**S05.5**

**BRAIN-REGION SPECIFIC MODULATION OF ETHANOL-INDUCED DEPRESSION OF GABAERGIC NEURONS IN THE REWARD SYSTEM BY THE NICOTINE RECEPTOR ANTAGONIST MECAMYLAMINE**

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**Background.** The nucleus accumbens (nAc) and the VTA are primary components of the mesolimbic dopamine system, which is activated by all drugs of abuse, including ethanol. GABAergic neurotransmission has repeatedly been shown to regulate the activity of dopaminergic neurons in the VTA, and the aim with this study was to define the involvement of nicotinic acetylcholine receptors (nAChRs) in mediating acute ethanol effects on GABAergic activity in the nAc and VTA of awake and freely moving mice.

**Methods.** Multielectrode in vivo electrophysiology was performed in the VTA and nAc of awake and freely moving C57BL6/J mice receiving intraperitoneal injections of saline, ethanol (2.0 g/kg), and the non-competitive nAChR antagonist mecamylamine (1.0 mg/kg).

**Results.** GABAergic firing rate and burst activity was significantly depressed following administration of ethanol as compared to saline in both the VTA and in the nAc. Pre-treatment with mecamylamine inhibited the ethanol-induced decrease in GABAergic firing rate in the VTA, while the reduced firing rate sustained following ethanol-administration in accumbal medium spiny projection neurons. The ethanol-induced decrease in VTA burst activity was not inhibited by mecamylamine.

**Conclusion.** The data presented here shows that ethanol depresses GABAergic activity in the VTA and nAc of mice, and that nAChRs contribute to this modulation in the VTA. This finding is in line with previous studies of ethanol-induced dopamine-release, and supports an involvement of GABAergic neurons in regulating the activation of the mesolimbic dopamine-system by ethanol.

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**S06.3**

**INFRALIMBIC MGLUR2 DEFICIT AS A KEY MECHANISM FOR ALCOHOL ADDICTION**

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Our recent data show that metabotropic glutamate receptor subtype 2 (mGluR2) loss in the rodent and human prefrontal cortex - post-mortem analysis of anterior cingulate cortex from alcoholic patients shows a significant reduction in mGluR2 transcripts - is a major consequence of alcohol addiction. This specific molecular alteration leads to a hyper-glutamatergic state as demonstrated by microdialysis and human spectroscopy studies and subsequently to increased propensity to relapse. Normalization of mGluR2 function may therefore be of therapeutic value. Indeed restoration of mGluR2 function by infusing a lentiviral vector expressing the mGluR2 into the infralimbic cortex attenuates relapse-like behavior. For pharmacological intervention studies we have used a DSm-V/V animal model for alcohol addiction and show that the application of the mGluR2/3 agonist LY379268 (0.1, 1 and 3 mg/kg) results in a pronounced reduction in relapse-like drinking behavior. This anti-relapse effect was paralleled by a reversal of altered expression levels of a set of glutamatergic signalling-related genes to the levels found in mGluR2/-naive age-matched control rats. We further tested the mGluR2/3 agonist LY487379 (0.1, 10, and 30 mg/kg) and found that the highest dose reduced relapse-like drinking behavior in male as well as in female rats. The combination of sub-threshold doses of LY379268 (0.3 mg/kg) and LY487379 (30 mg/kg) produced a super-additive anti-relapse effect. We suggest that a loss or reduction of mGluR2 in the infralimbic region is critical in mediating addictive-like behavior and show that either genetic or pharmacological interference with this pathomechanism can reduce relapse behavior.

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**S06.2**

**MODULATION OF SENSITIVITY TO ALCOHOL INTEROCEPTIVE CUES BY MGLURS**

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The discriminative stimulus (interoceptive/subjective) effects of drugs have the potential to contribute to drug taking behavior. Therefore, in order to obtain a comprehensive understanding of the contribution of metabotropic glutamate receptors (mGluRs) to alcohol drinking behaviors, it is critical to examine the functional role of these receptors in modulating the interoceptive alcohol cue. Here we present findings showing reduced sensitivity to the interoceptive effects of alcohol following mGluR5 antagonism and mGluR3 agonist treatment in Long Evans rats trained on a two-lever alcohol discrimination task. Systemic administration of mGluR5 antagonists (MPEP, MTEP) and the mGluR2/3 agonist LY379268 did not induce alcohol-like discriminative stimulus effects. However, pretreatment with these compounds altered sensitivity to alcohol. That is, significant reductions in alcohol-appropriate responding were observed, suggesting decreased sensitivity to the interoceptive effects of alcohol. The use of c-Fos immunoreactivity (IR) indicated differential brain regional response to these mGluR compounds and as such, the functional involvement of mGluR compounds in modulating interoceptive sensitivity to alcohol in the nucleus accumbens and the amygdala were evaluated. Intra-accumbens, but not intra-amygdala, mGluR5 antagonism inhibited the interoceptive effects of alcohol. In contrast, intra-amygdala, but not intra-accumbens, mGluR2/3 activation, inhibited the interoceptive effects of alcohol. Together these findings confirm a role for these receptors in modulating sensitivity to the interoceptive alcohol cue. Given that activity at these receptors has also been implicated in modulating alcohol drinking, the mGluR class of receptors may be an efficacious target for the development of therapeutics for the treatment of addiction.

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**S06.1**

**ARE METABOTROPIC GLUTAMATE RECEPTORS PROMISING TARGETS FOR ALCOHOL ADDICTION?**

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**S06.4**

**THE ROLE OF MGLURS IN NOVELTY-SEEKING BEHAVIORS AND THE ESCALATION OF ALCOHOL DRINKING AFTER ABSTINENCE IN MICE**

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Novel experiences activate the brain’s reward system in a manner similar to drugs of abuse, and high levels of novelty- and sensation-seeking behavior have been associated with increased susceptibility to alcohol and drug abuse. We find that metabotropic glutamate receptor 5 (mGluR5) signaling on dopaminoinceptive neurons is necessary for both novelty-seeking behavior and the abstinence-induced escalation of alcohol drinking. We have generated transgenic mice with selective knock-down of the mGluR5 in neurons expressing D1 dopamine receptors (mGluR5KD-D1). These mutant mice...
displayed no apparent behavioral deficits, and normal anxiety levels or learning ability. However, mGluR5KD-D1 animals showed reduced locomotor activity when placed in a novel environment and exhibited decreased interaction with a novel object. Moreover, unlike control animals, mGluR5KD-D1 mice did not perform instrumental responses under the operant sensation-seeking paradigm, although they learned to respond for food normally. When mGluR5KD-D1 mice were provided access to alcohol, they showed similar patterns of consumption as wild-type animals. However, mutant mice did not escalate their alcohol consumption after a period of forced abstinence, while control mice almost doubled their intake. These data identify mGluR5 receptors on D1-expressing neurons as a common molecular substrate of novelty-seeking behaviors and behaviors associated with alcohol abuse.

S07

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NONINVASIVE SCREENING TOOLS FOR LIVER CIRRHOSIS IN ADDICTED PATIENTS

S07.1

INDIVIDUAL CIRRHOSIS SCREENING VIA LIVER STIFFNESS: PRACTICAL APPROACH AND INTERPRETATION

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Noninvasive screening for liver cirrhosis in patients addicted to drugs or alcohol has been a continuing problem in internal and addictive medicine. This has dramatically changed with the recent introduction of transient elastography (Fibroscan) to directly measure liver stiffness (LS). This novel technique is expanding rapidly around the globe since it allows the diagnosis of liver cirrhosis in a true bed-side manner within minutes. LS is an excellent screening parameter for cirrhosis with a high negative predictive value. Thus, a normal LS < 6 kPa excludes ongoing liver disease while a LS of 8 kPa and 12.5 kPa represent generally accepted cut-off values for F3 and F4 fibrosis. Meanwhile, LS has also been successfully used to monitor treatment outcome of patients with alcoholic liver cirrhosis and as prognostic parameter for hepatic complications such as the risk of variceal bleeding or hepatocellular carcinoma.

However, it is important to conceive that several other factors apart from cirrhosis stage may affect LS. Such factors include liver inflammation, liver congestion, cholesterol and rare conditions such as amyloidosis or mastocytosis. Thus, although LS is an excellent screening tool for liver disease, it should always be interpreted in the clinical context. For such a hepatological expert interpretation of LS values, a concomitant ultrasound and laboratory parameters are required which will increase the diagnostic accuracy over 99%. Novel actual algorithms will be discussed especially with regard to alcoholic liver disease, how to interpret increased LS values within the clinical setting.

S07.2

LIVER STIFFNESS AND CONTROLLED ATTENUATION PARAMETER MEASUREMENTS USING SHEAR WAVE BASED QUANTITATIVE ELASTOGRAPHY

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Introduced in 2003, Vibration-Controlled Transient Elastography (Fibroscan®, Echosens, France) disruptive technology is now widely used in routine clinical practice and research. Indeed, a large body of evidence demonstrated that liver stiffness measurement significantly correlates with liver fibrosis. Other factors, such as chronic inflammation, mechanical obstruction and liver congestion have been shown to increase liver stiffness. Recently a new parameter has been introduced: Controlled Attenuation Parameter (CAP). CAP is a measure of ultrasound attenuation developed to assess steatosis. With CAP, Fibroscan® can help assessing both liver fibrosis and Steatosis. In this paper, the different quantitative shear wave based techniques will be presented. Advantages and limitations of each technique will be described in terms of technology, clinical results, examination procedure, etc. The performances of CAP will be detailed and we will present how these performances can be enhanced using a dedicated liver guidance tool.

S07.3

IS THERE A ROLE FOR HISTOLOGY IN THE PREDICTION OF LONG-TERM MORTALITY IN ALD?

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Patients with non-severe alcoholic liver disease (ALD) are at lower risk of short-term mortality but die at later time points. The aims of our study were to develop a non-invasive model for the prediction of long-term mortality in patients with ALD and to investigate if histological parameters of ALD can improve the diagnostic accuracy of the non-invasive model. A cohort of 189 consecutive patients with a history of significant alcohol consumption (> 40 g/d for women (n = 69); > 60 g/d for men (n = 120)) enrolled between 1985 and 2008 was studied. Other causes of liver disease were excluded. All patients underwent liver biopsy for staging. Cox regression models were used for univarid and multivariate analyses. Variables independently associated with mortality were used to build new prognostic models. The parameters included in the non-invasive clinical-biochemical (CB) model were sex, alkaline phosphatase, bilirubin, creatinine, INR and thrombocyte count. The clinical-biochemical-histological (CBH) model included fibrosis stage and pericellular fibrosis in addition to parameters of the CB model. Diagnostic accuracies of the CB, CBH and Child Pugh score (CPH) were evaluated by ROC analysis for prediction of mortality at one and five years. The performance of the CBH model (AUCCBH 1a: 0.87/5a: 0.85) was superior to the CB and CPH at both time points (AUCCB 1a: 0.83/5a: 0.81 and AUCCPH 1a: 0.77/5a: 0.74, respectively). Each of the prognostic models showed better performance in men as compared to women. The CBH model is a new prognostic tool for the prediction of long-term mortality particularly in men with ALD.

S07.4

WHAT SHOULD BE USED TO MEASURE LIVER STIFFNESS: TE, ARFI OR SSE?

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One of the most important benefits of elastographic methods (transient elastography TE, ARFI, spleens stiffness elastography SSE) is the non-invasive diagnosis of liver cirrhosis. The areas under the ROC curve for the prediction of cirrhosis by TE reach 0.90-0.99, for cutoff values between 9-26.6 kPa. In some patients, although the cirrhosis diagnosis is established on pathological and ultrasonographic criteria, with evident nodular regenration, liver stiffness may not reach cirrhotic values when the finer septa surrounding the nodules do not increase the overall fibrosis of the liver. Generally speaking, liver stiffness will be lower in macronodular than in micronodular cirrhosis. Liver stiffness increases as the liver disorder progresses, but the prognostic significance of liver stiffness values is still under evaluation. Liver stiffness alone is not reliable enough to be used as a screening method to detect the esophageal varices grade in liver cirrhosis patients, but using TE to measure both liver and spleen stiffness ensures a better prediction of the presence of esophageal varices.

Real-time ultrasonographic elastography is another technique assessing liver stiffness through different methods: either through interpretation of a colour map, or through numerical quantification of the shear wave speed in the liver parenchyma (ARFI technique). The performance of ARFI is similar with that of TE in predicting cirrhosis but lower in early fibrosis stages. This presentation is a comparative review of the performance of TE, ARFI and SSE, alone or in combination, for the diagnosis and monitoring of liver cirrhosis.

S07.5

LIVER STIFFNESS AND SERUM MARKER: COMPETITION OR SUPPLEMENTATION?

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Transient Elastography (TE, Fibroscan) and serum markers (Fibrotest, Fibrometer) can be used for non-invasive diagnosis of liver fibrosis in Alcoholic Liver Disease. Specific TE cut offs have been described for the diagnosis of alcoholic cirrhosis. Some cautions must be known, as a significantly higher elasticity for the diagnosis of alcoholic cirrhosis compared with cut offs used in viral cirrhosis, the impact of alcohol abstinence on reducing liver stiffness, or the presence of histological evidence of alcoholic hepatitis. Some serum markers are patented. Diagnostic accuracy is high for diagnosis of cirrhosis. The combination of serum markers with elastography improves