In summary, systemic treatment in alcoholism needs a multidisciplinary approach to consider all causes of tissue damage and the diverse therapeutic response, with specific individual assessment.

S19.5

ALCOHOL AND CARCINOGENESIS
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Following ingestion, ethanol (EtOH) exerts direct and indirect effects on hepatic physiology. Many deleterious consequences of ethanol relate to intrahepatic metabolism and changes in hepatic REDOX status. Previous studies report different hepatic responses to chronic EtOH intake between males and females. The aims of the current studies were to determine effects of EtOH on hepatic tumor progression in male (m) and female (f) mice. Neonatal (m) and (f) B6C3 mice were injected with single dose diethylnitrosamine (DEN, 1 mg/kg, i.p., 23 days old). Mice were weaned onto, and maintained on, an EtOH-drinking water regime (10/20% (v/v); alternate days) beginning at 16 or 40 weeks for 8 weeks. At 24 and 48 weeks, animals were sacrificed and blood-tissue harvested. Significantly more (m) DEN mice developed altered hepatic foci (foci) vs. (f) mice in pair-matched groups at 24 and 48 weeks in the absence of significant differences in BAC (range; 10.43 ± 3.96 to 14.13 ± 3.46 mm). Foci area were significantly greater in pair-matched DEN initiated (m) vs. (f) mice, and (m) DEN + EtOH (0.320 ± .033mm²) vs. (m) DEN (0.104 ± 0.042 mm²) at 24 weeks and (0.481 ± 0.029 mm²) (DEN) vs. 0.268 ± 0.061 mm² (DEN + EtOH) at 48 weeks. Measures of hepatic injury were assessed by scoring sections for steatosis, necrosis, inflammation and sirius red staining. Significantly increased liver pathology was identified in (m) vs. (f) DEN + EtOH mice at 24 and 48 weeks. Induction of CYP2E1 was significantly greater in (m) mice maintained on EtOH drinking water compared with (f), as were measurements of malondialdehyde (MDA; indicator of lipid peroxidation). Conversely, glutathione (GSH) was significantly higher in (f) vs. (m) mice in response to EtOH + DEN initiation. Immunohistochemistry for proliferating cell nuclear antigen (PCNA) identified a 121% increase in staining in (m) DEN + EtOH vs. a 16.9% decrease in (f) DEN + EtOH at 24 weeks and a 79.3% increase in (m) and 14.5% decrease in (f) mice at 48 weeks. Western blot identified corresponding, significant increases in cyclin D1 in (m) vs. (f) DEN + EtOH mice. Finally, inclusion of silibinin, a dietary anti-oxidant derived from the milk thistle plant, failed to significantly alter hepatic pathology or tumor progression in females. Conversely, dietary silibinin acted to further enhance EtOH-dependent increases in hepatocarcinogenesis in males. These data demonstrate chronic EtOH consumption in the setting of underlying hepatocyte transformation significantly enhances tumorigenesis in (m), but not in (f) mice. Additionally, (f) mice maintain higher GSH levels and appear to more effectively manage EtOH-associated REDOX changes, data further supported by inclusion of a natural, plant-derived dietary antioxidant.

S20

ESBRA-APSAAR JOINT SYMPOSIUM: MORPHOLOGICAL AND FUNCTIONAL ALTERATIONS IN THE BRAIN OF ALCOHOLICS

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S20.1

PHARMACOKINETICS AND THE BRAIN HEMODYNAMIC AND SUBJECTIVE PERCEPTION EFFECTS OF ACETALDEHYDE IN HETEROZYGOUS ALDH2*1/*2 ALCOHOLICS
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It has been well documented that although homozygosity of the variant aldehyde dehydrogenase-2 (ALDH2) gene allele, ALDH2*2, in Asians almost fully protect against developing alcohol dependence, the heterozygosity only affords a partial protection to varying degrees. The partial protection against alcoholism has been ascribed to the faster elimination of acetaldehyde by residual hepatic ALDH2 activity and the lower accumulation in circulation in nonalcoholic heterozygotes. The physiological basis for overcoming the protection in ALDH2*1/*2 alcoholics, however, remains unclear. To address this question, we recruited a total of 27 Han Chinese alcohol-dependent men, matched by age and body mass index, controlled for normal liver and cardiovascular functions, from a population base of 221 alcoholics. The subjects were divided into ALDH2*1/*1 homozygotes (n = 13) and ALDH2*1/*2 heterozygotes (n = 14). Following a moderate dose of ethanol (0.5 g/kg body weight), blood ethanol/acetaldehyde/acetate concentrations, cardiac and extracranial/intracranial arterial hemodynamic parameters, as well as self-rated subjective sensations were measured for 130 min. ALDH2*1/*2 alcoholics exhibited significantly higher blood acetaldehyde levels as well as prominent cardiovascular effects and the subjective perceptions, compared with the ALDH2*1/*1 alcoholics. Comparable profiles of blood acetaldehyde were found between heterozygotic alcoholics and the previously reported nonalcoholic heterozygotes intakeing the same dose of ethanol. ALDH2*1/*2 alcoholics revealed, however, significantly lower intensities in both physiologic and psychologic responses than did the nonalcoholic heterozygotes. These results indicate that acetaldehyde, rather than ethanol or acetate, is primarily responsible for the observed alcohol sensitivity reactions in heterozygotic alcoholics and suggest that physiological tolerance and/or innate low sensitivity may play a crucial role in overcoming the deterring response. A potential pharmacogenetic classification of acetaldehydism and alcoholism for alcoholics carrying the different ALDH2 genotypes is proposed.

S20.2

THE POSSIBLE THERAPEUTIC APPROACH FOR AMELIORATION OF ALCOHOL-INDUCED BRAIN DAMAGE
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Alcohol exposure causes various kinds of neuronal damage, resulting from increased cell death or decreased cell proliferation in several brain regions. Recent neuroimaging studies demonstrated brain atrophy in alcoholics, and these morphological changes are considered as the result of neural network impairment. The emerging evidences suggest that the impairment of neurogenesis is the key factor for the pathophysiology of alcohol-induced brain damage. Based on clinical studies, brain atrophy in alcoholics is partially reversible during abstinence. The promotion of neurogenesis for the repair of the damaged neural network is suggested to be a key strategy to improve cognitive and emotional problems. As the nervous system has a limited capacity for self-repair, there is great interest in the possibility of repairing the CNS by transplanting neural stem cells (NSCs). We have demonstrated the effectiveness of intravenous transplantation of NSCs to the fetal alcohol spectrum disorders (FASD) model rat against its behavioral deficits. NSC transplantation ameliorated behavioral abnormalities of FASD model rat in the evaluation of anxiety, memory, cognition and social interaction tasks. We also evaluated the expression of PSD95, one of the markers of synaptic density and synaptic maturation, in amygdala, and found that the reduced level of PSD95 in the FASD model rat was recovered by the treatment of NSC transplantation. These results suggest that NSC transplantation may contribute to repair the neural network in the synaptic level and then provide structural and functional recovery of the damaged brain.

S20.3

CHRONIC ALCOHOL INTAKE CAUSES NEUROINFLAMMATION AND MYELIN ALTERATIONS IN MOUSE BRAIN
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Neuroinflammation is involved in the pathophysiology of progressive neurodegenerative disorders, including those associated with white matter injury. Alcohol abuse and alcoholism are associated with neurocognitive deficits, reduction in brain weight, loss of white matter and myelin fiber disruption, neuronal injury and neurodegeneration. However, the underlying mechanisms of these alterations remain elusive. We have shown that chronic ethanol intake, by activating the innate immune system and TLR4 receptors in glial cells, triggers the production of cytokines and inflammatory mediators and causes neuroinflammation and brain damage. Because neuroinflammation can be associated with demyelination and neuronal damage, we evaluated whether the ethanol-induced brain pro-inflammatory environment could be involved in the myelin dysfunctions observed in alcoholics. To answer this
question, we have used brains of wild-type mice (WT, TLR4+/+) and of mice lacking TLR4 (KO, TLR4−/−) which were treated chronically with ethanol (for 5 months). Using western blot and qPCR analyses, we demonstrate that chronic ethanol treatment down-regulates various myelin proteins (proteolipid protein, PLP; myelin basic protein, MBP; myelin oligodendrocyte glycoprotein, MOG; myelin-associated glycoprotein, MAG; 2',3'-cyclic-nucleotide 3'-phosphodiesterase, CNPase) in different brain regions (frontal cortex, corpus callosum, hippocampus and cerebellum) of ethanol-treated TLR4+/+ mice. These changes in myelin proteins were not observed in brain regions of ethanol-treated TLR4−/− mice. Electron microscopy studies also revealed that, while in the corpus callosum (CC) of ethanol-treated WT mice, 47% of axons showed myelin sheath disruptions, either no significant alterations or small focal fiber dysruptions were observed in the CC of TLR4−/−-treated mice. Furthermore, some leukocyte infiltration was observed in the medial frontal cortex of ethanol-treated WT mice. In summary, the present results suggest that ethanol-induced neuroinflammation might be involved in the myelin disruptions and white matter loss observed in humans suffering alcohol abuse or alcoholism. (This study was supported by Institute Carlos III: PNSD and RTA-Network and SAP 2009-07503.)

S20.4
SYNAPTIC DYSFUNCTION IN THE BRAIN OF THE HUMAN ALCOHOLIC
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We have developed a set of molecular techniques to study transcript and protein expression human autopsy brain, and to explore the influence allelic variants. Cases with common comorbid diseases such as cirrhosis of the liver can give different outcomes, either because they tend to have higher levels of consumption, or because the failing liver cannot remove toxins such as ammonia. Well-known sex differences in susceptibility to alcoholism can also modify the expression of key components. We compared vulnerable and relatively spared cortical areas in brains taken at autopsy from alcoholics and matched controls. Synaptosomal and synaptic membrane preparations from well-characterized cases and controls were used to quantify receptor binding as well as the expression of receptor-subunit transcripts and proteins. GABAA receptor parameters differ markedly between alcoholics without comorbid disease and controls, whereas glutamate-NMDA receptor components are more prominently affected in cirrhotic alcoholics. Expression of α-synuclein varies with both alcohol status and gender. Both genotype and gender can modify these outcomes. For example, the A1 allele of the DRD2 gene modulates NMDA-subunit expression differentially in males and females; SNCA genotype may interact with subject factors to influence its expression. To supplement these hypothesis-driven approaches we use discovery-based techniques, including microarrays and mRNA profiling. These paradigms provide new, sometimes unexpected, leads. Proteomic studies detect selective changes in synaptic proteins, including displays of multiple isoforms of the same protein, which suggest that post-translational processes alter key components of the synaptic machinery. These alterations will have consequences for the efficacy of trans-synaptic signalling in damaged areas of the brain of the human alcoholic. (This study was supported by NIAAA AA12404 and NHMRC #40155.)

S20.5
PROLONGED CHANGES IN THE CNS DURING ALCOHOL ABSTINENCE
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Our research examines functional changes seen following the cessation of chronic alcohol intake, particularly changes which outlast the acute phase of alcohol withdrawal and which may underlie the protracted withdrawal syndrome. We have demonstrated alterations in the mesolimbic dopamine system and in neuronal calcium channels. We have also found increases in the effects of other drugs of dependence when these are given repeatedly after cessation of chronic alcohol. Our recent work has focused on the glucocorticoids. We demonstrated that chronic alcohol consumption and withdrawal increase the brain levels of glucocorticoid, a change that outlasts the withdrawal symptoms and which is not paralleled by increases in plasma glucocorticoid concentrations. These brain changes were regional specific and were particularly notable in prefrontal cortex and hippocampus. Glucocorticoids are neurotoxic in high concentrations, especially when there is increased excitatory amino acid transmission as occurs during alcohol withdrawal. Studies on organotypic cultures have shown that corticosterone increases the neurotoxic effects of alcohol withdrawal. We also have evidence that the cognitive deficits caused by chronic alcohol intake are due, at least in part, to neuronal damage during alcohol withdrawal. Behavioural studies showed that memory deficits, and also the brain glucocorticoid increases, can be reduced by specific drug treatments, including the glucocorticoid type II receptor antagonist, mifepristone and the calcium-channel antagonist, nimodipine. These results indicate that glucocorticoids play a role in the genesis of the cognitive deficits caused by chronic alcohol consumption. As corticosterone has reinforcing properties, the increases in central glucocorticoid levels could also be involved in the development of dependence.

S21
GENETIC DIFFERENCES AT THE SEROTONIN TRANSPORTER GENE PROMISE A PHARMACOGENETIC APPROACH TO THE USE OF ONDANSETRON WITH AND WITHOUT OTHER AGENTS TO TREAT ALCOHOLISM
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Hypothesis. We previously have shown that the L-carriers of the promoter region of SLC6A4 of the serotonin transport (5-HTT) or SERT gene, versus their SS counterparts, have higher alcohol cue-induced craving and possibly a higher propensity for drinking behavior. SNP rs1042173 (G/T) in the 3’-untranslated region (3’-UTR), was also shown to affect SLC6A4 expression levels. Building upon previous findings, we tested the hypothesis that rs1042173 polymorphism could affect cue-induced alcohol craving in alcohol-dependent Hispanics.
Methods. We tested this hypothesis by examining whether in 34 Hispanic male alcohol-dependent volunteers (34.8 years) whether there are differences in craving based upon subtyping for RS1042173 (i.e. TT vs. TG/GG or Gx). SAS PROC mixed was used for data analysis.
Results. On subjective ‘urge to drink’ and ‘crave for a drink’, we found a significant (P < 0.05) main effect of the cue experiment and a main effect of genotype and cue effects. TT allele has lower 5-HTT mRNA and protein expression levels than the G allele and was associated with higher intensity of drinking and alcohol craving.
Conclusion. These results provide support for the hypothesis that rs1042173, an SNP in the 3’-UTR of the SLC6A4 gene can affect cue-induced craving in alcohol-dependent male participants.

S21.1
POLYMORPHISM OF THE SNP IN THE 3’-UNTRANSLATED REGION OF THE SEROTONIN TRANSPORTER GENE DIFFERENTIALLY AFFECT ALCOHOL CRAVING
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Conclusion. These results provide support for the hypothesis that rs1042173, an SNP in the 3’-UTR of the SLC6A4 gene can affect cue-induced craving in alcohol-dependent male participants.

S21.3
PHARMACOGENETIC APPROACH AT THE SEROTONIN TRANSPORTER GENE AS A METHOD TO REDUCE SEVERE ALCOHOL CONSUMPTION
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Pharmacogenetic treatments can optimize therapeutic response and limit inter-individual variability. Hence, finding efficacious pharmacogenetic approaches is important in advancing the treatment of alcohol dependence. The serotonin (5-HT) transporter (5-HTT) gates approximately 60% of neuronal 5-HT function. Specific genetic variants at the 5-HTT gene have been associated with major differences in 5-HT expression and with the prediction of alcohol craving; as such, they might constitute an important target for clinical treatment. Our aim was to show that ondansetron is an efficacious pharmacogenetic approach in advancing the treatment of alcohol dependence. The serotonin (5-HT) transporter (5-HTT) gates approximately 60% of neuronal 5-HT function. Specific genetic variants at the 5-HTT gene have been associated with major differences in 5-HT expression and with the prediction of alcohol craving; as such, they might constitute an important target for clinical treatment. Our aim was to show that ondansetron is an efficacious pharmacogenetic approach in advancing the treatment of alcohol dependence. The serotonin (5-HT) transporter (5-HTT) gates approximately 60% of neuronal 5-HT function. Specific genetic variants at the 5-HTT gene have been associated with major differences in 5-HT expression and with the prediction of alcohol craving; as such, they might constitute an important target for clinical treatment. Our aim was to show that ondansetron is an efficacious pharmacogenetic approach in advancing the treatment of alcohol dependence. The serotonin (5-HT) transporter (5-HTT) gates approximately 60% of neuronal 5-HT function. Specific genetic variants at the 5-HTT gene have been associated with major differences in 5-HT expression and with the prediction of alcohol craving; as such, they might constitute an important target for clinical treatment. Our aim was to show that ondansetron is an efficacious pharmacogenetic approach in advancing the treatment of alcohol dependence.