalidomide reduced the LPS-induced 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 abrogation of KC sensitization to LPS. Besides thalidomide, pioglitazone, a PPARγ agonist, 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 actions, there appears to be a strong possibility that this type of drugs will prove beneficial to patients with severe alcoholic liver injury.