Conclusions. These data reflect local variations in and controls.

Methods. We used membrane binding to assay radioligands and modulators in five brain regions from alcoholics and matched controls (n = 6). Data were analysed by non-linear curve-fitting.

Results. [14C]Flunitrazepam affinity was invariant, [14C]Flumazenil affinity varied regionally, and [14C]Ro15-4513 affinity varied both regionally and between groups. [14C]Flunitrazepam and [14C]Flumazenil receptor densities were higher in several brain regions of alcoholics, whereas [14C]Ro15-4513 density was not. Zolpidem affinity in modulating [14C]Ro15-4513 and [14C]Flumazenil binding was lower in hippocampus and caudate. Regional differences in Hill slope (nH), notably in occipital cortex, precluded a one-site model. Zolpidem modulation of [14C]Flumazenil binding resolved into 2 sites in 4 regions. Affinity was lower in occipital cortex (52 ± 1 µM) than in other regions (range 9–12 µM), P < 0.01, between alcoholics and controls. Binding capacities varied regionally but not between cases. Zolpidem modulation of [14C]Ro15-4513 binding resolved into 2 sites in all areas. In controls, a proportion of binding to the high-affinity site was significantly lower (29%; p < 0.05) in alcoholics; in alcoholics, the fraction differed between hippocampus (27%) and occipital cortex (71%). Regional profiles of binding capacity differed significantly between alcoholics and controls.

Conclusions. These data reflect local variations in and controls.

PERSISTENT EFFECTS OF BINGE DRINKING ON ADOLESCENT BRAIN

S26.1

EFFECTS OF ADOLESCENT ETHANOL EXPOSURE ON BRAIN SYNCHRONY, BEHAVIOR, ITI VOLUMES, NEUROGENESIS AND CHOLINE ACETYL TRANSFERASE

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Background. Epidemiological data indicate that excessive alcohol consumption is prevalent among adolescents and may have lasting neurobehavioral consequences. Animal models provide the control necessary to investigate the potential neurotoxic effects of alcohol on adolescent brain development.

Methods. Wistar rats were exposed to ethanol vapor/air control 14 hrs/day for 35 days from P22-P57 (average BAC 163 mg%). Rats were withdrawn from vapor for 2-4 weeks and assessed for: electrophysiology, disinhibition, startle response and swim responses as adults. Rats were sacrificed at either day 72 or day 127 and either perfused for MRI and histochemical analyses (measures of neurogenesis, ChAT).

Results. Alcohol exposed rats displayed a significant reduction in the amplitude of their responses to prepulse stimuli during the startle paradigm, reduced energy and phase locking of ERO gamma activity, disinhibition in the open field conflict and decreased latency to immobility in the swim test. Volumetric analyses of MRI data indicated a reduction in the size of the hippocampus and an increase in the size of the ventricles in the ethanol exposed animals. Measures of neurogenesis were reduced in hippocampus and measures of ChAT were reduced in the basal forebrain in the ethanol exposed animals. Behavioral measures were found to correlate with structural brain findings.

Conclusions. These studies demonstrate that behavioral measures of arousal, affective state, disinhibitory behavior, brain synchrony, hippocampal volume and histochemical measures of ChAT and neurogenesis are all significantly impacted by periadolescent ethanol exposure and withdrawal in Wistar rats. (Supported by AA019969, AA020022).

S26.2

ADOLESCENT BINGE DRINKING INDUCES PERSISTENT HMGB1-TLR-RAGE SIGNALING

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High mobility group box 1 (HMGB1) is a nuclear cytokine-like protein that is an agonist for Toll-like receptors (TLR) and receptor for advanced glycation end products (RAGE). Ethanol treatment of brain slice cultures increased HMGB1 mRNA and protein, released HMGB1 into the media and increased expression of TLR. Our studies suggest that ethanol releases neuronal HMGB1 that stimulates TLR receptors inducing multiple NFкB proinflammatory cytokines and mediators, e.g., COX, NOX and iNOS. Ethanol, HMGB1 and LPS induction of cytokines are blocked by TLR4 antagonists, Minocycline, a microglial activation blocker, also blocks ethanol responses suggesting a critical role of microglial TLR4 receptors. Induction of proinflammatory signaling contributes to ethanol inhibition of neurogenesis and neurodegeneration. Induction of brain proinflammatory genes persists for long periods prompting the hypothesis that adolescent binge drinking will lead to persistent proinflammatory activation in the brain. We treated rats with an adolescent intermittent ethanol (AIE) protocol (5 gm/kg, i.g., 2 days on-2 off, P25-P55). Ethanol treatment increased HMGB1 and TLR4 receptor expression at P55 in prefrontal cortex. During maturation to young adulthood, P80, TLR receptors declined whereas HMGB1 increased, with the AIE induced increases persisting into adulthood but following maturational changes. RAGE in AIE treated rats increased with maturation, but not in controls. Assessments of human post-mortem alcoholic orbital frontal cortex find increased levels of HMGB1, TLR4, TLR3, TLR2, RAGE and NOX. These findings are consistent with adolescent binge drinking causing persistent proinflammatory gene induction that contributes to alcoholic psychopathology. Supported by NIH, NIAAA.
long-term cognitive deficits. We have assessed whether the activation of the innate immune response is mediated by TLRs signalling and if the up-regulation of inflammatory mediators trigger myelin disruptions and causes long-term cognitive effects in the adolescent rats. Adolescent and adult rats treated with intermittent binge-like ethanol exposure were used. We show that binge-like ethanol administration to adolescent rats increases both the gene expression of TLR4 and TLR2 and the levels of TNFα and IL-1β in the prefrontal cortex (PFC). These events were correlated with alterations in the levels of several myelin proteins such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), NG2 proteoglycan and myelin associated glycoprotein (MAG) in the PFC of adolescent rats. Notably, the above effects were associated with long-term cognitive dysfunctions. Conversely, the same ethanol treatment did not cause significant changes in either inflammatory mediators or myelin changes in the PFC of adult rats. The findings support the role of neuroinflammation and TLR4/TLR2 activation in ethanol-induced alterations in myelin maturation during adolescence, events that can have a long-lasting influence on the efficiency of the cognitive processes (Supported by SAF-2012, PNSD, ICIII and FEDER funds RD12/0028-Network).

S27

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INNOVATIVE TECHNIQUES IN HUMAN LABORATORY ALCOHOL RESEARCH

S27.1

THE COMPUTER-ASSISTED INFUSION SYSTEM (CAIS) FOR EXPERIMENTAL ETHANOL ADMINISTRATION IN HUMANS

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Ingestion of alcohol leads to substantial variability across subjects in the system-ic exposure to alcohol, including that of the brain. Using intravenous infusion bypasses these sources of variability and, together with a physiologically based pharmacokinetic (PBPK) model of alcohol distribution and elimination grants much better control over the time course of arterial blood alcohol concentration (aBAC). Infusion rates are adapted once every 30 seconds to follow an aBAC time profile prescribed by the experimenter. Besides improved control over aBAC, other advantages of i.v. alcohol administration over oral ingestion include safety, since aBAC declines immediately after the infusion is stopped and blinding, since alcohol can be administered without the subject’s awareness. Infusion also provides a reliable method for dissociating the response to alcohol administration from demand characteristics such as taste, smell, and familiarity/preference of the source of the alcohol. Initially, these methods were developed to achieve constant aBAC over several hours of experiments testing alcohol effects (“alcohol clamping”). With more refined methods of modeling, infusion was recently developed for self-administration experiments with free access or operant paradigms requiring work to gain access to alcohol. Results of two self-administration studies will be presented. The computer-assisted infusion system (CAIS), i.e. a software platform enabling for all these types of experiments is now made available to interested researchers.

S27.2

CHARACTERIZATION OF OPERANT INTRAVENOUS ALCOHOL SELF-ADMINISTRATION IN HUMANS

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Computer-Assisted Self-infusion of Ethanol (CASE) is a method of intravenous (IV) alcohol administration that allows alcohol self-administration in a laboratory setting, while controlling the breath alcohol concentration (BrAC) using a physiologically-based pharmacokinetic (PBPK) model-based algorithm. The objective of this study was to develop and characterize operant IV alcohol self-administration in non-dependent drinkers using two paradigms: free-access and progressive-ratio. Healthy non-dependent drinkers completed two IV alcohol self-administration sessions, each consisting of a 25-min priming phase followed by a 125-min phase where they could push a button for additional ethanol infusions, using a free-access (FA) schedule (one button press/infusion) and a progressive ratio (PR) schedule (increasing button presses for infusions). The number of rewards and peak and average BrAC were recorded, as well as the total and average number of button presses and button-press rate for the PR group. Regression analyses assessed effects of drinking history, alcohol sensitivity and personality. Results indicated high degree of within-session correlation among self-administration measures for both paradigms, indicating high internal consistency. In the FA paradigm, self-administration measures were significantly associated with drinks/drinking day, alcohol sensitivity, and reward sensitivity. Alcohol urges following priming were associated with self-administration measures for both OB and PR paradigms and rates of self-infusion were associated with subjective response to alcohol and measures of expectancy and impulsivity. Individuals with higher expectancy of negative alcohol effects and with higher impulsivity showed lower responses on both paradigms. These results indicate that IV self-administration measures reflect drinking history and alcohol sensitivity and expectancies effects.

S27.3

STUDYING THE ROLE OF FEEDING-RELATED PATHWAYS IN ALCOHOLISM VIA HUMAN LABORATORY STUDIES

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Feeding-related peptides may play a role in alcohol-related behaviors, including alcohol craving and consumption. We have reported, for example, that anorexigenic peptides such as insulin and thyroid hormones may be associated with alcohol craving and alcohol consumption. More recently, our lab started investigating the possible role of ghrelin in alcoholism. For example, human studies with alcohol-dependent individuals suggest that higher blood levels of the orexigenic gut-brain peptide ghrelin are associated with higher self-reported alcohol craving. Our most recent longitudinal study indicated a significant difference in baseline ghrelin levels between non-abstinent and abstinent subjects, as well as inverse ghrelin’s patterns between the two groups during the study. Furthermore, there was a positive significant correlation between baseline ghrelin levels and self-reported alcohol craving. More recently, we performed a human laboratory study in non-treatment seeking heavy drinking alcohol-dependent individuals where subjects are randomized to receive an intravenous (IV) administration of ghrelin 1 microg/kg, 3 microg/kg or saline solution (placebo). Then, subjects undertake an alcohol cue-sensitivity session to assess cue-induced urge to drink, as well as other subjective (e.g. attention to cues) and physiological (heart rate, salivation) measurements of alcohol cue-elicited craving. In this study, administration IV of ghrelin resulted in acute increase in craving (cue-induced urge to drink). Altogether, these data suggest that the ghrelin system may represent a novel pharmacological target to treat patients with alcoholism.

S27.4

SUBJECTIVE RESPONSES TO ALCOHOL IN THE LAB PREDICT NEURAL RESPONSES TO ALCOHOL CUES

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Subjective responses to alcohol represent biomarkers of alcoholism liability and treatment response. This study aimed to integrate the human laboratory with neuroimaging by testing whether subjective responses to alcohol during alcohol administration predict neural responses to alcohol cues in the scanner. Study design included a within-subjects controlled alcohol administration in the laboratory followed by an alcohol taste cues blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) task. Twenty alcohol dependent individuals from the Los Angeles community were recruited (6 females; 90% Caucasian; mean age = 29.4). Laboratory assessments of subjective alcohol high, liking, wanting (i.e., craving), positive reinforcement, and negative reinforcement during alcohol administration were entered as predictors of neural response to the presentation of alcohol versus control cues in the scanner (whole-brain cluster-corrected at Z > 1.96, p < 0.05). Alcohol craving during alcohol administration was found to predict less neural activity to alcohol cues in regions including the precentral and postcentral gyr, and supplementary motor area. Alcohol high, however, predicted greater neural activity to alcohol cues in regions including the anterior cingulate cortex, angular gyrus, occipital cortex, and superior parietal lobule. Consistent results were observed across the reinforcement factors such that greater positive and negative reinforcement in the lab predicted greater activation during alcohol cue presentation in regions including the precentral, anterior cingulate cortex, and cuneal cortex. This study provides initial evidence that subjective responses to alcohol during alcohol administration predict neural responses to alcohol cues.