

17<sup>TH</sup>

2019

# ESBRA

*European Society  
for Biomedical Research  
on Alcoholism Congress*



**21 - 24**

**September 2019**

**Lille Grand Palais (France)**

**Abstracts**



# WELCOME ADDRESS

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Dear Colleagues,

On behalf of the organizing committee of the ESBRA 2019 Congress, I am happy to welcome you to the 17th Congress of the European Society for Biomedical Research on Alcoholism in Lille, France. The congress takes place at the Lille Grand Palais, in the city of Lille.

Alcohol research is undergoing rapid development in all fields from basic research to clinical practice and treatments. This congress will bring insights about recent advances on basic research and innovations in the research field of alcohol use disorder and its comorbidities with all the alcohol-related pathologies. The meeting will provide the opportunity to share idea between researchers from the different fields and countries.

Lille, is a lively and welcoming city with many facets! Lille is the largest city of the Hauts-de-France region. With more than 6 millions inhabitants in 2014, the Hauts-de-France region is the third most populous region in France. You can discover the treasures of its architectural, historical heritage, and gastronomic specialties. Discover a unique way of life and a unique atmosphere.

The gala diner will take place at the Hôtel de Région Hauts-de-France very close to Lille Grand Palais.

A young investigator event is also scheduled on sunday night.

We are sure that Lille will be a magnifiscent location to host a memorable 17th ESBRA Congress in which high quality science and city will be blended. We will be very happy to welcome you to Lille.

**Mickael Naassila**

President 17th ESBRA Meeting



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# INVITED TALK & SYMPOSIUMS

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The abstracts are given in the order of the schedule.

Some abstracts are missing. A new version of this book of abstracts will be available after the conference.

## INVITED TALK

- [The gut microbiota, the inflammation and the brain in alcohol-use-disorders](#)  
*DE TIMARY Philippe (Department of Adult Psychiatry, Institute of Neuroscience, Brussels, Belgium)*

## SYMPOSIUM

### GPCRs & Alcohol-Seeking

LAWRENCE Andrew (*Florey Institute of Neuroscience & Mental Health*)

- **New lines of experimental evidence on the reducing effects of positive allosteric modulators of the GABAB receptor on alcohol-motivated behaviors**  
*Giancarlo Colombo and Paola Maccioni (Neuroscience Institute, Section of Cagliari, National Research Council of Italy, Monserrato (CA), Italy)*
- **Loss of control over alcohol drinking behaviour is linked to persistent changes in the dopaminergic and opioidergic systems**  
*Valentina Vengeliene, Rainer Spanagel, Anita C Hansson (for VV: Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania; for RS and ACH: Institute of Psychopharmacology, Central Institute of Mental Health, Faculty of Medicine Mannheim, Heidelberg University, Germany)*
- **The role of Nociceptin/orphanin FQ NOP receptor system in alcohol abuse**  
*Roberto Ciccocioppo, Anna Maria Borruto, Yannick Fotio, Alice Ilari, Michele Petrella, Alessio Masi, Nazzareno Cannella (University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy); Friedbert Weiss (The Scripps Research Institute, Department of Neuroscience, La Jolla, USA)*
- **Muscarinic acetylcholine receptors in alcohol use disorder**  
*WALKER Leigh (Florey Institute of Neuroscience and Mental Health)*

## **New lines of experimental evidence on the reducing effects of positive allosteric modulators of the GABA<sub>B</sub> receptor on alcohol-motivated behaviors**

Giancarlo Colombo, Paola Maccioni

Neuroscience Institute, Section of Cagliari, National Research Council of Italy, I-09042 Monserrato (CA), Italy

Among G-protein-coupled receptors, the GABA<sub>B</sub> receptor has recently gained considerable interest in the alcohol research field: the prototypic GABA<sub>B</sub> receptor agonist, baclofen, has repeatedly been reported to suppress several alcohol-related behaviors in laboratory animals as well as alcohol consumption and craving for alcohol in patients affected by alcohol use disorder (AUD). More recently, preclinical research in the alcohol field has focused on the positive allosteric modulators (PAMs) of the GABA<sub>B</sub> receptor, a new class of GABA<sub>B</sub> receptor agents with improved safety profile in comparison to baclofen. The present talk will summarize the several lines of experimental evidence unanimously indicating that GABA<sub>B</sub> PAMs retain the ability of baclofen to inhibit multiple alcohol-motivated behaviors in rodents; importantly, the effects of GABA<sub>B</sub> PAMs are selective for alcohol and occur at doses largely lower than those producing hypolocomotion and sedation. More specifically, all GABA<sub>B</sub> PAMs tested to date (i.e., CGP7930, GS39783, BHF177, *rac*-BHFF, CMPPE, ADX71441, COR659, and ORM-27669) have invariably been reported to reduce, or even suppress, several alcohol-motivated behaviors in rats and mice. These behaviors include excessive alcohol drinking, alcohol relapse-like drinking, alcohol binge-like drinking, operant oral alcohol self-administration (under both fixed and progressive ratios of reinforcement), reinstatement of alcohol seeking, alcohol-induced locomotor stimulation, and alcohol-induced conditioned place preference. The use of validated animal models of several aspects of AUD confers remarkable translational value to these findings. Among these GABA<sub>B</sub> PAMs, COR659 appears to be of particular interest because of a likely dual mechanism of action, involving – beside the positive allosteric modulation of the GABA<sub>B</sub> receptor – an action at the cannabinoid CB<sub>1</sub> receptor. This results in a unique behavioral pharmacological profile, including suppression of multiple behaviors motivated by alcohol and food (including highly palatable food) in rats. To summarize, data collected to date (i) confirm that the GABA<sub>B</sub> receptor is a major part of the neural substrate controlling alcohol drinking and mediating the reinforcing, motivational, stimulating, and rewarding properties of alcohol, and (ii) suggest that positive modulation of the allosteric binding site(s) is an effective mechanism, in addition to activation of the orthosteric binding site, to potentiate GABA<sub>B</sub> receptor-mediated neurotransmission and inhibit alcohol-motivated behaviors. The recent transition of the first GABA<sub>B</sub> PAMs to the initial steps of clinical testing makes investigation of efficacy of GABA<sub>B</sub> PAMs in AUD patients an important and feasible option.

381 words

## **Loss of control over alcohol drinking behaviour is linked to persistent changes in the dopaminergic and opioidergic systems**

Valentina Vengeliene, Rainer Spanagel, Anita C Hansson

Affiliations: (for VV) Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania and (for RS and ACH) Institute of Psychopharmacology, Central Institute of Mental Health, Faculty of Medicine Mannheim, Heidelberg University, Germany

Repeated exposure to deprivation phases in long-term alcohol drinking Wistar rats has proven to be a useful method to induce addictive features, such as loss of control over drinking behaviour. In this study we explored neurobiological mechanisms that underlie transition from controlled to compulsive alcohol consumption. For this purpose male rats were given concurrent ad libitum access to water, as well as to 5%, 10% and 20% alcohol solutions during an observation period of eight months. Loss of control over drinking behaviour was measured during post-abstinence drinking phase by adding quinine hydrochloride to alcohol solutions and by pairing mild foot-shock with instrumental responding for alcohol. Changes in mesocorticolimbic and nigrostriatal neurotransmission induced by long-term alcohol consumption were assessed by means of receptor autoradiography for dopamine and opioid receptor in rats withdrawn from alcohol for three weeks. Our results showed that loss of behavioural flexibility in voluntary alcohol drinking rats are, at least partly, caused by persistent upregulation of  $\mu$ -opioid receptor, downregulation of dopamine transporter and upregulation of dopamine receptor D1 in the ventral striatum and mPFC. Dopamine receptor D2 levels were unaffected by long-term drinking procedure. Our findings suggest that development of addictive behaviour could be a result of lost ability of the brain to adapt to a changing environment with respect to alcohol removal and re-exposure, which points at the importance of reversal of the lost brain plasticity in the development of new treatment strategies.

Contact: [valentina.vengeliene@zi-mannheim.de](mailto:valentina.vengeliene@zi-mannheim.de)

## **The role of Nociceptin/orphanin FQ NOP receptor system in alcohol abuse**

Roberto Ciccocioppo<sup>1</sup> Anna Maria Borruto<sup>1</sup>, Yannick Fotio<sup>1</sup>, Friedbert Weiss<sup>2</sup>, Alice Ilari, Michele Petrella<sup>1</sup>, Alessio Masi<sup>1</sup>, Nazzareno Cannella<sup>1</sup>

<sup>1</sup> *University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy*

*The Scripps Research Institute, Department of Neuroscience, La Jolla, USA*

The 17 amino acid peptide Nociceptin/Orphanin FQ (N/OFQ) is the natural ligand for the orphan G protein-coupled receptor (GPCR) Opioid Receptor Like-1 (ORL1), now known as NOP. Earlier studies in genetically selected Marchigian Sardinian (msP) alcohol preferring rats and in alcohol dependent Wistars demonstrated that activation of NOP by selective ligands reduced alcohol drinking and seeking. The effect was particularly pronounced following repeated drug administration. More recently, using selective NOP antagonists our laboratory found that NOP receptor blockade also reduced alcohol intake and relapse elicited by stress or by cues predictive of substance availability. Moreover, in a series of experiments aimed at characterizing the neurocircuitry mediating the effects of these drugs we found that the central amygdala (CeA) plays a critical role in the effect of NOP agonists. Whereas, the effects of the antagonists involve both the CeA and the ventral tegmental area (VTA).

Why both, NOP agonists and antagonists, reduces the motivation for alcohol is still unclear. However, based on evidence that NOP agonists like Ro 64-6198 and MT-7716 reduced alcohol drinking following chronic but not acute administration we propose that multiple dosing of NOP agonists, through receptor desensitization, may reduce N/OFQ transmission. This hypothesis is corroborated by binding data showing that in msP rats repeated injections of Ro 64-6198 down-regulated the expression of NOP receptors in various brain areas. Based on data showing that NOP receptors are upregulated in high alcohol drinking animal like msP and in postdependent Wistars we propose a heuristic model according to which increased activity of N/OFQ system facilitate alcohol use. This view is corroborated by data showing that rats with genetic deletion of NOP receptors drink less alcohol compared to wild type control *Grant support (AA017447 and AA014351).*

Contact: [roberto.ciccocioppo@unicam.it](mailto:roberto.ciccocioppo@unicam.it)

## Muscarinic acetylcholine receptors in alcohol use disorder

Leigh C Walker<sup>1,2</sup>, Alice E Berrizi<sup>3</sup>, Nicola A Chen<sup>1,2</sup>, Victoria Perreau<sup>1,2</sup>, Patricia Rueda<sup>3</sup>, Craig W Lindsley<sup>4</sup>, Carrie K Jones<sup>4</sup>, Christopher J Langmead<sup>3</sup> & Andrew J Lawrence<sup>1,2</sup>

<sup>1</sup> Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, <sup>2</sup> Florey Department of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, <sup>3</sup> Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Parkville, Victoria, <sup>4</sup> Departments of Pharmacology and Chemistry, Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, TN, 37232, USA

Despite the large socioeconomic burden of alcohol use disorders (AUD), therapeutic treatment options are limited. There is a need to characterize the neurochemistry underpinning alcohol seeking to aid identifying and evaluating novel targets. AUDs are characterised by a transition to compulsive alcohol seeking, which is hypothesized to involve a shift from ventral to dorsal striatum. In addition, a medial to lateral shift in the dorsal striatum is implicated in the transition from goal-directed to habitual alcohol seeking. Muscarinic and nicotinic acetylcholine receptors (AChRs) are potential targets for AUD treatment as they are expressed within the mesocorticolimbic reward system, including dense expression in the dorsal striatum. Here they modulate dopamine and glutamate release, which may regulate reward processing. To assess the role of AChRs in AUD, we first conducted genome-wide RNA sequencing in the caudate/putamen of 10 human alcoholics and 10 healthy controls and concurrently examined AChR expression in the corresponding regions in rat (dorsolateral and dorsomedial striatum) following chronic alcohol consumption/withdrawal using qPCR. Next we examined the role of select mAChR and nAChR subtypes in alcohol consumption and seeking using selective allosteric modulators. Finally, we probed the role of select mAChR and nAChR subtypes in the dorsal striatum in alcohol consumption and seeking. Collectively, our data show that specific mAChRs are potential novel target pharmacotherapies for the treatment of AUD.

Contact: [leigh.walker@florey.edu.au](mailto:leigh.walker@florey.edu.au)

## YOUNG INVESTIGATOR SYMPOSIUM

- Nicotine increases alcohol self-administration via  $\mu$ -opioid receptor activity in the ventral tegmental area  
*DOMI Esi (Center for Social and Affective Neuroscience, IKE, Linköping University, Linköping, Sweden)*
- Unveiling the alcohol-dependent alterations of local translation in the prefrontal cortex during adolescence  
*LAGUESSE Sophie (University of Liege)*
- Opposing effects of the hormones leptin and ghrelin on neural alcohol cue reactivity, craving and relapse in alcohol addiction: Two streams merge to one river?  
*BACH Patrick (Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University)*
- Altered GABA-signaling in the amygdala contributes to pathological alcohol choice over high value alternative rewards  
*AUGIER Eric (Linköping University, Sweden)*
- Neural correlates of implicit emotional processing in binge drinking  
*LANNON Séverine (Cognition Health and Society Laboratory (EA 6291), Université de Reims Champagne-Ardenne, Reims, France)*

## **Nicotine increases alcohol self-administration via $\mu$ -opioid receptor activity in the ventral tegmental area.**

E. Domi<sup>1</sup>, A. Hansson<sup>2</sup>, P. Marvin<sup>2</sup>, E. Barbier<sup>1</sup>, Xu Li, E. Augier<sup>1</sup> and M. Heilig<sup>1</sup>

<sup>1</sup> Center for Social and Affective Neuroscience, IKE, Linköping University, Linköping, 581 83 Sweden.

<sup>2</sup> Institute of Psychopharmacology, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, 68159, Germany.

Alcohol and nicotine are the most commonly co-abused drugs, with a large majority of alcoholics diagnosed with a comorbid addiction to nicotine. The endogenous opioid system is involved in the rewarding properties of both alcohol and nicotine. We previously found that CERC-501, a highly selective KOR antagonist reduced escalated alcohol self-administration induced by the intermittent access to alcohol 20%. In here we tested the effect of CERC-501 on escalation of alcohol drinking induced by nicotine. Chronic nicotine elicited a robust and specific escalation of alcohol drinking without affecting saccharin self-administration and locomotion. CERC-501 did not suppress nicotine-induced increased alcohol self-administration in opposite to naltrexone which blocked escalated drinking. Our in situ hybridization data showed a different pattern of expression and functional activation of MORs in alcohol escalation induced by nicotine, while KORs expression and activity was not affected by the combination of the two drugs. Specifically, our data showed an increased expression and a decreased function of MORs in the ventral tegmental area of alcohol-escalated rats. Nicotine induced escalation of alcohol self-administration was also accompanied by decreased p-DARPP32 in nucleus accumbens shell. This suggest that nicotine pretreatment reduces the rewarding value of alcohol and therefore mediates alcohol escalation. In conclusion our results also suggest that targeting  $\mu$  rather than  $\kappa$ -opioid receptors may represent a promising pharmacotherapeutic approach for the treatment of alcohol use disorders where alcohol consumption is driven by nicotine.

# **Unveiling the alcohol-dependent alterations of local translation in the prefrontal cortex during adolescence**

Laguesse, S., Van Hees, L., Nguyen, L.

Alcohol use disorder (AUD) is a devastating relapsing disease which represents the fourth leading cause of preventable death worldwide. AUD has mainly been considered as a pathological condition in adults, but recent evidence suggests that the roots of alcohol addiction begin to grow during adolescence. Adolescence is a critical developmental period characterized by significant changes in brain architecture and behaviors. Brain maturation begins in posterior regions and progresses towards anterior higher-order regions, including the prefrontal cortex (PFC). The PFC is implicated in executive functions and its immaturity in adolescents is associated with lack of inhibitory control over behavior, increased impulsivity and desire of risk-taking. It is widely believed that the enhanced ability of the adolescent PFC to undergo experience-dependent changes is associated with heightened vulnerability to exogenous agents, including alcohol. Adolescent Alcohol Exposure (AAE) may interfere with the ongoing maturation of frontal brain circuits, leading to profound long-lasting consequences on PFC structure and function. In addition, AAE is related to serious psychological problems, comorbid psychopathology and detrimental neurocognitive consequences, and clinical studies have shown that AAE significantly increases the risk of developing psychiatric and behavioral disorders later in life, including addiction. However, the precise cellular mechanisms underlying the alcohol-induced cognitive and behavioral impairments, the molecular mechanisms underlying defects in PFC maturation, and possible sex differences are still poorly understood. Alcohol addiction is considered as a maladaptive form of learning and memory. Indeed, alcohol is thought to “usurp” the molecular mechanisms underlying those processes, including synaptic plasticity, which depends on the local translation of mRNAs at synaptic sites. It has been shown in adult mice that excessive alcohol consumption modifies synaptic protein composition in brain regions associated with the mesocorticolimbic pathway, promoting the development and maintenance of addiction. Here we use a mouse model of voluntary adolescent binge drinking to study the alcohol-dependent structural and functional defects in the PFC as well as the behavioral consequences. We report that excessive alcohol consumption during adolescence leads to long-lasting behavioral impairments in adulthood, such as increased anxiety and alcohol intake as well as reduced cognitive performances, both in males and females. By using transgenic mouse lines and Ribotag profiling, we are comparing the synaptic transcriptome of specific neuronal populations in the PFC (i.e. glutamatergic neurons and interneurons) in order to identify candidate synaptic mRNAs whose translation levels are modified by AAE.

## **Neural correlates of implicit emotional processing in binge drinking**

Séverine Lannoy<sup>1</sup>, Fabien Gierski<sup>1,2,3</sup>, Laurence Dricot<sup>4</sup>, Farid Benzerouk<sup>1,2</sup>, Christophe Portefaix<sup>5</sup>, Sarah Barrière<sup>2</sup>, Véronique Quaglino<sup>6</sup>, Arthur Kaladjian<sup>1,2</sup>, & Mickaël Naassila<sup>3</sup>

<sup>1</sup> Cognition Health and Society Laboratory (EA 6291), Université de Reims Champagne-Ardenne, Reims, France.

<sup>2</sup> Psychiatric Department, EPSM-Marne, Reims, France.

<sup>3</sup> INSERM U1247 GRAP, Research Group on Alcohol and Drugs, Université de Picardie Jules Verne, Amiens, France.

<sup>4</sup> Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium.

<sup>5</sup> Imaging Center, University Hospital, Reims, France.

<sup>6</sup> CRP-CPO (EA7273), Université de Picardie Jules Verne, Amiens, France.

Binge drinking is a widespread alcohol consumption pattern in young people, defined by occasional but high alcohol intoxications (Courtney and Polich, 2009). It has been related to deleterious consequences such as brain modifications and cognitive dysfunctions (Carbia et al., 2018; Hermens et al., 2013; Maurage et al., 2013b). Recent studies have also underlined a difficulty to process emotions in young binge drinkers (e.g., Huang et al., 2017; Lannoy et al., 2018b), suggesting that this alcohol consumption pattern may also be associated with emotional impairments. Importantly, emotional impairments have been identified as key factors to describe severe alcohol-use disorders but also explain relapse risk (Rupp et al., 2017). Regarding the binge drinking alcohol consumption pattern (i.e. alternation between high intoxications and withdrawals), research proposed that it would induce similar brain alterations than severe alcohol-use disorders (Stephens and Duka, 2008). This proposal supports the continuum hypothesis, suggesting that binge drinkers would be characterized by qualitatively similar impairments than patients with severe alcohol-use disorders (Enoch, 2006; Sanhueza et al., 2011). Therefore, the existence of emotional impairments in binge drinking could precipitate the development of chronic and severe alcohol-related disorders (e.g., Wills et al., 2016). Accordingly, it appears central that future binge drinking studies explore emotional processing and their brain correlates to precisely determine their role in the maintenance of excessive alcohol use and the possible development of severe alcohol-use disorders.

The current literature mainly showed that when binge drinkers had to process emotional contents (e.g., identification, recognition), they performed poorly than control participants (Lannoy et al., 2018b; Maurage et al., 2013a). Disrupted brain activations and electrophysiological activities were also observed during the identification of emotional prosodies (Maurage et al., 2009, 2013a) and emotional crossmodal integration between facial and vocal expressions (Lannoy et al., 2018a). Moreover, altered electrophysiological activities were also found during the view of positive and negative affective scenes (Connell et al., 2015;

Huang et al., 2017). Nevertheless, it is unclear whether the simple view of emotions (i.e. when no specific processing is required on emotional stimuli) already lead to alterations at the brain level, as preliminary observed for the processing of affective scenes.

In the current study, we explored, beyond the ability to explicitly recognize emotions, the brain activations related to the simple view of emotional stimuli in binge drinkers and controls. Behavioral and neuroimaging findings were combined to explore brain responses during the view of emotional facial expressions (happiness, anger, sadness, fear, contempt) while participants performed a gender categorization task. Preliminary analyses showed specific patterns of activations in binge drinking, such as increased responses in the anterior cingulate cortex during the implicit processing of fear. By highlighting disrupted brain activations whereas no direct emotions processing is required, these results extend the understanding of emotional difficulties in binge drinking. They also support the continuum hypothesis regarding emotional alterations between binge drinking and alcohol-use disorders and reinforce that emotional impairments may be considered a central vulnerability factor in alcohol-related disorders.

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# Opposing effects of the hormones leptin and ghrelin on neural alcohol cue-reactivity, craving and relapse in alcohol addiction:

## Two streams merge to one river?

Patrick Bach MD<sup>1,2</sup>, Jan Malte Bumb MD<sup>1,2</sup>, Rilana Schuster MD<sup>1,2</sup>, Sabine Vollstädt-Klein PhD<sup>1,2</sup>, Iris Reinhard<sup>3</sup>, Marcella Rietschel MD<sup>4</sup>, Stephanie H. Witt PhD<sup>4</sup>, Klaus Wiedemann MD<sup>5</sup>, Anne Koopmann MD<sup>1,2</sup>, Falk Kiefer MD<sup>1,2</sup>

<sup>1</sup> Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University

<sup>2</sup> Feuerlein Center on Translational Addiction Medicine (FCTS), University of Heidelberg

<sup>3</sup> Department of Biostatistics, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University

<sup>4</sup> Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University

<sup>5</sup> Department of Psychiatry & Psychotherapy, University Medical Center, Hamburg; Martinistr. 52; 20246 Hamburg

### INTRODUCTION

Increasing evidence supports the role of appetite-regulating hormones in the pathophysiology of alcohol addiction. Amongst those, leptin and ghrelin and a “cross-talk” between both hormones were implicated in the pathophysiology of alcohol addiction, both modulating alcohol craving and drug-seeking (Hirth *et al.*, 2016). Preclinical and clinical data thus far indicate that leptin and ghrelin interact with each other and that both modulate the signaling rate of dopaminergic neurons in reward networks (Fulton *et al.*, 2006; Farooqi *et al.*, 2007; Haass-Koffler *et al.*, 2015). However, their role in alcohol addiction is far from being understood, especially the neurobiological underpinnings of their effects remains to be elucidated. To address this issue, we investigated the association between leptin and acylated ghrelin and mesolimbic brain response to alcohol cues, alcohol craving and relapse risk in a sample of seventy alcohol-dependent patients in the post-acute withdrawal phase.

### METHODS

A total of seventy abstinent alcohol-dependent patients were recruited from an in-patient setting in the Clinic of Addictive Behavior and Addiction Medicine of the Central Institute of Mental Health (Mannheim, Germany) after having completed detoxification treatment (mean abstinence = 11.6 days, range = 5 - 25). All patients underwent a combined psychometric and functional magnetic resonance imaging (fMRI) assessment of alcohol cue-reactivity and alcohol craving using a validated set-up (Vollstadt-Klein S, 2012). In addition, plasma levels of leptin, total and acetylated, active ghrelin were measured prior to the fMRI session after overnight fasting. Additionally, relapse data was collected during the three months following the assessment using a semi-structured interview, which incorporated the Alcohol Timeline Followback questionnaire (Sobell *et al.*, 1996). Brain imaging data were preprocessed and analyzed using the statistical parametric mapping software for Matlab (SPM), according to standard procedures and previous studies (Bach *et al.*, 2015). Associations between hormone levels and mesolimbic cue-reactivity were tested using multivariate regression models in SPM, using a combined voxel- and cluster-extent threshold that corresponds to a family wise error rate correction of  $p_{FWE} < 0.05$ . Cox regression analyses were performed to assess the associations between leptin, acylated ghrelin and relapse risk during the three months following the experiment.

### RESULTS

Analyses of psychometric data showed that leptin plasma levels were negatively correlated with the scores of the Obsessive Compulsive Drinking Scale ( $r = -0.305$ ,  $p = 0.020$ ,  $p_{FDR} = 0.040$ ). For ghrelin, there was a positive association between acylated ghrelin levels and changes in the intention to drink alcohol, such that higher acylated ghrelin levels were associated with an increase in the intention to drink alcohol ( $r = 0.331$ ,  $p = 0.006$ ,  $p_{FDR} = 0.024$ ). Multiple regression analyses in SPM showed a significant negative association between leptin plasma levels and alcohol cue-induced activation as the dependent variable in left (77.2% of cluster) and right caudate (18.3% of cluster), with a relevant proportion being located in the dorsal striatum (20.6 %), while only a small proportion was located in the ventral striatum (5.1%, combined threshold, corresponding to  $p_{FWE} < 0.05$ ). In addition, mean alcohol cue-induced activation extracted from bilateral caudate, negatively correlated with plasma leptin levels ( $r = -0.316$ ,  $p = 0.016$ ,  $p_{FDR} = 0.040$ ), corroborating the results of the whole brain analyses. In contrast, acylated ghrelin showed a significant positive association to alcohol cue-induced activation in several clusters of brain areas, including the bilateral insulae and parts of the superior and middle frontal gyri, as well as the middle cingulum. Further, the mean functional activation in the left and right insula significantly correlated with acylated ghrelin levels ( $r = 0.279$ ,  $p = 0.013$ ,  $p_{FDR} = 0.026$ ). Cox regression analyses showed a significant association between leptin and time to heavy-relapse, such that high leptin levels during the post-acute phase of withdrawal were associated with a longer time to first heavy-relapse ( $\text{Chi}^2$  overall model = 4.308, Hazard Ratio = 0.922, 95%CI 0.853 – 0.996,  $p = 0.039$ ), while acylated ghrelin and BMI did not contribute to the prediction of time to heavy-relapse ( $p > 0.684$ ).

## CONCLUSION

We could show that leptin and acylated ghrelin showed opposing associations with the extent of alcohol cue-induced mesolimbic cue-reactivity and alcohol craving. Our finding of a negative association between leptin and cue-reactivity in the bilateral caudate and striatum is in line with previous evidence that supplementation of leptin attenuates mesolimbic hyper-activation in the NAc, caudate and putamen of leptin-deficient patients (Baicy *et al.*, 2007; Farooqi *et al.*, 2007). The present results also mirror findings of animal studies showing that leptin modulates firing of dopaminergic neurons in the VTA that project to the striatum (Fulton *et al.*, 2006). We thus suspect that the reduced striatal cue-reactivity might be the neurobiological correlate of leptin's effect on relapse-risk. The findings of a positive association between acylated ghrelin and cue-induced brain response in the left and right insula, harmonize with previous studies that showed that intravenous administration of ghrelin to healthy volunteers during an fMRI food-cue task, increased brain response in the amygdala, orbitofrontal cortex, insula, and striatum (Malik *et al.*, 2008), further supporting the plausibility of BOLD response modulation by ghrelin. The reported results further support the relevance of appetite regulating hormones in the pathophysiology of addiction and their potential role as future treatment targets.

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# Altered GABA-signaling in the amygdala contributes to pathological alcohol choice over high value alternative rewards

## Full name and Affiliations:

Eric Augier, PhD  
Linköping University, Institute for Clinical and Experimental Medicine (IKE)  
Center for Social and Affective Neuroscience (CSAN)  
581 85 Linköping (Sweden)  
Mobile: +46 (0)722715017  
Visiting address: Cellbiologen pl12, ing 64, Campus US  
Email: [eric.augier@liu.se](mailto:eric.augier@liu.se)

**Introduction:** Alcohol addiction is characterized by a progressive shift of decision making, in which alcohol is increasingly chosen over healthy non-drug rewards. Only a subset of people transition from recreational to addictive alcohol use, a pattern that is similar to that of other addictions. By contrast, in commonly used animal models, nearly all rats learn to self-administer addictive drugs, including alcohol and animals have no alternative to drug use. These models fail to capture an important and common feature of human addiction: a continued use of the substance despite the opportunity to engage in important and meaningful social and recreational activities.

**Purpose:** The neurobiological underpinnings of choosing alcohol over a natural reward in the context of developing alcoholism are presently unknown. Here, we set out to identify molecular mechanisms underlying this choice behavior.

**Methods:** We first employed an exclusive choice-based method to identify rats that continue to self-administer alcohol at the expense of a high-value natural reward, a sweet solution, and assessed whether these animals show other characteristics reminiscent of clinical alcoholism. Specifically, we measured their motivation to obtain and take the drug (modeled using an elevated breakpoint on a progressive-ratio schedule) and their continued drug use despite its harmful and negative consequences (here modeled using continued alcohol self-administration despite quinine adulteration or delivery of a shock punishment contingent with drug delivery). We then carried out a discovery effort using gene expression profiling.

**Results:** Using this procedure in outbred rats, we were able to identify two populations characterized by distinct patterns of choice behavior. The vast majority of rats stop responding for alcohol when offered the opportunity to access a high-value alternative reward (SP, Saccharin-preferring). However, a subpopulation (15% across multiple batches of outbred Wistar rats, a proportion similar to human alcohol addiction rates) continues to choose alcohol despite the presence of the alternative (AP, Alcohol-preferring). Rats that choose alcohol display a constellation of behavioral traits reminiscent of clinical alcoholism, mimicking key clinical diagnostic criteria for alcoholism. Specifically, they show elevated motivation to obtain alcohol, as measured by progressive ratio breakpoints and continue to take alcohol despite negative consequences that make the majority of rats stop self-administration, namely adulteration with increasing concentrations of the bitter tastant quinine, or pairing of the alcohol delivery with foot-shock.

Furthermore, a differential gene expression screen using a custom NanoString nCounter panel found minimal evidence for differential gene expression between alcohol choosing vs non-choosing rats in several brain structures examined, but did identify that a marked dysregulation of a GABAergic

pathway within central amygdala (CeA) was associated with addiction-like phenotype. A >50% down-regulated expression of the g-aminobutyric acid (GABA) transporter GAT-3 (*Slc6a11*) in this structure was accompanied by similarly down-regulated transcripts encoding several GABA<sub>A</sub> receptor subunits. This indicated the possibility of elevated inhibitory GABA-tone due to impaired clearance of extracellular GABA by the transporter, a hypothesis that was confirmed using slice electrophysiology. Additionally, a viral-vector mediated knockdown of GAT-3 resulted in increased GABA-mediated inhibition in the CeA in a slice electrophysiology experiment, and converted SP rats into AP rats *in vivo*, demonstrating a causal role of GAT-3 for alcohol choice.

Finally, we assessed whether these results may have translational relevance and carried out an RNAseq transcriptome analysis in post-mortem tissue from alcohol dependent people and controls. We found that

GAT-3 expression was selectively decreased in the central amygdala of alcohol-dependent people compared to those who died of unrelated causes.

**Conclusion:** Our data provide strong support for a causal contribution of neuroadaptations affecting GABA signaling within the amygdala to the development of alcohol addiction. Furthermore, our findings suggest that pre-existing differences in GABAergic gene expression in the CeA may also influence susceptibility to developing alcohol addiction. Collectively, these experiments identify that impaired GABA clearance within the amygdala contributes to alcohol addiction, appears to translate between species, and may offer targets for new pharmacotherapies for treating this disorder.

Key words: individual vulnerability, choice, alcoholism, GAT-3, GABA

## INVITED TALK

- [Targeting Biased Decision Making in the Treatment of Alcohol Use Disorders](#)  
*WIERS Reinout (Addiction Development and Psychopathology (ADAPT) Lab, Dept of Psychology, Universiteit van Amsterdam)*

ESBRA 2019

Abstract

Reinout Wiers

### **Targeting Biased Decision Making in the Treatment of Alcohol Use Disorders**

Alcohol Use Disorder (AUD) and other addictions have been characterized as a chronic brain disease from the biomedical perspective and as the unfortunate outcome of adverse social conditions from the social science perspective. We emphasize biased decision making as a central characteristic in (alcohol) addiction. From a therapeutic perspective, the important question is to what extent these biases reverse after successful abstinence, and to what extent they can be reversed through targeted training. Two types of Cognitive Training (CT) can be distinguished: those in which general abilities are trained (e.g., working memory training) and those in which initial motivational reactions to alcohol are targeted, so called cognitive biases (Cognitive Bias Modification, CBM, Wiers 2018). I will review the state of affairs in both. Training of general abilities takes a long time, but does show promise for a subgroup of patients. CBM has shown to increase one-year abstinence in several large clinical trials, with effect sizes similar to medication for alcohol (NNT=12). It is also becoming clear for which individuals CBM shows most promise as an add-on treatment (those with a strong cue-reactivity and/or impulsivity), and we are beginning to understand the neurocognitive mechanisms underlying training effects (e.g., reduced cue-reactivity). CT shows modest but reliable effects as add-on to regular psychosocial treatment, but does not appear to work in the absence of psychosocial treatment, nor in the absence of motivation to change (e.g. in proof-of-principle studies in students). Finally, I will sketch ways forward, such as combining training with neurostimulation. Together these findings emphasize the malleability of the addicted brain and the promise of targeted CT in the treatment of AUD.

## SYMPOSIUM

### Cellular and Extracellular Effects of Alcohol Exposure in Liver and Extrahepatic Organs

*CASEY Carol (University of Nebraska Medical Center)*

- **TP-R Deletion Promotes Adipose Tissue Browning and M2 Macrophage Polarization with a Concomitant Reduction in Alcohol-associated Liver Injury in Mice**  
*VISWANATHAN Saras (Veterans Affairs Nebraska-Western Iowa Health Care System & Department of Medicine, University of Nebraska Medical Center, Omaha, NE)*
- **Down-regulation of Rab3D: Critical Role in Alcohol-induced Liver Injury**  
*CASEY Carol (University of Nebraska Medical Center)*
- **Complement Activation in Alcoholic Hepatitis: Possible Association with Acute Kidney Injury**  
*NAGY Laura (Northern Ohio Alcohol Center, Department of Inflammation and Immunity, Cleveland Clinic, Cleveland OH)*
- **Masked Hemolysis as Important Factor of Iron Overload in ALD**  
*RAUSCH Vanessa (Center for Alcohol Research and Salem Medical Center, University of Heidelberg, Heidelberg, Germany)*

## **TP-R Deletion Promotes Adipose Tissue Browning and M2 Macrophage Polarization with a Concomitant Reduction in Alcohol-associated Liver Injury in Mice.**

Saraswathi Viswanathan, Terrence Donohue and Carol Casey.

Veterans Affairs Nebraska-Western Iowa Health Care System & Department of Medicine, University of Nebraska Medical Center, Omaha, NE

Ethanol (EtOH) administration leads to changes in adipose tissue (AT) lipid storage function and metabolism. In a model of non-alcoholic fatty liver disease (NAFLD), mice lacking thromboxane-prostanoid receptor (TP-R) were protected against NAFLD with a concomitant increase in markers of AT browning. Here, we hypothesized that ethanol would induce AT lipolysis to promote AT browning and that blocking TP-R would enhance EtOH-induced AT browning and attenuate free fatty acid (FFA) flux to liver which would ameliorate alcohol-associated liver disease (AALD). Male TP-R knock out (KO) and wild type (WT) littermate controls were fed Lieber-DeCarli control or EtOH liquid diets for 4 wk. Chronic EtOH feeding increased mRNA for *Ucp-1*, a marker of AT browning, in visceral AT (VAT) of WT mice ( $P<0.05$ ) which was further elevated in TPR-KO mice ( $P<0.01$ ). Moreover, we found a remarkable increase in  $\beta$ 3-adrenergic receptor (*Adrb3*) mRNA expression, which stimulates AT browning, in VAT of KO mice but not in WT mice after EtOH exposure ( $P<0.01$ ). RNAs encoding M2 anti-inflammatory macrophages were significantly increased in EtOH fed-KO, but not WT mice. On the other hand, mRNA levels of *Ccl2* (encoding MCP-1), an inflammatory marker, rose significantly in EtOH-fed WT, but not KO mice. Plasma MCP-1 levels followed this same trend. Our data show that blockade of TP-R enhances the effect of EtOH in inducing browning markers in AT. Additionally, TP-R deletion leads to AT macrophage polarization and an M2 anti-inflammatory phenotype. Thus, blocking TP-R function in AT may be effective in ameliorating AALD.

## **Down-regulation of Rab3D: Critical Role in Alcohol-induced Liver Injury**

Carol Casey, Karuna Rasineni, Terrence Donohue, Paul Thomes, Armen Petrosyan

Departments of Internal Medicine and Biochemistry, University of Nebraska Medical Center, Omaha, NE and the Department of Veterans' Affairs, Omaha, NE, USA

The goal of our current work is to examine how ethanol exposure results in impaired function of the Golgi apparatus. The Golgi apparatus (also called the Golgi body or Golgi complex) packages proteins into membrane bound vesicles inside the cell before the vesicles are sent to their destination. As such, this organelle resides at the intersection of the secretory, lysosomal, and endocytic pathways; it is known to be of particular importance in processing proteins for secretion. Previous work from our laboratory has identified multiple defects in endocytosis, protein trafficking, and secretion, after alcohol administration, but we have not until now, examined a role for altered Golgi function in these processes. Of central importance to our study is the role of a small GTPase, Rab3D, which is involved in exocytosis, secretion and vesicle trafficking. We have shown that Rab3D protein content was significantly decreased after alcohol administration, and recently we have obtained exciting new preliminary data that ethanol-impaired Rab3D function plays an important role in Golgi disorganization and fragmentation. We are focusing our examination on a role for Rab3D in transport through the Golgi, and have data showing how alcohol-induced remodeling of Golgi morphology is a significant impairment of post-Golgi trafficking, and may lead to utilization of *trans*-Golgi membranes for the formation of autophagosomes. With our expertise and experimental models, we hope to provide novel insights as to how alcohol affects trafficking of proteins within the liver cells leading to toxicity.

# Complement Activation in Alcoholic Hepatitis: Possible Association with Acute Kidney Injury

Laura E Nagy,

Northern Ohio Alcohol Center, Department of Inflammation and Immunity, Cleveland Clinic, Cleveland OH

Complement contributes to liver injury in murine models of alcoholic liver disease (ALD); however, the role of complement in patients with alcoholic hepatitis (AH) is not well studied. Acute Kidney Injury (AKI) is a major predictor of mortality in severe AH. Since complement activation contributes to AKI in other kidney diseases, here we investigated the association between complement activation and AKI in ALD, making use of both murine models and patient samples. When mice are exposed to carbon tetrachloride (CCl<sub>4</sub>) in combination with chronic intra-gastric ethanol feeding, they develop an alcoholic liver fibrosis-associated AKI ([10.1016/j.taap.2016.09.011](https://doi.org/10.1016/j.taap.2016.09.011)). Using publically available data, we found that 23 of the 1996 differentially expressed genes in AKI mice were complement genes (GSE83529). Further, when DEGs were filtered into the protein-protein interaction (PPI) network complex, 8 of the top 30 hub DEGs were found to be complement genes. We also find evidence of complement activation in liver explants from patients with AH compared to healthy controls (tissue from the Clinical Resources for AH Investigations at Johns Hopkins University). Further, the concentrations of C5a, a potent anaphylatoxin, and factor Ba, an indicator of alternative pathway activation, were elevated in both the circulation and urine from patients with AH from the DASH consortium compared to healthy controls. Kidney Injury Marker-1 (KIM-1) increased in urine of severe AH patients compared to moderate AH. Taken together, these data suggest that complement activation is associated with AKI in AH and inhibition of complement activation may be a potential target for clinical intervention.

# Masked Hemolysis as Important Factor of Iron Overload in ALD

Rausch, Vanessa; Silva, Inês; Peccerella, Teresa and Mueller, Sebastian

Center for Alcohol Research and Salem Medical Center, University of Heidelberg, Heidelberg, Germany

**Background:** 50% of ALD patients develop hepatic iron overload (HIO) and anemia, however, the underlying mechanisms including hepcidin response are poorly understood. Herein, we introduce hemolysis as novel factor in disrupting hepcidin regulation and eventually causing iron overload.

**Method:** We here studied hepcidin, molecular and laboratory iron markers in ALD patients (n=831, mean alcohol consumption 192 g/day). The effect of hemolysis was further studied in C57BL/6 mice using phenylhydrazine (PHZ)-induced hemolysis model. Finally, *in vitro* erythrophagocytosis model was used to recapitulate *in vivo* findings and to investigate the underlying mechanisms.

**Results:** Indirect evidence for hemolysis (anemia, ferritin, LDH, MCV, CD163) as cause for HIO was found in 16.4% of heavy drinkers. Despite higher ferritin levels as compared to controls, hepcidin was not adequately upregulated in hemolytic patients suggesting a suppressive effect. In confirmation, PHZ-induced hemolysis (anemia, transaminases, LDH) suppressed hepcidin in mice leading to anemia, elevated transaminases and transferrin saturation and LDH levels. Phagocytosed erythrocytes were detected in the spleen and iron-loaded Kupffer cells in the liver. *In vitro*, erythrophagocytosis led also to the suppression of hepcidin at higher pathological levels of oxidized erythrocytes.

**Conclusion:** Our data suggest that suppression of hepcidin by masked hemolysis seems to be an important factor contributing to HIO in ALD patients.

## SYMPOSIUM

### SECOND HITS IN ALCOHOL-RELATED ORGAN DAMAGE

*KHARBANDA Kusum (VA Medical Center, University Nebraska Medical Center)*

- **Alcohol on fat: worse consequences for liver injury**  
*SETH Devanshi (Drug Health Services, Royal Prince Alfred Hospital, Camperdown, Australia. 3Faculty of Medicine, The University of Sydney, Sydney, Australia. 4Centenary Institute of Cancer Medicine and Cell Biology, The University)*
- **Liver stiffness as a novel prognostic marker in heavy drinkers: First data from a prospective 10-year follow-up study**  
*Sebastian Mueller, MD, PhD (Dept. of Internal Medicine, Salem Medical Center and Center for Alcohol Research, University of Heidelberg, Germany)*
- **Reactive aldehydes from Alcohol and Cigarette Smoke co-exposure impairs lung innate anti-microbial defense**  
*Todd A. Wyatt. PhD (University of Nebraska Medical Center, USA)*
- **Matrix Stiffness Regulates Fibrosis Progression in Alcohol-Induced Liver injury**  
*KIDAMBI Srivatsan (University of Nebraska-Lincoln)*

**Title:** Alcohol on fat: worse consequences for liver injury

**Speaker's name:** Devanshi Seth, PhD

**Devanshi Seth, PhD**

Drug Health Services

Royal Prince Alfred Hospital Camperdown

and Centenary Institute of Cancer Medicine and Cell Biology

Central Clinical School

The University of Sydney

Sydney, NSW, Australia.

Email: [d.seth@sydney.edu.au](mailto:d.seth@sydney.edu.au)

**Authors:** E Huang, AMP Duly, C Yee, SV McLennan and D Seth

**Abstract:**

**BACKGROUND AND AIMS:** Drinkers who are obese are more likely to develop liver cirrhosis than those within a healthy weight range implying the potential for an interaction between alcohol and obesity. Experimental models of alcohol and high fat diet (HFD) alone have proven difficult to induce severe liver injury even after several weeks of treatment. LPS is commonly required as a 'second hit' with alcohol to advance steatosis to steatohepatitis and in diet-related obesity models induction of diabetes accelerates liver injury. We studied the interaction between alcohol and HFD in liver injury mouse model comparable to human setting of episodic heavy drinking with fat-rich food.

**METHODS:** C57BL6 male mice were fed either chow or high fat diet (HFD) ad libitum for 12 weeks. A sub-set of mice from each group were also given alcohol (2g/kg body weight) twice/week via intra-gastric lavage. Liver injury was examined by histopathology and liver/serum biochemistry. Expression of molecules related to lipid metabolism, inflammation and fibrogenesis were examined (Q-PCR, immunofluorescence).

**RESULTS:** We show that chronic moderate alcohol exacerbates liver injury in a mouse model of HFD compared to either alcohol or HFD alone. Alcohol superimposed on HFD increased serum and liver lipids, triglycerides, cholesterol, circulating insulin, inflammation and several molecules related to lipid processing. In addition, immune cells also increased with Alcohol+HFD. Whereas alcohol alone moderately increased ethno-adducts, Alcohol+HFD fed animals showed a striking elevation of etheno-DNA adducts clusters within the liver suggesting a precancerous profile in our model.

**CONCLUSION:** Alcohol on fat worsens liver injury.

**Title:** Liver stiffness as a novel prognostic marker in heavy drinkers: First data from a prospective 10-year follow-up study

**Speaker's name:** Sebastian Mueller, MD, PhD

**Sebastian Mueller, MD, PhD**

Professor of Medicine

Vice Head, Medical Department (Gastroenterology, Hepatology), Salem Medical Center and Co-Director, Center for Alcohol Research, University of Heidelberg

Zeppelinstraße 11 - 33

69121 Heidelberg

Email: [sebastian.mueller@urz.uni-heidelberg.de](mailto:sebastian.mueller@urz.uni-heidelberg.de)

**Abstract:**

**Background and Aims:** We here present first data on the prognostic impact of liver stiffness (LS) on long-term survival of Caucasian heavy drinkers in a 10 year, prospective single center trial.

**Method:** Information of survival status was obtained in 675 (71.6%) of 943 screened patients that had presented for alcohol detoxification over a 10-year period from 2007-2017 with a mean daily consumption of alcohol of 178 g. Mean observation time was 3.7 years and mean duration of heavy drinking was 14.0 years. All patients had LS measurements by transient elastography and routine laboratory tests.

**Results:** During the observation time, 106 patients (15.7%) died. The cause of death could be clarified in 42 patients (39%) and it was liver-related in 16 (38%). Overall death was highest associated with LS ( $r=0.291$ ,  $P=1.3E-14$ ), followed by hemoglobin and alkaline phosphatase (AP). In a multivariate proportional hazard model, LS next to age, AP and serum albumin was the most significant independent predictor of survival with a hazard ratio of 1.013 (1.003 to 1.023,  $P<0.05$ ). Using ROC analysis, LS was the best predictor of death in general with an AUROC of 0.72 and a cutoff value of 14.0 kPa, followed by AP and albumin. Moreover, LS was the top predictor of death starting from 2 to 5 years. In contrast, LS was preceded by bilirubin and albumin in predicting one-year-survival.

**Conclusion:** We here identify LS as the best long-term prognostic parameter in patients who heavily consume alcohol. LS measurements should become an important parameter for the screening of alcoholics. **Description:** This presentation will be on the first preliminary data on the 10-year follow-up on the survival and risk factors in heavy drinkers.

**Title:** Reactive aldehydes from Alcohol and Cigarette Smoke co-exposure impairs lung innate anti-microbial defense

**Speaker's name:** Todd A. Wyatt. PhD

**Todd A. Wyatt, PhD**

Professor

Department of Environmental, Agricultural & Occupational Health

College of Public Health and

Professor of Medicine

Division of Pulmonary, Critical Care, Sleep & Allergy

Department of Internal Medicine

University of Nebraska Medical Center

Omaha, NE, USA

Email: [twyatt@unmc.edu](mailto:twyatt@unmc.edu)

**Abstract:** The vast majority of individuals with an alcohol use disorder smoke cigarettes. Alcohol misuse and cigarette smoking are co-morbidities resulting in the significant susceptibility to lung infections leading to pneumonia. Several innate lung defense mechanisms are altered by the combination of drinking alcohol and smoking cigarettes. Mucociliary clearance is negatively impacted by smoke and alcohol through cilia slowing and ciliated cell detachment caused by the co-exposure-induced activation of protein kinase C epsilon. Malondialdehyde and acetaldehyde are generated via both alcohol metabolism as well as the pyrolysis of tobacco leading to the formation of stable hybrid adducts known as MAA adducts. Lung surfactant protein is altered through MAA adduction resulting in decreased anti-microbial action by the collectin. Lastly, the secretion of lung mucosal immunoglobulin A is decreased through the action of MAA adducts in a TGF beta-dependent manner. Both airway epithelial cells and macrophages bind MAA adducted protein via CD204 suggesting that differential expression of CD204 polymorphisms may govern injury due to co-exposure. Thus, the suppression of lung innate defense is multi-faceted in alcohol misuse due to the comorbidity of cigarette smoking.

**Title:** Matrix Stiffness Regulates Fibrosis Progression in Alcohol-Induced Liver injury

**Speaker's name:** Srivatsan Kidambi, PhD

**Srivatsan Kidambi, PhD**

Associate Professor

Department of Chemical & Biomolecular Engineering

University of Nebraska - Lincoln

207 Othmer Hall, 820 North 16th Street

Lincoln, NE 68588, USA

Email: [skidambi2@unl.edu](mailto:skidambi2@unl.edu)

**Authors:** Senthilkumar Thulasingham, Michael Moeller, Madhusudanan Narasimhan, Carol Casey, Srivatsan Kidambi<sup>1</sup>

**Abstract:** Liver stiffness (LS) is widely used clinically to monitor, staging and diagnosis, of alcoholic fatty liver diseases (AFLD) and is also believed to predict improved outcome. The regulatory mechanisms of LS in regulating liver function during ALD, specifically, fibrosis, is incompletely understood. This study aims to uncover significant mechanisms underlying the role of increasing matrix stiffness during liver injury in the promoting fibrogenesis induced by alcohol. We hypothesize that LS is just not the readout of fibrosis but also an active contributor of the progression of liver fibrosis and stellate cell activation in AFLD. Using our innovative biomimetic liver fibrosis model that allows modulation of substrate stiffness (2 kPa, 9 kPa, 25 kPa and 55 kPa mimicking healthy, early fibrotic, fibrotic and extremely fibrotic substrates), we investigated the role of liver matrix stiffness in modulating primary hepatocytes and stellate cell function. In vitro experiments were designed using the conditioned medium (CM) of primary hepatocytes (isolated from alcohol fed rats) cultured on stiffness mimicking healthy and fibrotic environment supplemented to human stellate cells (HSC). A significant increase in HSC proliferation, and expression of fibrosis-related genes were observed in cells treated with CM from stiffer matrix. Together, all these data demonstrates the plausible role of stiffness in regulating hepatocytes function and contribute to stellate cells activation and progression of liver fibrosis during alcohol liver disease. Understanding the impact of stiffness on hepatocytes biology will provide significantly more nuanced data to aid drug development for AFLD and liver fibrosis.

## SYMPOSIUM

What can we learn from epidemiology in alcohol research? Recent findings from large population-based cohorts

*AIRAGNES Guillaume (AP-HP, Hôpitaux Universitaires Paris Ouest)*

- Communication 1: "Do addictive behaviors explain social inequality regarding depression? Findings from the Constances cohort."  
*MATTA Joane (Inserm, UMS11, Population-based Epidemiologic Cohorts, Villejuif, France.)*
- Communication 2: "Development of a predictive clinical tool of alcohol-related consequences: Results from the National Epidemiologic Survey on Alcohol and Related Conditions."  
*HOERTEL Nicolas (AP-HP, Hôpitaux Universitaires Paris Ouest, Department of Psychiatry and Addictology. Inserm, U894, Centre Psychiatrie et Neurosciences.)*
- Communication 3: "Associations of depressive symptoms with alcohol use: findings from the Constances cohort."  
*WIERNIK Emmanuel (Inserm, UMS11, Population-based Epidemiologic Cohorts, Villejuif, France.)*
- Communication 4: "Do personality traits predict alcohol consumption decades after their assessments? Findings from the Gazel cohort."  
*AIRAGNES Guillaume (AP-HP, Hôpitaux Universitaires Paris Ouest, Department of Psychiatry and Addictology, Paris, France. Inserm, UMS11, Population-based Epidemiologic Cohorts, Villejuif, France.)*

## **Communication 1: "Do addictive behaviors explain social inequality regarding depression? Findings from the Constances cohort."**

**Speaker: Joane Matta, PhD.**

**Authors:** Joane Matta, Nicolas Hoertel, Guillaume Airagnes, Emmanuel Wiernik, Frédéric Limosin, Marcel Goldberg, Marie Zins, Cédric Lemogne.

### **Abstract:**

**Objective.** To examine the associations between depression and alcohol, tobacco and cannabis, taking into consideration socioeconomic status (SES). **Methods.** We applied mediation and moderated mediation models stratified for sex to a nationally representative sample (N=37,192) of French men and women from the Constances cohort with baseline and follow-up measures regarding depressive symptoms. The structural equation model tested the associations between low SES status (income and education, separately) and depressive symptoms at follow-up mediated by alcohol, smoking and cannabis, while taking into consideration age and depressive symptoms at baseline. A second set of analyses tested the mediation and moderation models with interactions between SES and substances. **Results.** Mediation analyses using low education or low income did not explain the association between SES and depressive symptoms in either men or women. Mediation and moderation models showed that direct effects were not significant in the presence of interactions and that the moderation effect was largely significant. Direct associations between substances and SES or depressive symptoms were only retained for tobacco use. In the low education models, the estimate of moderation was  $0.278 \pm 0.076$  and  $0.182 \pm 0.09$  in men and women respectively. Strong moderation effects were also found in the low income models ( $0.348 \pm 0.076$  and  $0.247 \pm 0.08$  in men and women, respectively). **Conclusion.** Prevention strategies targeting at risk subgroups should consider SES and substance use not only as a cumulative risk factor but take into account their interplay; particularly regarding tobacco use, whatever the underlying mechanisms. Future studies should investigate mechanisms related to the observed associations.

**Communication 2: "Development of a predictive clinical tool of alcohol-related consequences: Results from the National Epidemiologic Survey on Alcohol and Related Conditions."**

**Speaker: Nicolas Hoertel, MD, PhD.**

**Authors:** Nicolas Hoertel, Marie Dosquet, Hugo Peyre, Carlos Blanco, Géraldine Ducoutumany, Philip Gorwood, Henri Leleu, Guillaume Airagnes, Cédric Lemogne, Henri-Jean Aubin, Frédéric Limosin.

**Abstract:**

**Objective.** To develop an individualized risk calculator tool of the 3-year risk of 6 important alcohol-related medical, psychological and social adverse outcomes based on predictor variables that are easily and routinely collected in primary healthcare settings. **Material.** A nationally representative sample of US adults aged 18 years or older was interviewed 3 years apart in the National Epidemiologic Survey on Alcohol and Related Conditions (wave 1, 2001-2002; wave 2, 2004-2005). Analyses concerned 22,009 respondents interviewed in both waves and using alcohol in wave 1. This sample was randomly split into a construction (N=11,013) and a validation sample (N=10,996). **Methods.** The 3-year risk of 6 important alcohol-related adverse outcomes (i.e., alcohol use disorder, withdrawal symptoms, occurrence of tremors or seizures, interpersonal relationship problems and legal problems) was modeled in the construction sample using logistic regression, with age, sex and the 3 AUDIT-C variables as predictors. Scoring was used to combine information derived from predictors and quantify alcohol-related risks for each subject. Discrimination and calibration were assessed in the validation sample based on the C-index and the Hosmer and Lemeshow (H-L) test. **Results.** The predictive values of the risk equations were good (C-index ranging from 0.75 to 0.83) and calibrated well (all H-L test p-values>0.44) in the validation sample, showing potential clinical usefulness. **Conclusions and Relevance.** This clinician-friendly individualized risk calculator can be useful to identify individuals with a short-term risk of developing alcohol-related adverse outcomes, encourage at-risk drinkers to cut down their drinking and facilitate the implementation of focused preventive interventions.

### **Communication 3: "Associations of depressive symptoms with alcohol use: findings from the Constances cohort."**

**Speaker:** Emmanuel Wiernik, PhD.

**Authors:** Emmanuel Wiernik, Nicolas Hoertel, Guillaume Airagnes, Joane Matta, Frédéric Limosin, Marcel Goldberg, Marie Zins, Cédric Lemogne.

#### **Abstract:**

**Background.** It remains unclear whether the risk of alcohol use is related to specific depressive symptoms (e.g. poor appetite), to specific dimensions underlying depression (e.g. somatic symptoms), to a general depression factor representing the shared effect of all depressive symptoms, or to a combination of these explanations. In addition, these effects could be specific to alcohol or common to other substances. **Methods.** From 14,117 men and 14,629 women included from January 2015 to December 2016 in the French Constance cohort, we applied structural equation modeling to examine the shared and specific effects of depressive symptoms (Center for Epidemiological Studies-Depression) on alcohol, tobacco, e-cigarette and cannabis use, while taking into account the co-occurrence between those substances. Analyses were stratified by sex and adjusted for age and education. **Results.** Heavy alcohol use was significantly associated with depression and this association was mostly mediated through a general depression factor (i.e. shared effect of all depressive symptoms). This was also the case for the other substances. Beyond and above the effect of that general factor, reduced positive symptoms (e.g. anhedonia) had an additional effect on heavy alcohol use, particularly in men. Somatic symptoms had an additional effect on cannabis use. **Conclusions.** Because the association between depressive symptoms and substance use was mainly mediated through a general depression factor, a better knowledge of biological and psychological mechanisms underlying this dimension may help reduce the burden of substance use. In addition, the importance of particular substance-specific dimensions of depression could help to better identify at-risk individuals.

**Communication 4: "Do personality traits predict alcohol consumption decades after their assessments? Findings from the Gazel cohort."**

**Speaker: Guillaume Airagnes, MD, MPH.**

**Authors:** Guillaume Airagnes, Cédric Lemogne, Alice Gueguen, Nicolas Hoertel, Marcel Goldberg, Frédéric Limosin, Marie Zins.

**Abstract:**

**Background.** Hostility has been found to be positively associated with alcohol intake in cross-sectional studies. Our aim was to examine prospectively the long-lasting association of hostility with alcohol consumption. **Methods.** We included 10,612 men and 3,834 women from the French Gazel cohort with mean ages in 1993 of 48.6 (SD=2.9) and 45.7(SD=4.2), respectively. Hostility (i.e. total, cognitive and behavioral) was assessed in 1993 with the Buss and Durkee Hostility Inventory. Alcohol consumption was self-reported annually from 1994 to 2014. Hostility scores were introduced successively in general linear mixed models with annual alcohol consumption in drinks per week as dependent variable. Multivariable analyses were adjusted for age, occupational status, marital status, retirement status and depression score. All the analyses were stratified by sex. **Results.** Among men(women), 83.0%(76.2%) completed at least 75% of all annual assessment of alcohol consumption over a 21-year follow-up. In univariate analysis, alcohol consumption was associated with total and behavioral hostility in both sex (all  $p < 0.001$ ). In multivariable analyses, these associations remained significant with a greater size effect for behavioral hostility. Estimated means of alcohol consumptions ranged from 10.50[95%CI:10.01-10.92] drinks per week to 13.32[95%CI:12.90-13.74] in men and from 4.09[95%CI:3.71-4.46] to 5.78[95%CI:5.39-6.17] in women, for the first and last quartiles respectively ( $p$  trends $<0.001$  and all pairwise comparisons $<0.01$ ). Similar effects were observed among participants with at-risk alcohol consumption at baseline. **Conclusions.** In both men and women, behavioral hostility predicted alcohol consumption over a 21-year follow-up. Interventions aiming at modulating behavioral hostility may help reducing its long-lasting influence on alcohol consumption.

## **SYMPOSIUM**

Beyond cognition: neuroscience correlates of affective and motivational processes in binge drinking

*MAURAGE Pierre (University of Louvain, Belgium)*

- Emotional impairments in binge drinking: insights through a behavioral and neuroscientific approach

*Severine Lannoy (University of Reims, France ; Stanford University, USA)*

- The role of interoception and emotional impulsivity in binge drinking

*Aleksandra Herman (University of Sussex, UK)*

- How do binge drinkers inhibit alcoholic images? A functional magnetic resonance imaging study

*Sónia S. Sousa (University of Minho, Portugal)*

- Neural correlates of an alcohol-cued Go/NoGo task: A dual process approach to binge drinking in college students”

*Javier Blanco-Ramos (University of Santiago de Compostella, Spain)*

**Aleksandra Herman** (University of Sussex, UK)

“The role of interoception and emotional impulsivity in binge drinking”

Despite our increasing knowledge of the cognitive as well as emotional alterations in binge drinking, the factors that actually predispose to binge drinking remain unclear. More recently the role of interoception, defined as the sense of the physiological state of the body, is being investigated in alcohol use and misuse. The talk will present our recent neuroimaging as well as behavioural findings focusing on the role of emotional impulsivity and interoception as well as emotional recognition alterations as driving factors for alcohol use in non-dependent binge drinkers. A model will be presented of how these factors interact with each other in the context of identifying potential endophenotypes associated with risk for the development of alcohol addiction.

**Severine Lannoy** (University of Reims, France; University of Stanford, USA)

“Emotional impairments in binge drinking: insights through a behavioral and neuroscientific approach”

Binge drinking is a widespread alcohol consumption pattern in young people. It has been related to deleterious consequences such as brain modifications and cognitive dysfunctions. Recent studies have also proposed that binge drinking may be associated with emotional difficulties. The talk will present behavioral, electrophysiological, and neuroimaging findings supporting this proposal. It will underline the existence of emotional deficits in binge drinkers as well as the heterogeneity of this population. Electrophysiological and neuroimaging results related to distinct paradigms involving the processing of emotional stimuli will also be presented to extend the understanding of emotional difficulties in binge drinkers. These results reinforce the importance of emotional processes in binge drinking and open new research avenues in the field of alcohol-use disorders.

**Sónia S. Sousa** (University of Minho, Portugal)

“How do binge drinkers inhibit alcoholic images? A functional magnetic resonance imaging study”

Binge Drinking (BD) is characterized by the consumption of large amounts of alcohol in a short time followed by a period of abstinence or very low consumption. This pattern of alcohol consumption is highly prevalent among adolescents and young adults, especially college students, and an important risk factor for substance abuse. Although BD is not considered an addictive disorder per se, binge drinkers (BDs) display cognitive deficits similar to addicted individuals. Namely, deficits in executive functions, such as in inhibitory control, impulse control and delay of gratification, in addition to functional alterations of the frontal networks involved in addictive behaviours. Thus, while behavioural differences are not usually observed between BDs and light social drinkers, increased activation in the BDs' frontal or fronto-parietal regions during the performance of executive-related tasks is relatively consistent across studies. This greater neural activation seems to reflect a brain compensatory mechanism that enables BDs to maintain task performance levels similar to controls. However, current knowledge on the BDs' neurofunctional response when inhibiting a motor response to alcoholic stimuli is scarce. In this sense, the main purpose of the present study was to assess functional activity in the brain networks associated with motor response inhibition to alcoholic stimuli in young BDs. Twenty College BDs and 16 age-matched non-alcohol consumers (NACs) (18-23 years-old) underwent a functional magnetic resonance imaging (fMRI) acquisition while performed a stop signal task with alcohol-related and non-alcohol-related stimuli. At the behavioral level, BDs exhibited lower reaction times than NACs in response to alcoholic stimuli. When analyzing neural activation, BDs displayed increased activity over the right dorsolateral prefrontal cortex during response inhibition to alcoholic stimuli. In addition, BDs showed augmented activation in limbic regions such as the parahippocampal gyrus, when observing alcoholic stimuli. Finally, brain activity of visual regions for alcoholic drinks was superior relative to non-alcoholic beverages in the BD group only. Overall, our results suggest that BDs may need additional cognitive resources to perform similar to NACs when inhibiting a response to alcoholic stimuli. These findings are in agreement with the only fMRI study conducted to date on motor inhibition to alcoholic stimuli in BDs (Amesa et al., 2014), pointing to a compensatory neurofunctional mechanism that may allow BDs to perform efficiently in inhibitory control-related tasks. Additionally, BDs seem to be more impulsive in their responses to alcoholic stimuli and to be attentionally biased towards this type of stimulus, which in turn could be activating emotional memories contributing to alcohol consumption reinforcement.

**Neural correlates of an alcohol-cued Go/NoGo task: A dual process approach to binge drinking in college students.**

Blanco-Ramos, Javier; Suárez-Suárez, Samuel; Caamaño, Francisco; Cadaveira, Fernando; Corral, Montserrat; Doallo, Sonia; Folgueira-Ares, Rocío; Pérez-García, José Manuel; Rodríguez Holguín, Socorro.

University of Santiago de Compostela (Spain)

Dual-process models have been proposed as an explanation of substance abuse and other risky behaviours, with inefficient decision-making abilities as the central core. Decision making abilities result from the balance between a reflective system and an automatic or affective system. The adolescent brain, in comparison with normal adults, presents an imbalance between earlier developed motivational systems and a still immature cognitive control system. As a result, adolescence is characterized by elevated risk-taking behaviours and seeking of novelty and rewarding sensations, associated with inefficient decision-making abilities. As the adolescent brain is still under critical development, alcohol misuse has serious deleterious effects, which may exacerbate the problem.

To explore brain activity associated with inhibitory control in motivational alcohol-related contexts in young binge drinkers (BD), a sample of college students performed a Go/NoGo task with beverage (alcohol vs. non-alcohol) stimuli while ERPs (80 controls, 71 BD) and fMRI (36 controls, 32 BD) were recorded.

Differences between BD and controls were found in the anterior N2 ERP wave and in the inferior frontal cortex activity during response inhibition. These differences were modulated by the alcohol-related content of stimuli. The results will be discussed in the frame of the dual-process models, providing new evidence on the understanding of excessive drinking habits in youth.

## INVITED TALK

- [How we got alcohol addiction wrong – one lever at a time](#)

*HEILIG Markus (Center for Social and Affective Neuroscience, Linköping Univ., Sweden)*

## **SYMPOSIUM**

How well do preclinical findings translate into the clinic?

*SPANAGEL Rainer (CIMH, Mannheim, Germany)*

- **Successes and pitfalls in translational alcohol research – an overview**  
*Rainer Spanagel (CIMH, Mannheim, GER)*
- **Improving the design and reporting of animal studies**  
*Nathalie Percie du Sert (NC3R London)*
- **Translational Neuroimaging: Convergent results from humans and animals**  
*Wolfgang Sommer (GER)*
- **Translational Genetics: Convergent results from humans and animals**  
*Stefanie Witt (GER)*

## SYMPOSIUM

Don't stress the amygdala—the role of pro- and anti-stress systems in AUDs

*KHOM Sophia (The Scripps Research Institute, Department of Neuroscience)*

- **Corticotropin releasing factor binding protein in the amygdala: the good, the bad and the ugly**  
*HAASS-KOFFLER CAROLINA L (Center for Alcohol and Addiction Studies Department of Psychiatry and Human Behavior and Department of Behavioral and Social Sciences, Brown University Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology NIAAA and NIDA, National)*
- **Dysregulated Endocannabinoid Signaling in the Central Nucleus of the Amygdala (CeA): Role in Anxiety and Excessive Alcohol Consumption**  
*SERRANO Antonia (Instituto de Investigación Biomedica de Malaga (IBIMA). UGC Salud Mental Laboratorio Medicina Regenerativa, pabellón gobierno, sotano Hospital Regional Universitario de Malaga)*
- **A role for amygdalar endocannabinoid signaling in excessive alcohol intake and comorbid anxiety in genetically selected Marchigian Sardinian alcohol preferring rats**  
*CANNELLA Nazzareno (University of Camerino, School of Pharmacy, Pharmacology Unit)*
- **Aberrant central amygdala Substance P/Neurokinin receptor 1 signaling in rodent models of alcohol dependence and anxiety**  
*KHOM Sophia (The Scripps Research Institute, Department of Neuroscience)*

***Corticotropin releasing factor binding protein in the amygdala:  
the good, the bad and the ugly.***

**CAROLINA L. HAASS-KOFFLER, PHARM.D**

Center for Alcohol and Addiction Studies

Department of Psychiatry and Human Behavior and Department of Behavioral and Social  
Sciences, Brown University

Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology NIAAA  
and NIDA, National Institutes of Health

To investigate the causal link between corticotropin releasing factor binding protein (CRFBP) in the central nucleus of the amygdala (CeA) and alcohol-seeking behaviors, we utilized a rat model trained to self-administer ethanol, designed with controlled regional expression of CRFBP in the CeA and electrophysiology data in transgenic mice expressing green fluorescence protein (GFP) in CRF Receptor 1 expressing neurons in chronic ethanol exposure. In rats, we found that CRFBP downregulation in the CeA reduces ethanol self-administration and CeA hemodynamic activity but does not attenuate yohimbine-induced ethanol self-administration. In mice, preliminary data suggest that CRFBP inhibition decreases postsynaptic GABA<sub>A</sub> receptor function through CRF R2 in the CeA. All these data support our hypothesis that CRFBP is not only a sequestering protein, but it may possess additional functions.

**Dysregulated Endocannabinoid Signaling in the Central Nucleus of the Amygdala (CeA):  
Role in Anxiety and Excessive Alcohol Consumption**

*ANTONIA M. SERRANO, PhD*

UGC Salud Mental- Hospital Regional Universitario de Málaga IBIMA

The endogenous cannabinoid system is a neuromodulatory system that plays a homeostatic role in the constraint and termination of stress responses. Disrupted endocannabinoid signaling is linked to maladaptive stress responses and affective disorders such as anxiety and depression. These negative emotional symptoms are associated with alcohol dependence and abstinence and may contribute to relapse drinking and excessive alcohol intake. We have shown that stress- and alcohol-induced increases in the endogenous levels of 2-arachidonoylglycerol (2-AG) in the CeA were blunted in rodents with a history of alcohol dependence. This effect was associated with anxiety-like behaviors and excessive alcohol consumption, and these dependence-associated behavioral effects were alleviated by enhancement of 2-AG tone. These results suggest that dysregulated 2-AG signaling in the CeA may contribute to dependence-related affective disorders and excessive alcohol intake.

**A role for amygdalar endocannabinoid signaling in excessive alcohol intake and comorbid anxiety in genetically selected Marchigian Sardinian alcohol preferring rats.**

*NAZZARENO CANNELLA, PhD*

University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy.

Addiction is a chronic disease characterized by compulsive drug seeking and taking. Transition from recreational drug use to excessive consumption and eventually drug addiction is shaped by the interaction of excessive drug consumption with genetic, stress, and environmental factors. The Marchigian Sardinian alcohol-preferring (msP) rat line, genetically selected for excessive alcohol consumption, have a hyperactive amygdalar corticotropin releasing factor (CRF) to CRF receptor-1 signaling resulting in increased Fatty Acid Amide Hydrolase (FAAH) activity and reduced N-arachidonylethanolamine (AEA) levels, compared to non-selected Wistar controls. MsP rats show innate traits resembling generalized anxiety and post-traumatic stress disorder (PTSD) in humans. Negative environmental conditions exacerbate this disorder, which can be attenuated by voluntary alcohol drinking or by enhanced endocannabinoid transmission. Here we provide evidences that FAAH inhibition in the central amygdala (CeA) decreases alcohol self-administration and stress-induced anxiety in msP but not in non-preferring Wistar rats. We also compared alcohol intake and comorbid anxiety in male and female msP and Wistar rats demonstrating that female msPs consume a larger amount of alcohol than male. Negligible alcohol consumption and no sex differences were observed in Wistars. Both male and female msP show comorbid anxiety, however, while in male alcohol alleviates generalized anxiety assed by an elevated plus maze paradigm, in female alcohol reduces the expression of a PTSD-like syndrome in a fear conditioning paradigm. In addition, inhibition of FAAH activity mimic the effect of alcohol reducing PTSD-like behavior in female msP rats. Considering the link between reduced endocannabinoid transmission, excessive stress response, and expression of PTSD-like traits, our data suggest that msP rats consume high amounts of alcohol to normalize their amygdalar endocannabinoid transmission in the attempt to medicate their innate negative state.

## **Aberrant central amygdala Substance P/Neurokinin receptor 1 signaling in rodent models of alcohol dependence and anxiety**

*SOPHIA KHOM, PhD*

The Scripps Research Institute

Department of Neuroscience

Substance P (SP) and its preferred target –neurokinin 1 (NK-1) receptors- have emerged as critical players in stress-elicited alcohol seeking and alcohol consumption. However, the underlying cellular mechanisms are still poorly understood. SP and NK-1 receptors are widely expressed in the brain and SP is released in response to stressful or painful stimuli. Here, we will discuss the effects of SP and a specific NK-1 receptor antagonist on GABA<sub>A</sub> receptor-mediated neurotransmission in the CeA of different rodent models of alcohol dependence as well as innate anxiety. Briefly, SP increases transiently frequency and amplitudes of spontaneous inhibitory postsynaptic currents (sIPSCs) in the CeA of alcohol-naïve rats suggesting increased GABA release and enhanced postsynaptic GABA<sub>A</sub> receptor-mediated neuronal inhibition. The NK-1 receptor antagonist decreases sIPSCs frequency indicative of SP release into the CeA under basal conditions and a SP participation in regulating neuronal activity. Most notably, SP effects on CeA GABA transmission are more pronounced and more sustained in alcohol-dependent rats. These functional effects are accompanied by decreased SP and NK-1 receptor expression pointing towards increased NK-1 receptor sensitivity. Similarly, larger and more sustained SP-induced increases of CeA GABAergic transmission are observed in rats undergoing alcohol withdrawal.

Collectively, these data support the hypothesis that both alcohol dependence- and withdrawal-associated stress sensitize SP/NK-1 receptor signaling.

## SYMPOSIUM

### THE ENDOCANNABINOID SYSTEM AND ALCOHOL-RELATED OUTCOMES: GENES, BRAIN AND BEHAVIOR

*HENDERSHOT Christian (Departments of Psychiatry and Psychology, University of Toronto)*

- **ENDOCANNABINOID GENES AND ALCOHOL-INDUCED REWARD PHENOTYPES**  
*Vijay A. Ramchandani, Matthew E. Sloan, Emily L. Vogt, Melanie L. Schwandt, Hui Sun, Peter Herscovitch, Markus Heilig, Nancy Diazgranados, David Goldman (Section on Human Psychopharmacology, National Institute on Alcohol Abuse & Alcoholism, Bethesda, USA)*
- **A LABORATORY-BASED INVESTIGATION OF FAAH C385A AND ALCOHOL-RELATED PHENOTYPES IN YOUTH**  
*Christian S. Hendershot, Jeffrey D. Wardell, Laura M. Best, Rachel F. Tyndale, Isabelle Boileau (Centre for Addiction and Mental Health and University of Toronto, Canada)*
- **ENDOCANNBINOID-MEDIATED EFFECTS OF ACUTE ALCOHOL AMINISTRATION IN HEALTHY HUMANS**  
*Leah M Mayo, Elisabeth Paul, Robin Kämpe, Niclas Stensson, Bijar Ghafouri, Markus Heilig (Center for Social and Affective Neuroscience, Linköping University, Sweden)*
- **ARE DEFICITS IN BRAIN ENDOCANNABINOID METABOLISM LINKED TO HEAVIER ALCOHOL USE? NEUROIMAGING STUDIES OF THE ENZYME FATTY ACID AMIDE HYDROLASE**  
*Laura M. Best (Centre for Addiction and Mental Health and University of Toronto, Canada)*

## ENDOCANNABINOID GENES AND ALCOHOL-INDUCED REWARD PHENOTYPES

Vijay A. Ramchandani, Matthew E. Sloan, Emily L. Vogt, Melanie L. Schwandt, Hui Sun, Peter Herscovitch, Markus Heilig, Nancy Diazgranados, David Goldman

Section on Human Psychopharmacology, National Institute on Alcohol Abuse & Alcoholism, Bethesda MD 20892, USA

**BACKGROUND:** Previous studies indicate that endocannabinoid (eCB) signaling may be related to the etiology of alcohol use disorder (AUD). Preclinical studies indicate that eCB administered during alcohol intake increases mesolimbic dopamine release. Cannabinoid receptor (CNR1) and fatty acid amyl hydrolase (FAAH) gene variations have been associated with alcohol response and AUD severity. However, the broader relationship between eCB and alcohol use has yet to be elucidated in humans.

**AIM:** The aim of this study was to examine the impact of endocannabinoid system polymorphisms *CNR1* rs2023239 and *FAAH* rs324420 following IV alcohol infusion on striatal dopamine release measured using [<sup>11</sup>C]-raclopride position emission topography (PET).

**METHODS:** Twenty-six healthy males underwent two, randomized PET scans with [<sup>11</sup>C]-raclopride, one concurrent with intravenous alcohol (target breath alcohol level of 80mg%) and the other with placebo. The difference in [<sup>11</sup>C]-raclopride binding potential between alcohol and placebo sessions was used to quantify alcohol-induced dopamine release.

**RESULTS:** Results show that *CNR1* T/T homozygotes had significantly greater alcohol-induced dopamine release in the posterior ( $p=0.001$ ) and anterior ventral striatum ( $p=0.025$ ) than C-allele carriers. There was no impact of the *FAAH* polymorphism on alcohol-induced dopamine release. To explore interactions between eCB and opioidergic mechanisms, a linear model combining *CNR1* and *OPRM1 A118G* genotype demonstrated a significant additive effect of both genotypes on alcohol-induced dopamine release ( $R^2=0.43$ ).

**CONCLUSION:** These results suggest an additive effect of opioid and cannabinoid systems on striatal dopamine release following alcohol. Future studies should explore the effect of eCB polymorphism on alcohol measures across the spectrum of AUD.

## A LABORATORY-BASED INVESTIGATION OF *FAAH* C385A AND ALCOHOL-RELATED PHENOTYPES IN YOUTH

Christian S. Hendershot, Jeffrey D. Wardell, Laura M. Best, Rachel F. Tyndale, Isabelle Boileau

Centre for Addiction and Mental Health and University of Toronto, Canada

**Background:** The *FAAH* gene encodes the fatty acid amide hydrolase (FAAH) enzyme, which metabolizes the endocannabinoid anandamide. The A allele of the *FAAH* C385A variant (rs324420) has been linked to reduced enzyme activity, higher anandamide, and increased severity of alcohol use in adults.

**Aims:** To extend this finding to a younger sample, we investigated associations of C385A with alcohol-related phenotypes among heavy-drinking youth.

**Methods:** Participants (N=283, mean age = 19.74 years) completed a self-report battery. A subset of participants completed alcohol administration sessions involving the alcohol clamp (target BrAC = 80mg%; n=88) and intravenous alcohol self-administration (IVASA) (n=61).

**Results:** Relative to youth with the C/C genotype, those with the A allele (37%) reported greater alcohol consumption (AUDIT-C;  $p=0.047$ ), hazardous use (AUDIT;  $p=0.075$ ), and stronger coping motives for drinking ( $p=.014$ ). Coping motives mediated the association of genotype with consumption and alcohol-related problems. During the alcohol clamp, within-subjects analyses of subjective responses revealed a significant, positive association between sedation and craving for A allele carriers ( $p=.045$ ), but not CC participants ( $p=.138$ ). Among CC participants, within-person increases in sedation during IVASA predicted within-person decreases in craving, which in turn predicted lower self-administration (indirect effect  $p=.002$ ). This effect was not observed for A allele carriers. Between-subjects analyses showed no genotype differences on peak BrAC during IVASA.

**Conclusion:** Findings support an association of the C385A variant with self-reported consumption in young drinkers. Results also implicate negative reinforcement processes, including the motivational salience of acute sedative effects, as candidate behavioral mechanisms for this association.

**Funding:** Supported by the Canadian Institutes of Health Research, the Ontario Mental Health Research Foundation, and NIH P60AA007611.

## ENDOCANNBINOID-MEDIATED EFFECTS OF ACUTE ALCOHOL ADMINISTRATION IN HEALTHY HUMANS

**Authors:** Leah M Mayo, Elisabeth Paul, Robin Kämpe, Niclas Stensson, Bijar Ghafouri, Markus Heilig

**Affiliation:** Center for Social and Affective Neuroscience, Linköping University, Sweden

**Background:** Chronic alcohol use is associated with dysregulation of the endocannabinoid system (eCB), a neuromodulatory system implicated in stress and reward processing. However, little is known regarding the acute effects of alcohol on the eCB system in humans.

**Aims:** Our goal was to assess the consequence of acute alcohol administration on circulating eCBs and subsequent relationship to the anxiolytic and rewarding effects of alcohol as assessed via functional magnetic resonance imaging (fMRI).

**Methods:** Thirty-two healthy adults (16 each men, women) participated in a within-subject pharmacological fMRI study consisting of two sessions (placebo, alcohol) on separate days. After drink consumption, they completed an fMRI scan assessing threat reactivity and reward processing. Blood samples were collected at baseline and before, during, and after the scan to assess plasma eCB levels. Breath-alcohol concentrations (BrAC; target = 0.06g%) and subjective mood and drug effects were assessed repeatedly throughout sessions. In addition, participants were genotyped at eCB-relevant loci (e.g. *FAAH* rs324420 and *CNR1* rs2023239).

**Results:** Alcohol significantly influenced circulating eCBs, but this effect differed between sexes. Specifically, alcohol consumption increased eCBs in women, but decreased eCB levels in men. Preliminary analyses indicate that the anxiolytic effects of alcohol (e.g. attenuation of amygdala reactivity to threat cues) appear to be mediated, in part, by eCB levels. Current analyses are underway to determine if eCBs similarly modulate reward processing during alcohol intoxication.

**Conclusion:** These data provide insight into acute biochemical consequences of alcohol that may contribute to individual differences in alcohol use and misuse.

## **ARE DEFICITS IN BRAIN ENDOCANNABINOID METABOLISM LINKED TO HEAVIER ALCOHOL USE? NEUROIMAGING STUDIES OF THE ENZYME FATTY ACID AMIDE HYDROLASE**

Laura M. Best, Bernard Le Foll, Esmaeil Mansouri, Richard Bazinet, Dina Lagzdins, Pablo Rusjan, Rachel F. Tyndale, Ph.D., Christian S. Hendershot, Markus Heilig, Junchao Tong, Stephen J. Kish, Isabelle Boileau

Centre for Addiction and Mental Health and University of Toronto, Canada; Department of Clinical and Experimental Medicine (IKE) / Division of Neuro and Inflammation Sciences (NIV) / Center for Social and Affective Neuroscience (CSAN), Linköping University, Linköping, Sweden.

**BACKGROUND:** Fatty acid amide hydrolase (FAAH) is the catabolic enzyme for the major endocannabinoid neurotransmitter anandamide and a target for medication development. Preclinical and genetic studies of a functional polymorphism in the FAAH gene (C385A, rs342240) suggest that lower FAAH levels might be associated with risk for alcohol use disorder (AUD).

**AIM:** To investigate whether lower brain FAAH level is associated with AUD, family history of AUD and /or behavioural phenotypes related to risk for AUD.

**METHODS:** FAAH brain levels were measured with positron emission tomography using the FAAH radioligand [C-11]CURB in healthy controls (n = 25), in heavy-drinking youth with positive (n = 14) or negative family history of AUD (n = 17) and in subjects with AUD at two time points (~5 and ~25 days of monitored abstinence: n = 14; n = 11). Heavy-drinking youth completed an intravenous alcohol infusion session and blood samples were taken in all participants to assess FAAH C385A genotype and plasma endocannabinoid levels at multiple time points.

**RESULTS:** [C-11]CURB binding was globally lower than controls during early but not protracted abstinence and significantly correlated with drinks per week and with plasma concentrations of anandamide. Family history of AUD did not affect [C-11]CURB binding, however higher alcohol consumption and hazardous use (Alcohol Use Disorders Identification Test (AUDIT)) in heavy-drinking youth, as well as lower sedative effects of alcohol during intravenous administration (Biphasic Effects of Alcohol Scale) was related to lower [C-11]CURB binding.

**CONCLUSION:** In line with preclinical studies our findings that lower FAAH is related to higher alcohol consumption (in AUD and in non-AUD heavy drinking youth) may be an acute consequence of recent chronic alcohol use (in AUD) and or a preexisting factor increasing vulnerability for hazardous use. Although clinical significance of low FAAH in AUD remains to be established, treatment approaches targeting FAAH should consider that increased endocannabinoid tone during early abstinence could drive drinking however some aspects could be beneficial.

**Funding:** Supported by the Canadian Institutes of Health Research, the Ontario Mental Health Research Foundation, and NIAAA 1R21AA022246-01A1.

## SYMPOSIUM

Progress in alcoholic liver disease: from benchside to bedside

*MATHURIN Philippe (Hôpital Huriez, CHRU Lille)*

- What are the best pathways to target in AH : from animal models to clinical setting  
*Hidekazu Tsukamoto, University of South California, Los Angeles, USA*
- How to stage disease severity in patients with compensated ALD: from serum markers to physical methods  
*Eric Nguyen-Khac, Service HepatoGastroentérologie, CHU Amiens-Picardie, Amiens, France*
- Diagnosis and staging of disease-severity in symptomatic alcoholic hepatitis  
*Christophe Moreno, Department of Gastroenterology, Hôpital Erasme, Brussels, Belgium*
- Medical management of severe forms of alcoholic hepatitis  
*Alexandre Louvet, Service Maladies de l'Appareil Digestif, CHRU Lille, Lille, France*
- From early transplantation to future development in alcoholic hepatitis  
*Philippe Mathurin, Service Maladies de l'Appareil Digestif, CHRU Lille, Lille, France*

## **SYMPOSIUM**

### **Insights into Alcohol-associated Cancers**

*MUELLER Sebastian (Center for Alcohol Research and Salem Medical Center, University of Heidelberg)*

- **Alcohol and Cancer – the epidemiological evidence**  
*Elisabete Weiderpass, IARC Lyon*
- **The role of adaptive immune system, particularly IgA+ and CD8+ T cells, in alcoholic steatohepatitis and its progression to hepatocellular carcinoma**  
*Shabnam Shalpour of University of California at San Diego*
- **Molecular Mechanisms of Alcohol-associated Cancers: from Metabolism and Oxidative Stress to Stem Cells and Genomic Instability**  
*Vasilis Vasiliou, Yale*
- **Local acetaldehyde its key role in alcohol-related upper GI tract carcinogenesis**  
*Miko Salaspuro*

## **Alcohol and Cancer: The Epidemiological Evidence**

Elisabete Weiderpass, Carolina Espina, Pietro Ferrari

*International Agency for Research on Cancer, World Health Organization, IARC-WHO, Lyon, France.*

Alcohol consumption is one of the top-10 health risks, contributing to circa 3.9% of worldwide burden of disease and 3.3 million annual deaths. Alcohol use increases overall cancer incidence and overall and cancer-specific mortality.

The 2012 IARC Monograph reviewed the epidemiological evidence on the possible association between alcoholic beverage consumption and cancer risk at 27 anatomical sites, and reported that cancers of the upper digestive tract (UADT; oral cavity, pharynx, larynx, esophagus), liver, colorectum and female breast are causally related to the consumption of alcoholic beverages. In 2018 the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) report additionally showed that alcohol intake was inversely associated with the risk of kidney cancer and described a suggestive positive relationship with pancreatic cancer.

There are several mechanisms through which alcohol induces cancer: a direct effect on the cells where conversion to acetaldehyde such as primarily in the liver cells, where it can induce cirrhosis; conversion also occurs in the saliva and the large intestine. Ethanol promotes production of highly reactive oxygen species, which can damage DNA, alter DNA methylation, and has hormonal effects, including increasing oestradiol levels, which may affect the risk of breast cancer. Ethanol facilitates uptake of carcinogens in the mouth and throat, thus also increasing the risk of tobacco-induced cancers. It may also propel already existing cancers through immunosuppression, angiogenesis, and decrease the effect of chemotherapeutics. Alcohol use increases overall cancer incidence and overall and cancer-specific mortality.

Epidemiological and experimental Research on alcohol and cancer remains limited. Research priorities include 1) investigation of the role of alcohol in a wider list of cancers; 2) better analytical strategies to elucidate the role of drinking patterns and of specific alcoholic drinks; and 3) elucidation of the role of smoking in alcohol-related carcinogenesis, particularly in those cancer sites that are tobacco-related.

## **Role of adaptive immune system, particularly IgA<sup>+</sup> and CD8<sup>+</sup> T cells, in alcoholic steatohepatitis and its progression to hepatocellular carcinoma**

Shabnam Shalpour, PhD

*University of California San Diego School of Medicine, La Jolla, CA, USA*

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide and a leading cause of cancer-related deaths. Despite major growth in fighting hepatitis virus B and C, the epidemic of liver disease continues to grow with clear links to obesity and alcohol abuse. Non-alcoholic and alcoholic steatohepatitis (NASH/ASH) are the major drivers of HCC, but the mechanisms underlying the progression to HCC are poorly known. Progress in this field depends on the availability of reliable preclinical models amenable to genetic and functional analyses and exhibiting robust NASH-to-HCC progression. Using MUP-uPA mouse models, we found that accumulation of IgA<sup>+</sup>PD-L1<sup>+</sup>CD138<sup>+</sup>IL-10<sup>+</sup> plasmacytes in NASH-afflicted human and mouse livers results in localized immunosuppression that fosters HCC development by attenuating the activation of a protective, tumor-directed cytotoxic CD8<sup>+</sup> T-cell response. Moreover, we found that alcohol feeding increases tumor development through a profound induction of gut barrier disruption, and systematic changes in adaptive immune cells' development and responses. Although high fat diet (HFD) supports CD8<sup>+</sup> T cell infiltration in the liver, alcohol suppresses it. Our work provides new insights on how HFD and alcohol regulate adaptive immune cells and thereby affect fibrosis and the response to immunotherapy. Specifically, consumption of alcohol or HFD regulates the response to anti-PD(L)1 therapy in a different manner, namely due to its distinct ability to regulate CTL function and induction of dysbiosis, both locally and systemically.

## **MOLECULAR MECHANISMS OF ALCOHOL-ASSOCIATED CANCERS: FROM METABOLISM AND OXIDATIVE STRESS TO STEM CELLS AND GENOMIC INSTABILITY**

Vasilis Vasiliou<sup>1</sup> and David C. Thompson<sup>2</sup>

<sup>1</sup>Department of Environmental Health, Yale School of Public Health and Yale Cancer Center, New Haven, CT, USA. <sup>2</sup>Department of Clinical Pharmacy, University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, CO, USA.

Chronic ethanol abuse is associated with, and may be involved with the development of, several types of cancers in humans, including liver, colon, pancreas and, in women, breast. Although the exact cause of alcohol-induced cancer is currently unknown, several possible underlying mechanisms have been suggested and are currently under investigation. It is well established that alcohol metabolism promotes oxidative stress. Of the many molecules formed during ethanol metabolism, the aldehydes exhibit activities that can promote cancer. Acetaldehyde and lipid peroxidation aldehydes (generated by reactive oxygen species during CYP2E1-mediated alcohol metabolism) can form DNA adducts, and thereby cause genetic mutations, inhibit DNA repair, and disrupt DNA replication processes, all of which cause genomic instability - a hallmark of cancer. In addition, aldehydes may adduct proteins and inhibit cellular processes, such as folate and retinoid metabolism. They can also regulate redox-sensitive signaling pathways and transcription factors that sustain inflammation. Alcohol consumption affects the composition and function of the gut microbiome, leading to inflammation and changes in the function of the immune system, both of which are known to promote and exacerbate cancer. Importantly, gastrointestinal bacteria can produce acetaldehyde from ethanol. Finally, reactive oxygen species and lipid aldehydes may also affect immunometabolism that is now recognized as an important factor in cancer. As such, there are many mechanisms by which aldehydes can contribute to alcohol-associated cancers. As the primary enzymes responsible for detoxifying aldehydes, aldehyde dehydrogenases (ALDHs) have the potential to influence alcohol-associated carcinogenesis. Indeed, we and others have shown ALDHs to be involved in the pathophysiology of cancer and cancer stem cells. We now propose two novel hypotheses regarding the role of ALDHs in alcohol-mediated carcinogenesis. First, ALDHs may shield tumor-initiating or tumor cells against genomic instability by protecting their DNA repair mechanisms from damage by acetaldehyde and lipid-derived aldehydes. Second, ALDH-derived acetate could serve as a precursor of acetyl-CoA, a fuel used to form biomolecules in cancer cells. In conclusion, the mechanisms by which alcohol metabolites and associated oxidative stress may contribute to carcinogenesis are complex and often interconnected. Their elucidation provides new opportunities for the prevention or mitigation of alcohol-associated cancers.

## **LOCAL ACETALDEHYDE -- ITS KEY ROLE IN ALCOHOL-RELATED UPPER GI TRACT CARCINOGENESIS**

Mikko Salaspuro

*Research Unit on Acetaldehyde and Cancer, University of Helsinki, P.O. Box 63, 00014 Helsinki, Finland.*

Ethanol molecule is neither genotoxic nor mutagenic. However, its first metabolite acetaldehyde (ACH) associated with the consumption of alcoholic beverages is classified as carcinogenic in humans. ACH concentrations present in saliva instantly after alcohol drinking result in the generation of mutagenic ACH-DNA adducts in human mouthwash samples and in oral mucosa of rhesus monkeys. Recently, similar ACH levels have been shown to play a key role in ethanol-dependent telomere shortening in primary human foreskin fibroblasts.

Strongest evidence for the local carcinogenicity of ACH in man provides a point mutation in the aldehyde dehydrogenase 2 gene, which has randomized millions of alcohol consumers to markedly increased ACH exposure via saliva and gastric juice. This novel human cancer model is associated with a manifold risk for upper GI tract cancer and proves conclusively the causal role of local ACH in alcohol-related upper digestive tract carcinogenesis. Most importantly, the model minimizes the role of confounding factors hampering most epidemiological studies on alcohol and cancer.

Normal human saliva does not contain measurable levels of ACH. However, alcohol ingestion results within seconds in a concentration-dependent accumulation of ACH in saliva, which continues up to 10-15 minutes after each sip of alcoholic beverage. The prominent instant increase of salivary ACH level is followed by a long-term phase lasting for as long as ethanol stays in the saliva. Bacteria and yeasts representing normal upper GI tract microbiome play a major role in local ACH formation from ethanol. This is contributed locally by organ specific expression and gene polymorphisms of ethanol- and ACH-metabolizing enzymes.

Lachenmeier DW, Salaspuro M, Regul Toxicol Pharmacol 2017;86:128-136.

Nieminen MT, Salaspuro M, Cancers 2018,10,11; doi:10.3390/cancers10010011

## INVITED TALK

- [ASH-NASH Synergism and Its Underlying Mechanisms](#)

*TSUKAMOTO Hidekazu (Professor of Pathology and Director for Res Ctr for ALPD and Cirrhosis- University of Southern California, Los Angeles, USA)*

## ASH-NASH Synergism and Its Underlying Mechanisms

Hidekazu Tsukamoto

Southern California Research Center for ALPD and Cirrhosis  
Keck School of Medicine of the University of Southern California,  
Los Angeles, CA91011, USA

Alcohol misuse and obesity are two leading independent risk factors for alcoholic and non-alcoholic steatohepatitis (ASH and NASH) around the globe. Synergistic interactions by these factors have also increasingly been recognized. In fact, the emerging evidence indicates the average BMI of some ASH patient populations in the U.S. may be around 30. ASH and alcoholic cirrhosis occur as the consequences of alcohol addiction which dictates heavy drinking and sustained blood alcohol levels (BAL) due to physical dependence. This condition can be reproduced in rodents by intragastric feeding of ethanol diet which also allows precise reproduction of the synergism between sustained BAL and overfeeding-induced obesity. This model exhibits heightened steatohepatitis, M1 macrophage activation, nitrosative stress driven by Notch-dependent mitochondrial metabolic reprogramming. Moderate alcohol intake may also synergistically work with NASH to promote liver cancer development. Evidence suggests social drinking is sufficient enough to promote liver cancer incidence in NASH-cirrhosis patients. This synergism is reproduced in mice injected with the hepatocarcinogen DEN and fed alcohol-containing Western diet. Tumor promotion in this model is dependent on activation of hepatic stellate cells (HSC) driven by Wnt- $\beta$ -catenin-mediated overexpression of stearoyl-CoA-desaturase (SCD), which in turn establishes a SCD-LRP5/6-Wnt positive loop to amplify Wnt- $\beta$ -catenin pathway in HSC and tumor microenvironment, leading to tumor-promoting lipid metabolic reprogramming. These findings highlight the causal roles of morphogen-driven metabolic reprogramming in steatohepatitis and liver tumor development promoted by ASH-NASH synergism.

## **SYMPOSIUM**

### Novel concepts in the evaluation of fibrosis in alcohol-related liver disease

*LACKNER Carolin (Graz, Austria)*

- Non-invasive fibrosis assessment in compensated and decompensated alcohol-related liver disease and clinical implications  
*R. Stauber, Graz (Austria)*
- Staging of alcohol-related liver disease and clinical implications  
*C. Lackner, Graz (Austria)*
- Morphologic features of fibrosis progression and regression in alcohol-related liver disease  
*R. Miquel, London (UK)*
- Histologic fibrosis patterns associate with disease severity and short-term mortality in patients with acute decompensation of cirrhosis  
*Y. Wang, Guangzhou (China)*

*R. Stauber/Graz, Austria:*

*Non-invasive fibrosis assessment in compensated and decompensated alcohol-related liver disease and clinical implications*

Alcohol-related liver disease (ALD) is the most common liver disease in the Western World. Especially in Europe ALD is a substantial burden as the European population has the highest per capita alcohol consumption world-wide. However, awareness about the risks of heavy drinking is low among patients with ALD and they usually present at an advanced stage with clinical symptoms such as jaundice and/or ascites. Early/compensated ALD does not manifest itself clinically and patients rarely seek medical advice, unless ALD is detected during work-up of other illnesses. Screening for early ALD is warranted since it is readily reversible under abstinence and treatment is available (psychological interventions, anti-craving drugs).

Recent data show that prognosis of early ALD, apart from abstinence, is mainly determined by histological fibrosis stage; advanced fibrosis (F3, bridging fibrosis; F4, cirrhosis) carries dismal outcome (10-year mortality 45%). However, due to the high prevalence of ALD, universal liver biopsy is not feasible.

Noninvasive fibrosis tests have shown high diagnostic accuracy for advanced fibrosis in a variety of liver diseases including ALD. Among these, simple fibrosis tests such as FIB-4 are based on routine clinical and laboratory parameters and provide relatively high diagnostic accuracy at low cost. Proprietary fibrosis panels based on direct fibrosis markers including Enhanced Liver Fibrosis (ELF™) test, FibroMeter™ and FibroTest show improved diagnostic accuracy. Vibration-controlled transient elastography (VCTE, FibroScan®) enables noninvasive estimation of liver stiffness (LS) and has shown superior diagnostic accuracy in comparative studies. However, LS is not only determined by fibrosis but also inflammation, cholestasis and/or hepatic congestion. Importantly, several studies have demonstrated a rapid decline of LS during alcohol detoxification, which presumably reflects the resolution of steatohepatitis. A recent comparative study showed best performance for VCTE (per protocol) and ELF score. While the latter tests are mostly restricted to referral centers, simple tests such as FIB-4 are suitable for screening in primary care.

The clinical impact of noninvasive fibrosis tests in decompensated ALD is rather limited since most patients already have clinical signs of cirrhosis.

*C.Lackner/Graz, Austria:*

## *Staging of alcohol-related liver disease and clinical implications*

### Abstract

Histological stage has emerged as one of the most important predictors of outcome in most chronic liver diseases including non-alcoholic fatty liver disease (NAFLD), chronic viral hepatitis and autoimmune liver diseases. Surprisingly, despite the fact that ALD is among the most frequent liver diseases, to date no universally accepted histological staging system has been devised. Because NAFLD and ALD share broad morphological overlap, also with respect to type of fibrosis and its centrilobular predominance, several authors have proposed that histological staging systems for NAFLD may also be used for ALD. However, there are points of concern with such an approach. Firstly, most patients with compensated as well as decompensated ALD are cirrhotic at first presentation. There are different histological stages of severity of cirrhosis which are not reflected in most staging systems used in NAFLD. Secondly, there are data suggesting that pericellular and septal fibrosis types may have different impact on outcome in patients with ALD. An ALD-specific staging system has recently been devised by the Consortium for the **Study of Alcoholic LiVer Disease in Europe (SALVE) Histopathology Group**. This ALD-specific staging system (SALVE stage) reflects different histological stages of severity of cirrhosis and the degree of pericellular fibrosis in ALD. The potential clinical implications of SALVE staging will be discussed.

## Histologic fibrosis patterns associate with disease severity and short-term mortality in patients with acute decompensation of cirrhosis

Yan Wang MD, PhD. Institute of Hepatology, Southern Medical University, GZ China

### Abstract

Histologic fibrosis has an important prognostic value in natural history of chronic liver disease. However, there are few evidences for its role in the end stage of liver disease. For the purpose of fully describing the morphologic features of cirrhotic fibrosis with diverse etiology, we developed a dual-photon microscopy-based computerized image analysis tool to detect collagen architectural features including both collagen geometry and collagen spatial network within septa, nodule, and sinusoidal regions of interest. A profile of these collagen features composed the quantitative collagen pattern (QCP) of the detected biopsy specimen. By using this tool, we analyzed a retrospective cohort, which included 225 hospital patients diagnosed with acute decompensation of biopsy-proven cirrhosis, with clinical collaboration from the Institute of Liver and Biliary Science, New Delhi, India. We found that QCP faithfully captured the morphologic difference in fibrosis patterns between etiologies of fatty liver diseases and viral hepatitis and between Laennec stages. It also related to MELD and HVPG. When using for predicting the short-term mortality in these patients, QCP had a robust prognostic performance superior to MELD, CTP, Laennec stage and collagen proportionate area. On multivariate regression, it was identified as an independent prognostic factor. All the data indicate that histologic fibrosis captured by QCP could have an important prognostic value even in the end stage of liver disease. In addition, because by using the same measurement strategy, we previously found that cirrhosis remodeling in viral hepatitis can be detected in high accuracy, we would propose that QCP may be helpful for assisting the investigation of undiscovered meaning of histologic fibrosis with diverse chronic liver disease.

## **SYMPOSIUM**

### Biomechanic signaling and alcoholic liver disease

*KIDAMBI Srivatsan (University of Nebraska-Lincoln)*

- Hepatic sinusoidal pressure as driving force in alcoholic liver disease  
*MUELLER Sebastian (University of Heidelberg)*
- Modulation of liver and spleen stiffness to vasoactive drugs in rodents: potential therapeutic implications?  
*ELSHAARAWY Omar (University of Heidelberg)*
- Stiffness induces Hepatocytes Metabolic reprogramming during Alcoholic Liver Disease  
*KIDAMBI Srivatsan (University of Nebraska-Lincoln)*
- Role of glycolysis in mechanosensing of liver sinusoidal endothelial cells  
*GREUTER Thomas*

## SYMPOSIUM

### ETHANOL-INDUCED NEUROINFLAMMATION AND OXIDATIVE STRESS: CHRONIC ETHANOL INTAKE AND BINGE-LIKE INTOXICATION. TRANSLATIONAL OPTIONS

*ISRAEL Yedi (Pharmacology Program, Faculty of Medicine-ICBM, University of Chile)*

- POSITRON EMISSION TOMOGRAPHY (PET) OF TRANSLOCATOR PROTEIN-18KDA IMAGING TO STUDY THE DYNAMICS OF ETHANOL-INDUCED NEUROINFLAMMATION  
*SABA Wadad (Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Service Hospitalier Frédéric Joliot)*
- EVALUATION OF N-ACETYLCYSTEINE EFFECTS ON TWO PRECLINICAL RAT MODEL OF ALCOHOL USE DISORDERS: THE OPERANT BINGE DRINKING MODEL AND THE "POST-DEPENDENT" STATE RAT MODEL  
*VILPOUX Catherine (Université Picardie Jules Verne, INSERM ERI-24 GRAP, Groupe de Recherche sur l'Alcool et les Pharmacodépendances)*
- ETHANOL INDUCED OXIDATIVE STRESS-NEUROINFLAMMATION SELF-PERPETUATING CYCLE IS BLUNTED BY N-ACETYL CYSTEINE INHIBITING CHRONIC ALCOHOL INTAKE AND RELAPSE BINGING. TRANSLATIONAL OPTIONS  
*ISRAEL Yedi (Pharmacology Program, Faculty of Medicine-ICBM, University of Chile)*
- ADMINISTRATION OF ANTI-INFLAMMATORY MESENCHYMAL STEM CELLS OR ITS SECRETOME INHIBITS ALCOHOL SELF-ADMINISTRATION AND BLOCKS RELAPSE INTAKE. MECHANISMS AND TRANSLATIONAL OPPORTUNITIES  
*EZQUER Fernando (Center for Regenerative Medicine, School Medicine, Universidad del Desarrollo, Santiago)*

## **TSPO PET imaging to study the dynamics of ethanol-induced neuroinflammation**

Wadad SABA

Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Service Hospitalier Frédéric Joliot

*UMR1023 Inserm/CEA/CNRS/Université Paris Sud/Univ. Paris Saclay*

*4, place du Général Leclerc 91400 Orsay, France.*

Imaging techniques play an important role for the non-invasive determination of the effects of alcohol in vivo, mainly focusing on brain structure and neuronal function. Positron Emission Tomography (PET) imaging using radioligands of the translocator protein 18 kDa (TSPO), a biomarker of glial activation, is useful to address the importance of neuroinflammation in various pathophysiological states and offers a unique tool to study the dynamics of the neuroimmune impact of alcohol exposure on the brain.

In adolescent monkeys, TSPO PET imaging using  $^{18}\text{F}$ -DPA-714 revealed that an acute and initial ethanol exposure (0.7-1.0 g/L) induced an immediate and prolonged (7-12 months) glial activation, suggesting a priming of glial function after initial alcohol exposure. In rats chronic alcohol exposure over a 14-day period induced an increase in the binding of  $^{18}\text{F}$ -DPA-714 which was reduced by nalmefene (0.4 mg/kg, s.c, 1 hour prior to ethanol injection). This unexpected effect could be linked to the antagonist property of nalmefene on Toll-like receptor 4 (TLR4). Our results are consistent with neuroinflammation associated with acute/chronic alcohol exposure. This contrasts with the decreased binding of  $^{11}\text{C}$ -PBR28, another TSPO ligands, observed in the brain in patients with alcohol use disorders, which is consistent with a blunted peripheral proinflammatory response compared with controls.

TSPO PET imaging provides a novel insight into the dynamics of glial function related to alcohol exposure and may be useful to i) address the neuroimmune component of alcohol-related neurotoxicity and addiction and ii) evaluate immunotherapeutic strategies for neuroprotection or the treatment of alcohol dependence.

**Evaluation of N-acetylcysteine effects on two preclinical rat model of Alcohol Use Disorders: the operant binge drinking model and the “post-dependent” state rat model**  
Vilpoux C. Sophie Lebourgeois, María Carmen González-Marín, Mickael Naassila

Université Picardie Jules Verne, INSERM ERI-24 GRAP, Groupe de Recherche sur l'Alcool et les Pharmacodépendances, Centre Universitaire de Recherche en Santé (CURS). Chemin du Thil, 80025 Amiens cedex 1, France

Many components of ethanol addiction such as reinforcement, withdrawal, extinction, and relapse are known to involve glutamate transmission, and N-acetylcysteine (NAC) is thought to counteract glutamatergic dysregulation underlying ethanol addiction. We tested NAC effect on two different rat models of Alcohol Use Disorders (AUD).

In a rat model of operant binge drinking, we demonstrated the efficacy of acute 100 mg/kg NAC treatment to reduce ethanol self-administration, alcohol-seeking behavior and to reduce relapse on rats that were abstinent for 17 days.

In a “post-dependent” state rat model (induced by chronic intermittent ethanol (CIE) vapour exposure for 10 weeks in male Wistar rats), we evaluated the effects of NAC during acute withdrawal, 8 hours after inhalation chambers were turned off. We showed that a lower dose of NAC (25 mg/kg) was enough to reduce ethanol self-administration and motivation to consume ethanol, evaluated in a progressive ratio paradigm, while the 50 mg/kg NAC reduced extinction responding and reacquisition of self-administration after 1 month abstinence.

Overall, our results demonstrate that NAC is able to limit rat's ethanol self-administration, extinction responding, and relapse, making it a potential new treatment for the maintenance of abstinence via an anti-craving effect. We will discuss further the possibility to evaluate NAC potential as a new treatment of AUD in patients.

Keywords: N-acetylcysteine Alcohol Use Disorders, ethanol self-administration, rat, relapse, seeking.

## **“ETHANOL-INDUCED NEUROINFLAMMATION AND OXIDATIVE STRESS: CHRONIC ETHANOL INTAKE AND BINGE-LIKE INTOXICATION. TRANSLATIONAL OPTIONS”**

**Chair: Dr. Yedy Israel**

**Date: ESBRA Congress Symposium September 22 at 13:30**

### **SYMPOSIUM ABSTRACT**

It is well accepted that chronic alcohol intake or its administration lead to neuroinflammation; seen as morphological changes in astrocytes and microglia. These are clearly seen in alcohol-consuming rodents, while microglial changes in humans presenting alcohol-use-disorders (AUD) may depend on the noninvasive detection method employed. Neuroinflammation and oxidative stress are self-perpetuated for prolonged periods in a vicious-like cycle and appear to be the basis for protracted increases in chronic ethanol intake and relapse-like binge drinking. The presentations in this Symposium show (i) clear microglial changes, as determined by PET imaging in AUD patients; (ii) demonstrate marked reductions in ethanol intake induced by antioxidant N-acetyl cysteine both in operant and chronic ethanol administration paradigms in rodents, including the abolition of binge-like drinking in the post-deprivation and re-access condition (ADE) (iii) show that N-acetyl cysteine normalizes the both oxidative stress and the glial changes induced by ethanol, in line with the vicious cycle hypothesis (iv) demonstrate that the systemic or intranasal administration of mesenchymal stem cells (MSC) or MSC-products, presenting marked anti-inflammatory and antioxidant properties, strongly inhibit ethanol-induced and relapse drinking. These studies further show that MSCs normalize the ethanol-reduced levels of the glial glutamate transporter GLT-1. Overall the Symposium ties neuroinflammation/oxidative-stress/hyper-glutamatergic conditions as the likely mechanism that perpetuates chronic alcohol intake and promotes intoxicating relapse. Translational options are envisioned.

### **Speakers:**

**Wadad Saba**, INSERM/CEA/CNRS/Université Paris Sud/Univ. Paris.

**Catherine Vilpoux**, Université Picardie Jules Verne, INSERM ERI-24 GRAP, Groupe de Recherche sur l'Alcool et les Pharmacodépendances, Amiens.

**Yedy Israel**, Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago.

**Fernando Ezquer**, Center for Regenerative Medicine, School Medicine, Universidad del Desarrollo, Santiago, Chile.

ADMINISTRATION OF ANTI-INFLAMMATORY MESENCHYMAL STEM CELLS OR ITS SECRETOME INHIBITS ALCOHOL SELF-ADMINISTRATION AND BLOCKS RELAPSE INTAKE. MECHANISMS AND TRANSLATIONAL OPPORTUNITIES.

<sup>1</sup>Fernando Ezquer, <sup>2</sup>Maria Elena Quintanilla, <sup>2</sup>Paola Morales, <sup>1</sup>Daniela Santapau, <sup>1</sup>Pablo Berrios-Cárcamo, <sup>1</sup>Marcelo Ezquer, <sup>2</sup>Mario Herrera-Marschitz, <sup>2</sup>Yedy Israel.

<sup>1</sup>Center for Regenerative Medicine, School Medicine, Universidad del Desarrollo, Santiago, <sup>2</sup>Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Santiago, CHILE

Chronic alcohol consumption leads to neuroinflammation and brain oxidative stress, which inhibit the astrocyte Na-glutamate transporter (GLT-1), proposed to perpetuate alcohol intake and relapse. Mesenchymal stem cells have been postulated as a therapeutic option for the treatment of different diseases since they can produce potent anti-inflammatory molecules and reduce oxidative stress. Studies presented evaluate the addictive-like suppression exerted by (i) intracerebroventricular administration of anti-inflammatory mesenchymal stem cells (MSCs), (ii) intravenous administration of MSC-spheroids or (iii) intranasal administration of MSC-derived secretome in a rat model of chronic ethanol intake and relapse drinking.

Studies show that administration of a single intracerebroventricular or intravenous dose of MSCs or the administration of three intranasal doses of MSC-derived secretome: (a) inhibited chronic ethanol intake and relapse binge drinking by 80–90%, displaying protracted effects over 4-5 weeks; (b) fully normalized alcohol-induced neuroinflammation, shown<sup>[1]</sup> as a reduced astrocyte and microglia activation in hippocampus; (c) reduced brain oxidative stress evidenced by the restoration of the normal hippocampal GSH/GSSG ratio and (d) markedly increased the levels of the GLT-1 transporter in prefrontal cortex and nucleus accumbens.

Knockdown of GLT-1 transporter by administration of an antisense oligonucleotide fully abolished the inhibitory effect of MSC-derived secretome on ethanol intake, suggesting that the glutamate transporter GLT-1 mediates the addictive-like MSCs inhibitory effects.

Overall, studies indicate that the administration of anti-inflammatory mesenchymal stem cells or its secretome affords translational opportunities for the treatment of alcohol-use disorders. Supported by FONDECYT #1180042 to YI.

## SYMPOSIUM

Translational studies on ethanol effects in adolescence

*EHLERS Cindy (Department of Neurosciences, The Scripps Research Institute)*

- NEUROPHYSIOLOGICAL SYNCHRONY AND SLEEP ARE BOTH DISRUPTED IN YOUNG ADULT HUMANS AND RATS WITH A HISTORY OF ADOLESCENT ALCOHOL EXPOSURE  
*Cindy L. Ehlers Ph.D. The Scripps Research Institute US*
- ADOLESCENT ALCOHOL EXPOSURE ALTERS ADULT NEUROBIOLOGY THROUGH NEUROIMMUNE AND EPIGENETIC SIGNALIN  
*Fulton Crews Ph.D. University of North Carolina US*
- Impulsive action and decision making in young adults binge drinkers; brain mechanisms  
*Theodora Duka M.D. Ph.D University of Sussex UK*
- EPIGENETIC, NEUROINFLAMMATION AND GluN2B PARTICIPATE TO COGNITIVE DEFICITS AFTER ETHANOL BINGING IN ADOLESCENT RAT  
*O. Pierrefiche Ph.D. University of Picardie FR*

Symposium title: Translational studies in adolescent alcohol use and misuse  
Organizers chairs: Cindy L Ehlers Ph.D. and Antonio Noronha Ph.D.

Speaker C.L. Ehlers

NEUROPHYSIOLOGICAL SYNCHRONY AND SLEEP ARE BOTH DISRUPTED IN YOUNG  
ADULT HUMANS AND RATS WITH A HISTORY OF ADOLESCENT ALCOHOL EXPOSURE

The present study aimed to document the young adult consequences of adolescent alcohol exposure, in humans and rodents, as assessed by waking EEG and sleep. Synchrony of phase (phase-locking, PL) of event-related oscillations (EROs) between frontal and parietal cortex and sleep data, were evaluated. The human participants were young adults (age 18-30 yrs, n=1041), with and without a history of adolescent binge drinking (5 drinks for boys 4 for girls per occasion at least once per month), and 74 young adult rats with and without a history of 5 weeks of adolescent alcohol vapor exposure (PD 23-55). Human binge drinkers were found to have lower PL in the beta and theta frequencies between frontal and parietal cortex. PL was also decreased in the rats exposed to ethanol vapor in the theta band across the two cortical regions. A history of adolescent regular binge drinking was also associated with reduced sleep quality as indexed by: longer sleep latencies, more problems with breathing, bad dreams and an overall higher Pittsburgh sleep quality index total score. Adolescent vapor exposure in the rat was found to result in decreases in theta power (4-8 Hz) and delta (1-4 Hz) and theta (4-8 Hz) power during slow wave sleep (all  $p < 0.05$ ). These findings suggest that alcohol exposure during adolescence may result in deficits in sleep quality in humans and slow wave sleep in animals and decreases in synchrony between cortical neuronal networks, in both species. (Supported by R37 AA010201, AA026248, RO1 AA027316, U01 AA019969)

**Crews Title:****ADOLESCENT ALCOHOL EXPOSURE ALTERS ADULT NEUROBIOLOGY THROUGH NEUROIMMUNE AND EPIGENETIC SIGNALING.**

Adolescence brain develops in parallel with maturation of self-control. To investigate the impact of adolescent binge drinking on brain development Wistar rats were exposed to adolescent intermittent ethanol (AIE, 5gm/kg/day-2 days on-2 off) across puberty and assessed in adulthood for cognitive deficits and changes in neurobiology. Post-mortem human brain of controls or AUD patients were also determined. AIE increased adult brain expression of innate immune genes HMGB1, CCL2, Toll-like receptors, and RAGE. AIE also increased activation of the transcription factor NFkB and altered glial morphology in parallel with increases in histone methylation, H3K9me2, a marker of epigenetic silencing. AIE also increased adult risky decisions and blunted behavioral flexibility in parallel with reduced adult hippocampal neurogenesis, reduced forebrain cholinergic and midbrain serotonergic neurons as well as increases in markers of neurodegeneration. Post-mortem AUD human brain also indicated increases in HMGB1, toll-like receptors, RAGE, interferon, CCL2 and other signaling genes. Further, increases in human AUD brain gene expression correlated with age of drinking onset and lifetime alcohol consumption. Emerging studies find exercise, indomethacin, donepezil and galantamine prevent and/or reverse AIE pathology. These findings support the hypothesis that adolescent alcohol exposure increases expression of HMGB1, TLR, RAGE, NFkB, and other genes are modified through epigenetic gene silencing or enhancing signals that persist into adulthood long after alcohol exposure ends, but are in some cases are reversible. These findings identify targets for consideration of treatment of adolescent onset AUD. Supported by NIAAA AA020024, AA020023, AA011605.

## Impulsive action and decision making in young adults binge drinkers; brain mechanisms

Theodora Duka

Behavioural and Clinical Neuroscience, School of Psychology, University of Sussex, Brighton UK

Binge drinking is associated with increased impulsivity. Data will be presented to elaborate on the role of impulsivity facets and brain function in alcohol abuse. In young adult binge drinkers (BD; aged 18 to 25) the relationship between 'motor'- impulsivity in the form of "can't stop" and "can't wait" as well as 'temporal' impulsivity- (failure to delay gratification) is examined. In parallel, "can't wait" impulsivity is tested in two inbred strains of mice known to differ in alcohol intake (alcohol preferring and alcohol averse mice). In addition functional brain activity and resting state functional connectivity is tested in BD.

Binge drinkers showed robust impairments in "can't wait" impulsivity under increased attentional load; alcohol preferring mice also showed impairments in "can't wait" impulsivity compared to alcohol-averse mice before any exposure to alcohol. Brain imaging revealed that higher BD severity is associated with enhanced activation in precentral gyrus and superior parietal lobule during successful stop responses, indicating a compensatory mechanisms. Delayed gratification was associated with lower frontopolar activation. Resting-state functional connectivity revealed that the higher the incidence of BD, the lower the coupling of the right supramarginal gyrus to the Ventral Attentional Network (VAN).

These findings support the assumption that aspects of cognitive impairments seen in binge drinkers in particular those associated with "can't wait" impulsivity may precede drinking behaviour. Disrupted functional connectivity within the Ventral Attention Network in more bingeing individuals may suggest disrupted attentional processing providing supporting evidence for the brain signature associated with binge drinking.

Symposium title: Translational studies in adolescent alcohol use and misuse

Organizers chairs: Cindy L Ehlers Ph.D. and Antonio Noronha Ph.D.

**Speaker** O. Pierrefiche

## **EPIGENETIC, NEUROINFLAMMATION AND GluN2B PARTICIPATE TO COGNITIVE DEFICITS AFTER ETHANOL BINGING IN ADOLESCENT RAT**

Binge drinking induces memory impairment and recently, we showed that only Two Ethanol Binge-like Exposure (TEBE) in adolescent rats are sufficient to transiently abolish long-term synaptic depression (LTD) in the hippocampus leading to mnemonic deficits after 48h. To understand the mechanism of such long-lasting action of EtOH, we investigated the role of epigenetic and neuroinflammation after TEBE in the hippocampus of young adult rats. Neuroinflammation was revealed through an increase in TLR4 immunolabelling in CA1 area and in vimentin + GFAP co-labelling showing astrogliosis in the dentate gyrus (DG). Neurogenesis was revealed with an increase in doublecortin labelling in the subgranular zone of the DG. In *stratum oriens* of CA1, synaptic pruning probably occurs since synaptophysin labelling decreased. Expression level and activity of Histone Deacetylase 2 (HDAC2), involved in epigenetic, increased while acetylated Histone 4 (Ac-H4) decreased. Further, TEBE increases mRNA level for GluN2B subunit of the NMDA receptor while ChIP analysis revealed that HDAC2 modulates the GluN2B gene promoter. Further, TEBE altered GluN2A/GluN2B balance in synaptic transmission. Finally all cellular effects of TEBE were prevented with sodium butyrate, an HDAC inhibitor.

In conclusion, two EtOH exposures induces long-lasting memory-impairment because it overexpressed HDAC2 resulting in GluN2A/GluN2B imbalance that leads to LTD blockade. In parallel, neuronal injury and altered morphologic plasticity in the hippocampus take place. It is now important to study the link between inflammation and epigenetic in the effects of ethanol since this would help developing new therapeutic strategy.

**Key Words:** Ethanol, binge, hippocampus, rat, immunohistochemistry, epigenetic, neuroinflammation

## SYMPOSIUM

New arguments for a role of the gut microbiota and inflammation in alcohol-use-disorders  
*DE TIMARY Philippe (Department of Adult Psychiatry, Institute of Neuroscience, Brussels, Belgium)*

- The Gut Microbiome in Binge Alcohol Drinking: Recent Translational Efforts  
*LEGGIO Lorenzo (NIAAA DICBR, NIDA IRP, Brown University)*
- Implication of the gut microbiota in the behavioral changes linked to alcohol-dependence: mechanistic approach  
*LECLERCQ Sophie (Université catholique de Louvain, Institute of Neurosciences, Belgium)*
- The gut microbiota as a new target in the treatment of disinhibition in alcohol-dependence: a clinical study  
*QUOILIN Caroline (Institute of Neuroscience, Université catholique de Louvain, Belgium)*
- Does inflammation lead to changes in brain anatomy in alcohol use disorder (AUD)? The effects of alcohol withdrawal in MRI scans  
*DE TIMARY Philippe (Institute of Neuroscience and Department of Adult Psychiatry, Université catholique de Louvain and Cliniques universitaires Saint-Luc)*

**Lorenzo Leggio, MD, PhD, MSc**

**NIAAA DICBR  
NIDA IRP  
Brown University**

**Title: The Gut Microbiome in Binge Alcohol Drinking: Recent Translational Efforts**

We provided the first descriptive analysis of the gut microbiome in a unique non-human primate model of alcohol binge-drinking. We analyzed the gut microbiome on fecal samples from male baboons chronically exposed to either alcohol or a non-alcoholic isocaloric beverage (tang). There were three treatment groups: G1=tang (controls); G2="short-term" alcohol binge drink (2-3 years); G3="long-term" alcohol binge drink (10 years). Fecal samples were collected in two conditions: A=early abstinence (days 3-5) and B=during 3 days of ongoing drinking. Microbial alpha-diversity was significantly lower in the G3 group vs. the G1/G2 groups. The two genera *Lactobacillus* and *Streptococcus* showed high relative abundances in G3. *Fecalibacterium* was reduced in G3 only. For G2, the order Clostridiales and the family Ruminococcaceae showed high relative abundances compared to G1 and G3. Cohort G1 showed members of the family Anaeroplasmataceae to be more abundant. No significant difference was found between Conditions A and B. Our findings suggest that in alcohol binge drinking baboons, long-term exposure to alcohol binge drinking (G3) leads to significant changes in the gut microbiome, whereas short-term (G2) does not. These changes were not affected by acute short-term forced abstinence. This was confirmed in a rat model of binge alcohol drinking, where we see similar reduction in microbiome diversity after prolonged exposure, and this whether the rats were knock-out or not for the receptor of the feeding-related hormone ghrelin. Our result support a role for the gut microbiome in binge drinking.

Title: Implication of the gut microbiota in the behavioral changes linked to alcohol-dependence: mechanistic approach

Authors: Sophie Leclercq<sup>1,2</sup>, Tiphaine Le Roy<sup>2</sup>, Laure Bindels<sup>2</sup>, Caroline Quoilin<sup>1</sup>, Audrey Neyrinck<sup>2</sup>, Peter Stärkel<sup>3</sup>, Philippe de Timary<sup>1</sup>, Nathalie M. Delzenne<sup>2</sup>.

Affiliations:

1. Université catholique de Louvain, Institute of Neurosciences, Belgium
2. Université catholique de Louvain, Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Belgium
3. Université catholique de Louvain, Institute of Experimental and Clinical Research, Belgium

Abstract:

It is well established that alteration of the gut microbiota composition can disturb many aspects of host physiology, including metabolism, immunity and peripheral and central nervous system with consequences for brain functions and behavior. In a previous study, we showed that alterations of the gut microbiota composition of alcohol-dependent (AD) patients were associated with high score of depression, anxiety and alcohol craving, suggesting the existence of a gut-brain axis in AD patients.

Here, we demonstrated the causal role of the gut microbiota in the development of the psychological symptoms associated with alcohol dependence, by using fecal microbiota transplantation. The microbiota of AD patients and healthy controls (CT) were transferred into two groups of mice which were subsequently tested for behavior. We found that mice transplanted with the gut microbiota of AD patients exhibited increased depression-like behavior and decreased social behavior compared to CT-recipient mice. Furthermore, AD-recipient mice showed increased inflammatory cytokines and activated microglia markers in the striatum, decreased expression of myelin-related genes in the frontal cortex and unbalance of GABA/glutamate neurotransmission. Metabolomics analysis revealed that a specific metabolite might be responsible for these changes in brain functions and behavior observed in AD-recipient mice.

These results strongly reinforce the existence of gut-brain interactions in mental disorders, and highlight the gut microbiota as a new potential target in the management of alcohol addiction.

Title: The gut microbiota as a new target in the treatment of disinhibition in alcohol-dependence: a clinical study.

Authors: Caroline Quoilin<sup>1</sup>, Julie Duque<sup>1</sup>, Philippe de Timary<sup>1</sup>, Sophie Leclercq<sup>1,2</sup>.

Affiliations :

1. Institute of Neuroscience, Université catholique de Louvain, Belgium.
2. Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, Belgium

Alcohol dependence is usually seen as a disinhibitory disorder, notably characterized by a state of central nervous system hyperexcitability and a lack of inhibitory control. In particular, by applying transcranial magnetic stimulation (TMS) over primary motor cortex (M1) to assess the excitability of the motor corticospinal pathway during action preparation, we have shown that alcohol-dependent (AD) patients suffer from a deficit in physiological motor inhibition when planning a behaviour. Recently, among the potential mechanisms underlying this inhibitory deficit, an alteration of the gut microbiota composition has been identified as a promising candidate. Here, we aimed at determining whether a treatment with prebiotics, by restoring the microbiota, modifies motor cortex excitability and inhibitory abilities of AD patients. To do so, 50 AD patients participated in a randomized, double blind, placebo-controlled clinical trial, in which they received a supplementation with dietary fibers (inulin) or a placebo during a 3-week detoxification program. Motor cortex excitability was examined at the end of the treatment, using a range of TMS measures, including the resting motor threshold, recruitment curve, short and long intracortical inhibition and intracortical facilitation within M1. Moreover, physiological motor inhibition was assessed during action preparation by applying TMS in a choice reaction time task. Finally, all patients completed questionnaires and performed neuropsychological tasks to evaluate their level of impulsivity and behavioral response inhibition. This double-blinded clinical study will allow to elucidate whether the gut microbiota is an effective target in the treatment of a core symptom of alcohol dependence.

**Philippe de Timary, Géraldine Petit, Laurence Dricot, Sophie Leclercq, Pierre Maurage**

**Institute of Neuroscience and Department of Adult Psychiatry**

**Université catholique de Louvain and Cliniques universitaires Saint-Luc**

**Does inflammation lead to changes in brain anatomy in alcohol use disorder (AUD)?  
The effects of alcohol withdrawal in MRI scans.**

*AUD is characterized by large brain morphological alterations. Alcohol-withdrawal, is attended by large behavioral and inflammatory changes that have been related to altered microbiota composition. Here we tested by MRI scans the hypothesis of a drinking related edema that would resolve during withdrawal. 19 AUD inpatients undergoing a detoxification, were tested on the first and 18th day of withdrawal, for MRI brain anatomy and DTI. Results : Using paired T tests, alcohol withdrawal was attended by significant decreases in volume of 4th ventricle, choroid plexus, white matter, while the cortical volume was increasing. In parallel to these changes, we also observed a decrease in mean diffusivity in all the white matter regions tested, and these decreases were significant in 21 out of the 36 regions tested. A decrease in mean diffusivity was also observed in grey matter in 160 of the 180 regions tested and significant in 50 of these regions, but globally significant changes were observed in regions corresponding to the central executive, motor, salience, auditory, and most significant in the visual and default-mode grey matter regions. In only one region we observed a significant increase in mean diffusivity : the right pallidum. Conclusion : Alcohol withdrawal is related to changes in brain anatomy and mean diffusivity in both white and grey matter regions. These changes will be compared to plasma inflammatory markers, to evaluate whether these changes could express brain inflammation and to changes in functional connectivity and behavior.*

## **SYMPOSIUM**

### **CIFASD Advances in the Pathophysiology and Diagnosis of FASD**

*CHARNESS Michael (VA, Harvard Medical School, Boston University School of Medicine)*

- **Molecular Mechanisms Underlying Ethanol Teratogenesis and its Antagonism**  
*CHARNESS Michael (VA Boston Healthcare System)*
- **Synergistic gene-environment interactions in a zebrafish model of Fetal Alcohol Spectrum Disorders**  
*EBERHART J.K. (University of Texas at Austin)*
- **Ethanol and Cannabinoids Interact to Induce Birth Defects through a Mechanistic Pathway Converging on Primary Cilia**  
*PARNELL SE (Bowles Center for Alcohol Studies, University of North Carolina)*
- **Utilising 3D Facial Analysis for the Early Identification of FASD Associated Facial Dysmorphism at Neonatal and Infant Stages**  
*SUTTIE Michael (Nuffield Department of Women's & Reproductive Health, University of Oxford)*

Molecular Mechanisms Underlying Ethanol Teratogenesis and its Antagonism.  
X Dou, JY Lee, and ME Charness; VA Boston Healthcare System; Harvard Medical School; Boston University School of Medicine; West Roxbury, MA; USA

Ethanol teratogenesis is caused in part by ethanol disruption of the L1 neural cell adhesion molecule (L1). Ethanol inhibits L1 adhesion by interacting with a binding site in the extracellular domain of L1 at the Ig1-Ig4 interface. NAPVSIPQ (NAP) an octapeptide, blocks ethanol inhibition of L1 adhesion and prevents ethanol teratogenesis in mouse embryos at femtomolar concentrations by an unknown mechanism. Ethanol inhibition of L1 adhesion requires L1 binding to ankyrin-G and the spectrin/actin cytoskeleton. Dephosphorylation of selected residues on the L1 cytoplasmic domain (L1-CD) leads to uncoupling of L1 from ankyrin-G, rendering L1 insensitive to ethanol. Polymorphisms in the genes that encode p90<sup>sk</sup>, a kinase that phosphorylates the L1-CD, and ankyrin-G were associated with facial dysmorphism in children with heavy prenatal alcohol exposure. Ankyrin-G binding to L1 requires dephosphorylation of tyrosine in the FIGQY<sup>1229</sup> ankyrin-binding motif of the L1-CD. Femtomolar concentrations of NAP activated the phosphorylation of L1-Y1229 and the dissociation of L1 and ankyrin-G. NAP phosphorylation of L1-Y1229 was mediated by EphB2, and knockdown of EphB2 abolished ethanol inhibition of L1 adhesion. Thus, NAP antagonizes ethanol inhibition of L1 adhesion by activating the phosphorylation of L1-Y1229, inducing the dissociation of L1 and ankyrin-G, and stabilizing an ethanol-insensitive conformation of L1.

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Synergistic gene-environment interactions in a zebrafish model of Fetal Alcohol Spectrum Disorders.

J. K. Eberhart, S. Tucker, Y. Fernandes and N. McCarthy, University of Texas at Austin, Department of Molecular Biosciences, Austin, TX 78712

Susceptibility to FASD is genetically modulated, but the nature of these modifying loci is poorly understood. We have used genetic screens to identify mutants that are sensitive to normally sub-teratogenic doses of ethanol. Embryos mutant or heterozygous for *platelet-derived growth factor receptor alpha (pdgfra)* are exquisitely sensitive to ethanol-induced facial defects. Under control conditions, proper neural crest cell migration, but not survival, requires Pdgfra function. Following ethanol exposure, Pdgfra promotes the survival of neural crest cells. The PI3K pathway is the major Pdgfra effector, and the PI3K/mTORC1 pathway modulates ethanol teratogenesis. We have used CRISPR/Cas-9 to generate mutants altering mTORC1 function and are currently assaying the ethanol sensitivity of these mutants. Disruption of the essential mTORC1 complex member *raptor* sensitizes embryos to ethanol-induced defects, whereas elevating mTORC1 function by disrupting *tsc1a* restores facial development in ethanol-treated *pdgfra* mutants and heterozygotes. The mTORC1 pathway is also associated with social behavior, and ethanol alters social behavior in zebrafish. Consistent with our findings in the face, reduced *tsc1a* gene dosage appears to protect against ethanol-induced social behavioral reductions. Collectively, our findings implicate the mTOR pathway in multiple aspects of FASD.

*This work is supported by NIH/NIDCR R01DE020884, NIH/NIAAA and NIH/NIAAA U01AA021651 as a component of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) to J.K.E*

## Ethanol and Cannabinoids Interact to Induce Birth Defects through a Mechanistic Pathway Converging on Primary Cilia

SE Parnell, EW Fish, KE Boschen; Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC; USA

Ethanol has long been known to be teratogenic, inducing significant craniofacial and brain abnormalities during embryogenesis. We have shown that exposure to both synthetic cannabinoids and the natural cannabinoids found in cannabis - THC and cannabidiol (CBD), during early gestation in mice results in abnormalities similar to those caused by ethanol, including midfacial hypoplasia, holoprosencephaly, and cleft lips/palates. These effects are synergistically produced by the combination of small doses of ethanol and cannabinoids. The teratogenic effects of these drugs are mediated through reductions in the sonic hedgehog (Shh) signaling pathway within the neural tube and can be ameliorated in two ways: amplification of Shh signaling; or inhibition of cannabinoid signaling with cannabinoid receptor 1 (CB1) antagonists. Ethanol inhibits Shh signaling through disruptions of primary cilia function (Shh requires cilia for normal signaling). We also show that cannabinoids inhibit Shh signaling through inhibition of smoothed (Smo), the main effector molecule of the Shh pathway, and CB1 binds to Smo in the primary cilium, acting as an endogenous regulator to fine-tune Shh signaling and downstream mTOR pathway activity. Together, these data demonstrate a mechanistic pathway through which ethanol and cannabinoids act synergistically to impair embryonic development.

*Supported by NIAAA grants R01AA026068 and U01AA021651 as a component of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) (S.E.P.)*

## **Utilising 3D Facial Analysis for the Early Identification of FASD Associated Facial Dysmorphism at Neonatal and Infant Stages**

Michael Suttie<sup>1,2</sup>, Peter Hammond<sup>1,2</sup>, Neil Aiton<sup>3</sup> and the CIFASD<sup>4</sup>

<sup>1</sup> *Nuffield Department of Women's & Reproductive Health, University of Oxford, UK*

<sup>2</sup> *Big Data Institute, University of Oxford, UK*

<sup>3</sup> *Bright and Sussex University Hospitals NHS Trust, UK*

<sup>4</sup> *Collaborative Initiative on Fetal Alcohol Spectrum Disorders ([www.cifasd.org](http://www.cifasd.org)), USA*

*Supported by NIAAA Grant U01AA014809 as a component of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)*

At the most severe end of the FASD spectrum is Foetal Alcohol Syndrome (FAS), where diagnosis is reliant on the identification of a complex set of neurocognitive deficits and identifiable facial features. To attain diagnosis, individuals would typically have been brought to the attention of care professionals or by guardians or parents at early stages of education when behavioural and neurodevelopmental deficiencies become apparent. Individuals with FAS facial criteria make up only a small proportion of those prenatally exposed to alcohol, and cases are often missed. At the neonatal stage, cognitive assessment is not possible, and an individual without the characteristic facial features will not receive diagnosis until later on in childhood.

Previous studies in adolescent populations have utilised 3D imaging, accurately identifying FAS individuals, and objectively recognising subtle facial dysmorphism across the FASD spectrum. For this study, 3D images of infants taken at one month and one year were collected from a South African population, and neonatal images were obtained from a Caucasian population from a clinic in Brighton, UK. Using surface-based analysis of facial form, we observe subtle changes in dysmorphism that occur at the two time points. In addition, we identify subtle dysmorphology in those with a record of prenatal alcohol-exposure in the neonatal population.

At neonatal and infant stages, 3D imaging may accurately and objectively assist facial analysis in those exposed prenatally to alcohol, particularly where features are subtle and more challenging to identify. Early diagnosis is paramount to providing early support and improving outcomes.

## SYMPOSIUM

### Gut microbiota and alcohol: present and future

*LOPEZ-MORENO Jose Antonio (Dept. of Psychobiology and Behavioral Sciences Methods, Complutense University of Madrid)*

- **Gut microbiome may predispose to alcohol use disorder in rats**  
*BOUTREL Benjamin (Center for Psychiatric Neuroscience, Department of Psychiatry, Lausanne University Hospital, Switzerland)*
- **TRANSLATIONAL STUDIES IN ALCOHOL-INDUCED CHANGES IN GUT MICROBIOTA**  
*LÓPEZ-MORENO Jose Antonio (Dept. of Psychobiology and Behavioral Sciences Methods, School of Psychology, Complutense University of Madrid, Madrid, Spain.)*
- **Gut Microbiota in Alcohol Related Disease**  
*KESHAVARZIAN Ali (Rush University Medical Center, Department of Internal Medicine, Division of Digestive Diseases, Chicago)*
- **INTESTINAL MICROBIOTA IN ALCOHOLIC PATIENTS**  
*CIOCAN Dragos (AP-HP, Hepato-gastroenterology and Nutrition, Antoine-Béclère Hospital; INSERM UMRS U996 – Intestinal microbiota, macrophages and liver inflammation, DHU Hepatinov; University Paris-Sud / Paris Saclay, Clamart, France)*

## Gut microbiome may predispose to alcohol use disorder in rats

Kshitij S. Jadhav<sup>1</sup>, Veronica L. Peterson<sup>2,3</sup>, John F. Cryan<sup>2,3</sup>, Benjamin Boutrel<sup>1,4</sup>

<sup>1</sup> Center for Psychiatric Neuroscience, Department of Psychiatry, Lausanne University Hospital, Switzerland

<sup>2</sup> APC Microbiome Ireland, University College Cork, Cork, Ireland

<sup>3</sup> Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland.

<sup>4</sup> Division of Adolescent and Child Psychiatry, Department of Psychiatry, Lausanne University Hospital, Switzerland

Alcohol use represents a significant public health cost, accounting for 4.5% of global disease burden. With current pharmacotherapies largely unsatisfying, discovering novel alternatives to prevent alcohol use disorder becomes a priority. Hence, identifying biological markers predicting vulnerability to develop excessive alcohol consumption may lead to a real improvement of clinical care. With converging evidence suggesting that gut microbiota is capable of influencing brain and behavior, we aimed at investigating the gut microbiome in rats exhibiting uncontrolled alcohol seeking behaviors defined as: 1) inability to abstain during a signaled period of reward unavailability, 2) increased motivation assessed in a progressive effortful task and 3) persistent alcohol seeking despite aversive foot shocks. Based on addiction criteria scores, rats were assigned to either Vulnerable or Resilient group. Not only Vulnerable rats displayed increased impulsive and compulsive behaviors, but also displayed increased relapse after abstinence and increased sensitivity to baclofen treatments compared to resilient animals. Following a 2-month wash out period, rats were sacrificed; dorsal striatum was collected to assess dopamine receptor mRNA expression, and 16S microbiome sequencing was performed on caecal contents. Analyses revealed significant correlations between gut microbiome and impulsivity measures, as well as augmentations in striatal Dopamine 1 receptor (D1R) and reductions in D2R as vulnerability to AUD increased. Therefore, using a singular translational approach based on biobehavioral dispositions to excessive alcohol seeking without heavy intoxication, our observations suggests an association between gut microbiome composition and the vulnerability to lose control over alcohol seeking behaviors.

**Acknowledgements:** This work was supported by APC Microbiome Ireland and Science Foundation Ireland (SFI) [Grant No. 12/RC/2273], the Swiss National Science Foundation (Grant No. 310030\_185192) and the Department of Psychiatry, Lausanne University Hospital. KSJ is recipient of a Swiss Government Excellence Scholarship.

## TRANSLATIONAL STUDIES IN ALCOHOL-INDUCED CHANGES IN GUT MICROBIOTA

López-Moreno J.A.<sup>1</sup>, Rincón-Pérez I<sup>2</sup>, Echeverry-Alzate V<sup>1,3</sup>, Bühler K<sup>1</sup>, Calleja-Conde J<sup>1</sup>, Segovia L<sup>1</sup>, Giné E<sup>4</sup>, Rodríguez de Fonseca F<sup>1,3</sup>, Albert J<sup>5</sup>, Hinojosa J.A.<sup>2</sup>

<sup>1</sup> Dept. of Psychobiology and Behavioral Sciences Methods, School of Psychology, Complutense University of Madrid, Madrid, Spain.

<sup>2</sup> Instituto Pluridisciplinar, Complutense University of Madrid, Madrid, Spain.

<sup>3</sup> Instituto IBIMA, Unidad de Gestión Clínica de Salud Mental, Hospital Regional Universitario de Málaga, Universidad de Málaga, Spain.

<sup>4</sup> Department of Cellular Biology, School of Medicine, Complutense University of Madrid, Madrid, Spain.

<sup>5</sup> School of Psychology, Autonomous University of Madrid, Madrid, Spain.

The intestinal microbiota is an own ecosystem within our body that evolved with us and that changes with our diet and other environmental influences. In a sample of 507 university students we have found that the consumption of alcohol during weekends is associated with a change in the composition of feces and intestinal bacteria. The most plausible hypothesis would be to consider that alcohol changes the intestinal microbiota although a second hypothesis cannot be ruled out: that the differences in the composition of our gut microbiota would explain, at least in part, individual differences in the amounts of alcohol consumed. To verify this second hypothesis, we conducted a series of experiments with animal models (Wistar rats). A group of rats were subjected to alcohol intoxications and another group of rats were treated only with water. These two groups of rats were donors of fecal microbiota transplantation. Other groups of rats received the fecal microbiota transplantation. Animals that received the fecal microbiota transplantation from animals treated with alcohol drank more alcohol than animals that received the microbiota transplantation from animals treated with water or treated with vehicle. We also found that the use of a cocktail of antibiotics to sterilize the intestine produced a reduction in alcohol consumption. During the presentation we will show the main changes that we found in the population of intestinal bacteria analyzed by Next Generation Sequencing and the similarities / differences found between humans and animals.

**Acknowledgements:** This work was supported by the Ministerio de Sanidad, Consumo y Bienestar Social (Plan Nacional sobre Drogas 2018/050 to J.A.L.M) and the Fondo de Investigación Sanitaria (Red de Trastornos Adictivos, FEDER, RD16/0017/0008 to J.A.L.M).

## **European Society for Biomedical Research on Alcoholism (ESBRA)**

"Gut microbiota and alcohol: present and future" Oral Presentation Proposal

### **Title: Gut Microbiota in Alcohol Related Disease**

\*Ali Keshavarzian, Christopher B. Forsyth, Robin M. Voigt, Garth R. Swanson, Faraz Bishehsari, Maliha Shaikh, Stefan Green.

Rush University Medical Center, Department of Internal Medicine, Division of Digestive Diseases, Chicago, IL 60612 USA

The gut microbiota is currently one of the most active research areas in medicine, including alcohol-related diseases. However, this has not always been the case. Our laboratory has been a leader in investigating the complex relationship between the gut microbiota and diseases related to alcohol consumption/abuse. In early studies from our lab we demonstrated that increased intestinal permeability to gut microbial contents was associated with development of alcoholic liver disease (ALD) in human patients. We then went on to carry out studies in rats to show that disruption of the normal pattern of the gut microbiota (so called dysbiosis) by chronic alcohol feeding was associated with intestinal hyperpermeability and ALD in those models. In addition we went on to show that dietary microbiota-directed therapy with probiotics and prebiotic fiber could reverse the changes in the gut microbiota as well as prevent intestinal hyperpermeability and ALD. We then published the first comprehensive study of the gut microbiota in alcoholics with and without liver disease and found an association of specific microbiota dysbiosis with ALD in human subjects. Our lab was also the first one to show that disruption of circadian rhythms resulted in dysbiosis of the gut microbiota. Significantly, we went on to show that disruption of circadian rhythms together with chronic alcohol feeding exacerbated intestinal hyperpermeability to gut microbial contents and resulted in increased liver inflammation and steatosis. Using the APC mouse model of colon cancer, we showed that circadian disruption increased chronic alcohol feeding promotion of colon polyps and invasive polyps in that model and that microbiota directed intervention with prebiotic fiber feeding reversed these effects. We then went on to show that human shift workers with disrupted circadian rhythms exhibit dysbiosis and are more susceptible (than day workers) to alcohol-induced gut leakiness with only moderate consumption of alcohol. Finally in our most recent studies we utilized intestinal organoids from chronic alcohol fed mice to show that decreased colonic microbiota short chain fatty acids (dysbiosis) is associated with epigenetic changes in colonic stem cell fate and junctional proteins resulting in intestinal hyperpermeability and liver inflammation. Our current studies are focused on further investigating these circadian and intestinal stem cell effects of the microbiota and alcohol related diseases and on how microbiota directed therapies can be used to treat and prevent alcohol-related disease.

\*Denotes presenter

## **INTESTINAL MICROBIOTA IN ALCOHOLIC PATIENTS**

Dragos Ciocan

AP-HP, Hepato-gastroenterology and Nutrition, Antoine-Béclère Hospital; INSERM UMRS U996 – Intestinal microbiota, macrophages and liver inflammation, DHU Hepatinov; University Paris-Sud / Paris Saclay, F-92140, Clamart, France

Chronic harmful alcohol consumption can induce a large spectrum of conditions including liver, pancreatic, neurologic and psychiatric diseases. The intestinal microbiota is recognized as an important player in the development and the severity of different diseases related to alcohol. Alcohol induces changes in the composition and functions of microbiota (called dysbiosis). Also, alcohol increases the intestinal permeability which allows translocation of bacteria, bacterial components, and bacterial metabolites (such as short chain fatty acids, bile acids and tryptophan metabolites) into the portal and the systemic circulation. These elements can reach the liver or the brain, establishing a gut-liver or gut-liver-brain axis, and can trigger local and systemic inflammation. Targeting microbiota has been shown to improve liver injury both in animal models of diseases. Different strategies were tested: live bacteria (probiotics), microbiota transplantation, or the consumption of dietary fibres, such as pectin. All these methods can alter the ratio of bacterial species and thus their functions. But although the connections between the microbiota and the host are well established, the underlying mechanisms, including key components that might serve as potential therapeutic targets, remain to be elucidated. From a clinical perspective, well-designed studies that target microbiota in order to modify the clinical course, reverse disease or prevent complications are needed in patients with harmful alcohol consumption.

## SYMPOSIUM

### From Alcohol Initiation to Disorder: Perspectives and Treatment Across the Lifespan

*SILVERI Marisa (McLean Hospital / Harvard Medical School)*

- **Adolescent Neurobiology and the Impact of Alcohol Use Initiation**  
*SILVERI Marisa M. (Neurodevelopmental Laboratory on Addictions and Mental Health, McLean Hospital, Belmont, MA; Department of Psychiatry, Harvard Medical School, Boston, MA)*
- **Evaluating combined treatment versus single-focused treatment for Depression and Heavy Episodic Drinking in College Students**  
*PEDRELLI Paola (Massachusetts General Hospital, Boston, MA; Department of Psychiatry, Harvard Medical School, Boston, MA)*
- **Mindfulness-based Relapse Prevention for Drinking Reduction Among Community-Dwelling Older Adults Seeking Treatment for Alcohol Use Disorder**  
*WITKIEWITZ Katie (Department of Psychology, University of New Mexico, Albuquerque NM)*
- **Pharmacological Treatments for Alcohol Use Disorder: Riding the Waves of Medications Development**  
*LEGGIO Lorenzo (Division of Intramural Clinical and Biological Research, National Institute on Alcohol Abuse and Alcoholism, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD; Department of Behavioral and Social Sciences, Brown University)*

## **From Alcohol Initiation to Use Disorder: Perspectives and Treatment Across the Lifespan**

### **Presentation 1: Adolescent Neurobiology and the Impact of Alcohol Use Initiation**

Marisa M. Silveri, Ph.D.

Neurodevelopmental Laboratory on Addictions and Mental Health, McLean Hospital, Belmont, MA; Department of Psychiatry, Harvard Medical School, Boston, MA

Early initiation of alcohol use is considered an important risk factor for the later development of an alcohol use disorder. While behavioral and neurobiological alterations have been observed in relation to early onset alcohol use, it is not been fully elucidated whether differences reflect antecedents to, or consequences of, early alcohol initiation. In our longitudinal study, alcohol- and drug-naïve adolescents (n=81, 13-14years-old) completed neuroimaging at baseline, magnetic resonance imaging (MRI) and functional MRI during spatial navigation and emotional response inhibition task performance. Based on quarterly follow-up assessments of alcohol use, those who initiated use by age 16 exhibited smaller hippocampal volumes and larger posterior cingulate and superior frontal volumes than non-initiators. During fMRI, hippocampal activation during memory retrieval was negatively correlated with age of first use, while hippocampal activation during negative response inhibition trials was positively correlated with age of first use. These findings suggest that distinct regional brain volumes in areas involved in learning and memory (hippocampus) and adaptive decision-making (frontal lobe), and functional differences in hippocampal efficiency and sensitivity across emotional contexts may be important predictors for the timing of alcohol use initiation. Characterizing brain structure and function may therefore be useful in the search for biomarkers of risk for hazardous adolescent behaviors, such as alcohol consumption. Further, such neurobiological patterns, established early in the second decade of life, may then escalate into adulthood, conferring neurobiological risk of developing an alcohol use disorder later in life.

## **From Alcohol Initiation to Use Disorder: Perspectives and Treatment Across the Lifespan**

### **Presentation 2: Evaluating combined treatment versus single-focused treatment for Depression and Heavy Episodic Drinking in College Students**

Paola Pedrelli, Ph.D.

Massachusetts General Hospital, Boston, MA; Department of Psychiatry, Harvard Medical School, Boston, MA

Heavy Episodic Drinking (HED) and depressive symptoms often co-occur among college students and are associated with significant personal and societal problems. However, evidence-based treatments are not available for these comorbid conditions. The current study compared the effectiveness of a treatment combining Cognitive-Behavioral-Therapy for Depression and Brief Motivational Interviewing (CBT-D+BMI) and CBT-D alone among 94 college students with depressive symptoms and HED. Both treatment programs were associated with significant reduction in HED, alcohol-related problems (ARP), and depressive symptoms, at the end of treatment and at one-month follow-up, of similar magnitude. Moderators of outcomes were also examined. Among students with fewer depressive symptoms at baseline CBT-D was associated with greater preservation of gains relative to CBT-D+BMI long-term. Furthermore, among students with higher baseline frequency of HED, those who received CBT-D+BMI had a higher ARP reduction between baseline and the end of treatment than their peers receiving CBT-D. While the study did not include a no-treatment condition, the magnitude of improvement during treatment was higher than usually observed with time, suggesting a benefit of psychosocial treatments for this population. Depending on the main focus of treatment, HED or depression, different approaches may be implemented.

## **From Alcohol Initiation to Use Disorder: Perspectives and Treatment Across the Lifespan**

### **Presentation 3: Mindfulness-based Relapse Prevention for Drinking Reduction Among Community-Dwelling Older Adults Seeking Treatment for Alcohol Use Disorder**

Katie Witkiewitz, Ph.D.

Department of Psychology, University of New Mexico, Albuquerque NM

Treatments for alcohol use disorder (AUD) have progressed considerably over the past 30 years. Yet, relatively few studies have been conducted to evaluate treatments that target drinking reduction for less severe drinkers who are interested in reducing alcohol consumption and even fewer studies have examined treatments for older adults interested in drinking reduction. Mindfulness-based relapse prevention is a treatment for addiction that holds considerable promise for targeting cognitive and affective processes, and has recently been found in two randomized clinical trials to be superior to gold standard cognitive behavioral treatment in reducing heavy drinking and drug use days. Yet, prior work has not examined mindfulness-based relapse prevention (MBRP) for heavy drinking reductions as primary outpatient treatment and has not examined MBRP in older adults. In the current study, we examined the effectiveness of rolling group MBRP among treatment seeking individuals (n=84) with alcohol use disorder who were interested in reducing their heavy drinking. We also examined the effects of treatment among older adults (age 60+, n=25). Results indicated a significant effect of treatment on drinking reductions. The magnitude of drinking reductions also correlated with the number of MBRP group sessions attended. Results from the subgroup analyses indicated the effects were also significant in those over the age of 60. Taken together, these results suggest drinking and craving reductions are achievable among people with AUD, including older adults. The current findings also suggest a dose response relationship between MBRP sessions attended and drinking reductions.

## **From Alcohol Initiation to Use Disorder: Perspectives and Treatment Across the Lifespan**

### **Presentation 4: Pharmacological Treatments for Alcohol Use Disorder: Riding the Waves of Medications Development**

Lorenzo Leggio, M.D., Ph.D., M.Sc.

Division of Intramural Clinical and Biological Research, National Institute on Alcohol Abuse and Alcoholism, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD; Department of Behavioral and Social Sciences, Brown University, Providence, RI

Harmful alcohol use is a risk factor in more than 60 diseases and injuries resulting in approximately 2.5 million deaths per year worldwide, therefore effective treatments for alcohol use disorder (AUD) are needed. Among them, medications development represents a high priority in the alcohol field. However, only a few medications (“first wave”) are currently approved by the appropriate regulatory bodies (e.g., FDA, EMEA) for AUD. Research has been conducted with the goal of testing medications already approved for other clinical indications as potential effective treatments for AUD (“second wave”). Among several medications that may be mentioned in this group, two examples include GABA<sub>B</sub> receptor agonism via baclofen, a medication already approved for spasticity; and alpha-1-blockade via prazosin and doxazosin, two medications already approved for hypertension and benign prostatic hyperplasia. More recently, additional efforts have been made toward the identification of new neurobiological pathways involved in the development and maintenance of AUD and possibly useful as new therapeutic targets (“third wave”). Among them, neuroendocrine pathways such as ghrelin, GLP-1 and oxytocin seem promising and are currently under investigation. The overall goal of the clinical and translational research in the field of medications development for AUD is to increase the armamentarium of effective pharmacological treatments that health care providers may use to treat patients with AUD and provide evidence toward patient-oriented precision medicine.

## **SYMPOSIUM**

The changing face of clinical trials for alcohol use disorders

*AUBIN Henri-Jean (Université Paris-Sud)*

- Precision medicine in Alcohol Dependence: matching phenotypes to pharmacotherapy  
*KRANZLER Henry (Univ Pennsylvania School of Medicine)*
- Harm reduction: an alternative avenue for alcohol use disorder pharmacotherapy  
*Karl Mann, Katie Witkiewitz and the ACTIVE group*
- Placebo response in clinical trials for alcohol use disorders: can we improve clinical trial designs?  
*AUBIN Henri-Jean, FARINA Claire (Université Paris-Sud)*

Precision medicine in Alcohol Dependence: matching phenotypes to pharmacotherapy  
Henry Kranzler

Department of Psychiatry, University of Pennsylvania Perelman School of Medicine,  
Philadelphia, PA, USA

Early efforts to identify predictors of response to alcohol use disorder (AUD) treatment focused largely on patient characteristics that moderate the response to psychotherapy. Although some significant moderators were identified in Project MATCH, a large, multi-center trial that examined a series of features as predictors of response to three different psychotherapies for AUD, the findings have not substantially altered clinical care. More recent efforts have examined genetic predictors of medication response, with the most widely studied gene variant-medication pair being the Asn40Asp single nucleotide polymorphism in the mu-opioid receptor gene as a moderator of naltrexone treatment response. In a recent meta-analysis of that literature, we found no effect of the Asp40 variant on the response to naltrexone. Mann et al. recently reported that a subgroup of individuals from the PREDICT Study identified as reward drinkers showed an 83% reduction in the likelihood of any heavy drinking when treated with naltrexone compared to placebo. In a secondary analysis of a randomized trial of naltrexone among problem drinkers, reward drinkers treated with naltrexone reported significantly less frequent and less heavy drinking, and desire to drink mediated the effect of naltrexone on daily drinking. If validated prospectively, the reward drinker phenotype could serve as a clinically useful self-report measure that predicts the subgroup of AUD patients who benefit most from naltrexone treatment.

Harm reduction: an alternative avenue for alcohol use disorder pharmacotherapy  
Karl Mann, Katie Witkiewitz and the ACTIVE group

Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, J 5, 68159, Mannheim, Germany.

Only 10 – 20 % of individuals with a diagnosis of alcohol dependence subscribe to a specific treatment. Empirical data show that the most important reason for this treatment gap is the requirement to subscribe to total abstinence. The scientific discussion of this issue gained significant momentum when the European Medicines Agency accepted Reductions in alcohol consumption as an interim harm reduction strategy in the treatment of alcohol use disorder. Earlier studies have found that significant reductions in alcohol use are common among individuals with alcohol use disorder, however it remained unclear whether reductions in drinking risk levels are associated with significant improvements in health, quality of life, and other consequences of alcohol use disorder, and also whether reductions in drinking are stable over time. The goal of our approach was to examine the correspondence between levels of alcohol consumption and experiences of drinking-related consequences, mental health, blood pressure, and liver function tests during treatment among individuals receiving treatment for alcohol dependence in the COMBINE study. Results indicated reductions in WHO drinking risk levels were associated with significantly fewer alcohol related consequences, greater mental health, and improvements in physical health functioning, including reduced blood pressure and better liver function. Importantly, reductions in risk levels were also stable over time. The results provide evidence of reductions in WHO risk levels as a viable alternative to abstinence as an endpoint for alcohol clinical trials associated with meaningful reductions in alcohol related consequences and improvements in mental and physical health.

## Placebo response in clinical trials for alcohol use disorders: can we improve clinical trial designs?

Henri-Jean Aubin, Claire Farina

INSERM 1018, University Paris-Sud, Paul-Brousse Hospital, Villejuif, France.

The placebo response in Alcohol Use Disorder (AUD) trials has been shown to be negatively correlated to the treatment effect size. This systematic meta-analytic approach gives insight on how the choice of various endpoints and time points affect the placebo response in AUD trials. Moderator and meta-regression analyses can provide valuable information regarding the impact of variables such as patient characteristics and trial design options on the placebo response. Depending on endpoints and time points, placebo response effect size (standardized mean difference) may vary from values as high as 1.5 to 2.2. Return to any drinking at 12 weeks was observed in more than 70% individuals in trials selecting abstinent subjects at baseline. Placebo response moderators differed according to endpoints. Nevertheless, journal impact factor appeared to be consistently related to a lower placebo response. Interestingly psychiatric comorbidity did not appear to significantly affect the placebo response effect size. Also, studies published in recent years showed a higher placebo response effect size for alcohol reduction-related endpoints, whereas earlier studies showed a higher placebo response effect size for abstinent-related endpoints. Some of these findings can be valuable for future AUD randomized clinical trials protocols.

## **SYMPOSIUM**

Examining Psychosocial Predictors of Alcohol Outcomes: Implications for Etiology, Prevention, and Treatment

*PEARSON Matthew (University of New Mexico)*

- An Ecological Test of Stress- and Cue-Induced Alcohol Craving  
*PEARSON Matthew (University of New Mexico)*
- Alcohol Use among College Students: Lessons Learned from the Protective Strategies Study Team  
*STUDY TEAM Protective Strategies (University of New Mexico)*
- Beyond One Size Fits All: Deviance Regulation Theory-Based Interventions Targeting Use of Alcohol Protective Behavioral Strategies  
*DVORAK Robert (University of Central Florida)*
- I Am What I Am: Defining the Nomological Network of Alcohol Identity Using Meta-Analysis  
*MONTES Kevin (California State University, Dominguez Hills)*

Symposium: Examining Psychosocial Predictors of Alcohol Outcomes: Implications for Etiology, Prevention, and Treatment

An Ecological Test of Stress- and Cue-Induced Alcohol Craving

Matthew R. Pearson (University of New Mexico)

Katie Witkiewitz (University of New Mexico)

Eric Claus (Mind Research Network)

A sample of 68 participants were recruited and randomized to a mindfulness-based intervention (n=24), attentional bias modification (n=21), or a health education/attentional bias assessment control condition (n=23). Our design included the collection of drinking data for 2 weeks via ecological momentary assessment (EMA) before, during, and after the intervention. Our hypotheses were built from a craving-based model of drinking such that both exposure to alcohol cues and experience of negative affect/stress are expected to predict increased alcohol craving, which in turn leads to alcohol use. Although our data EMA data failed to support expected intervention-specific effects, we found independent effects of self-reported stress (2 items: stressed and overwhelmed), reports of experiencing specific stressors (financial/job-related, relationship, health-related, daily hassles), and negative affect on craving. For example, we found that relationship-related stressors and daily hassles predicted level of craving at the within-subject level, whereas financial/job-related stressors and health-related stressors predicted level of craving at the between-subject level. These kinds of nuanced associations between stress-related factors and alcohol-related outcomes have yet to be examined using ecologically valid data. Further, we found independent effects for four out of five cue exposure measures. Specifically, at the within-subjects level, we found that exposure to alcohol advertising, seeing alcohol, being proximal to a drinking establishment, and hearing conversations about alcohol were all uniquely predictive of alcohol craving (though being around other individuals with whom they have consumed alcohol was not).

Symposium: Examining Psychosocial Predictors of Alcohol Outcomes: Implications for Etiology, Prevention, and Treatment

Alcohol Use among College Students: Lessons Learned from the Protective Strategies Study Team  
Protective Strategies Study Team

Protective behavioral strategies (PBS) are specific behaviors one can utilize to minimize the harmful consequences of alcohol consumption. There has been an increasing amount of interest in use of PBS among college students, especially as an intervention target. In 2013, Pearson reviewed all PBS studies conducted at that time. This review demonstrated that despite the fact that a large number of studies have been conducted on PBS, there have been few replication attempts. To advance the PBS field, the Protective Strategies Study Team (PSST) was formed to conduct large multisite studies investigating PBS. In our first study, we collected over 7,307 across 10 different states in the United States. In this presentation, we highlight our major findings to date. Chiefly, we replicated and extended past findings by exploring a wide range of more distal antecedents (i.e., mental health symptoms, drinking motives, impulsivity-like traits) that may affect alcohol-related outcomes via PBS use (i.e., mediation) as well as a number of factors that interact with PBS use to predict outcomes (i.e., moderators). We also compare and contrast alcohol PBS findings with cannabis PBS findings. Finally, we discuss the implications of these findings for prevention and intervention efforts.

Symposium: Examining Psychosocial Predictors of Alcohol Outcomes: Implications for Etiology, Prevention, and Treatment

Beyond One Size Fits All: Deviance Regulation Theory-Based Interventions Targeting Use of Alcohol Protective Behavioral Strategies

Robert D. Dvorak (University of Central Florida)

The prototypical college student alcohol intervention is norm-based. Most of these interventions use personalized normative feedback to correct college students' overestimations of their peers' alcohol use. Unfortunately, these interventions tend to have small, short-lived effects on alcohol use. Protective behavioral strategies (PBS) are behaviors one can engage in before, during, or after drinking to help reduce alcohol use, intoxication, and/or alcohol-related harms. PBS have been shown to be directly related to lower alcohol consequences even when controlling for levels of use, making it a promising intervention target. There is reason to suspect that traditional normative feedback will not translate well to targeting PBS use. In this presentation, we review a social psychological theory, deviance regulation theory (DRT), that posits individuals engage in behaviors to stand out in positive ways or avoid standing out in negative ways. We review the findings from 5 studies conducted to date evaluating DRT-based interventions targeting alcohol PBS. Taken together, these interventions have been shown to increase alcohol PBS use, which in turn reduces alcohol use and/or alcohol-related problems. We discuss the implications of matching intervention content (in our case, positively framed vs. negatively framed messages) to individuals' preexisting beliefs (in our case, their normative perceptions) in the broader context of alcohol interventions.

I Am What I Am: Defining the Nomological Network of Alcohol Identity Using Meta-Analysis  
Kevin S. Montes (California State University, Dominguez Hills)  
Matthew R. Pearson (University of New Mexico)

Alcohol identity refers to the centrality of alcohol use to an individual's self-concept (e.g., the extent to which alcohol use is important to one's self-image or self/personal identity). Theory (e.g., PRIME theory of motivation; social identity theory, theory of planned behavior) suggests that alcohol identity may serve as an important source of motivation that guides alcohol use. Empirical research has also been conducted to examine the associations between alcohol identity and alcohol-related outcomes (e.g., frequency, quantity, consequences, and dependence). Traditionally, alcohol identity has been assessed using self-report measures although more recent investigations have assessed alcohol identity using implicit measures (e.g., implicit alcohol identity). Over 40 studies have been conducted to examine the associations between alcohol identity and alcohol-related outcomes along with narrative reviews that summarized findings from many of these studies. Missing from this extant literature is a systematic review that includes a meta-analytic component that synthesizes effect size estimates across these studies in order to derive a single, weighted effect size estimate between alcohol identity and each alcohol-related outcome. In the current study, we meta-analytically examined the associations between alcohol identity and alcohol-related outcomes. We also examined the associations between alcohol identity and known correlates of alcohol use (e.g., motives, expectancies, negative affect, norms, cravings). In addition, we summarize measurement approaches used in the assessment of alcohol identity. Findings from this meta-analytic study will inform a discussion surrounding opportunities to improve the measurement of alcohol identity as well as how best to target alcohol identity in future prevention/intervention efforts.

## INVITED TALK

- Immunotherapeutic strategies for Alcoholic Liver Disease – why, how and when?  
*CHOKSHI Shilpa (Viral Hepatitis and Alcohol Research Group; Kings College London; Institute of Hepatology, Foundation for Liver Research)*

## **SYMPOSIUM**

Do you feel your body or your emotions? Drinking alcohol in the context of body-mind communication

*WOJNAR Marcin (Medical University of Warsaw)*

- Drink until you cannot feel feelings: The role of physical pain in predicting relapse to alcohol use following treatment for alcohol use disorder

*Katie Witkiewitz*

- Can you feel your own body? - the role of interoception in alcohol use disorder

*JAKUBCZYK Andrzej*

- Can you feel what I am feeling – childhood adversity, alcohol use and mentalization

*KOPERA Maciej*

- Heavy Drinking, Depression, and Treatment Seeking for Mental Health Concerns in Physicians

*BROWER Kirk J. (University of Michigan Medical School)*

## **Drink until you cannot feel feelings: The role of physical pain in predicting relapse to alcohol use following treatment for alcohol use disorder**

Katie Witkiewitz

Identifying factors that predict a return to heavy drinking (i.e., relapse) following alcohol treatment is critical for the development of relapse prevention interventions. Physical pain is common among individuals with alcohol use disorders (AUDs), yet few studies have examined associations between pain and alcohol relapse. Data from the COMBINE study (n=1383) and the United Kingdom Alcohol Treatment Trial (n=743) were used to examine the associations between physical pain and alcohol relapse in randomized clinical trials for alcohol use disorders. Results indicated a significant association between physical pain and alcohol relapse, which was mediated by experiences of negative affect. Targeting acceptance and management of physical pain in the treatment of alcohol use disorder may help reduce relapse risk.

## **Can you feel your own body? - the role of interoception in alcohol use disorder**

Andrzej Jakubczyk

It has been suggested that interoception (which reflects the way one perceives somatic stimuli from the body) may contribute to alcohol use disorder (AUD) as it relates to the body's experience of substance use or withdrawal. Moreover, there is growing evidence that interoceptive responses are associated with immediate, discrete emotions. However, only a few studies have directly investigated associations between interoception, emotion regulation and alcohol use. The study conducted in a group of sober alcohol-dependent individuals showed that when controlling for level of anxiety, sleep problems, age, sex and education, individuals with AUD scored significantly higher on self-reported interoceptive sensibility and lower on interoceptive accuracy in comparison to healthy controls. Moreover, in the group of subjects with AUD measures of emotional utilization remained a significant correlate of interoceptive accuracy, whereas lack of own emotional awareness, difficulties controlling impulsive behaviors when experiencing negative emotions, and appraisal of emotions remained significantly associated with interoceptive sensibility. These results have to be treated as preliminary and need to be replicated; however, findings indicate that interoception may present a novel therapeutic target for treatment of AUD.

## **Can you feel what I am feeling – childhood adversity, alcohol use and mentalization.**

Maciej Kopera

Although theoretical link between childhood trauma and mentalization has been established empirical evidence for it is still limited. Current data shows that the direction of this relationship might be individually shaped in selected at-risk populations. Childhood trauma is highly prevalent in treatment-seeking subjects with AUD and may play a significant role in the development and severity of AUD. The first goal of the presented studies was to see if the presence of risky alcohol use during the developmental age would influence the relationship between childhood adversity and mental states recognition in early adulthood (Study 1). Additionally, we wanted to see if the transgression from risky alcohol use to AUD would influence the trauma-mentalization relationship in another treatment seeking AUD sample (Study 2). Our findings highlight an important and lasting role for variations in early life stress on individual differences in adult social cognitive functioning.

# Heavy Drinking, Depression, and Treatment Seeking for Mental Health Concerns in Physicians

Kirk J. Brower, MD

Professor of Psychiatry

Chief Wellness Officer

University of Michigan Medical School

## Introduction

The relationship between heavy drinking and depression in physicians is worthy of further study. A large survey of U.S. physicians found that 15.3% screened positive for an alcohol use disorder using the AUDIT-C, which correlated significantly with depression. The objective of this study was to look at the influences of heavy drinking (HD) and depression on treatment seeking for mental health concerns in physicians. It was hypothesized that each would have independent effects and reduce the likelihood of treatment seeking.

## Methods

A faculty and physician health survey was sent by an email link to 3657 faculty members at a medical school. Anonymity was assured by using an external survey center, so that only de-identified data were analyzed. A total of 1710 (46.8%) of surveys were returned, including 1089 physicians who constituted the study group. The sample consisted of 46% females with a modal age group between 36 to 45 (34.5%). The NIAAA single question screen and criteria for HD, and the PHQ9 for depression were used.

## Results

Rates of any HD and for at least monthly HD during the past year were 23.1% and 7.3%, respectively. Moderate-to-severe depression and any suicidal ideas during the past 2 weeks were endorsed by 13.6% and 3.7%, respectively. Physicians with at least monthly HD had significantly increased severity of depression. Frequency of suicidal thoughts were significantly correlated with both any HD and at least monthly HD. Those who drank heavily at least monthly were less willing to seek treatment for a mental health concern (60.8% vs. 67.2%,  $p=0.02$ ).

## Conclusion

Frequent heavy-drinking physicians had greater depression severity, but were less willing to seek treatment for a mental health concern than other physicians. Possible reasons for these findings will be discussed.

## **SYMPOSIUM**

Social cognition in severe alcohol-use disorders: from emotional decoding to dehumanization experience

*MAURAGE Pierre (University of Louvain, Belgium)*

- It's complicated: Different tasks lead to different conclusions as to the severity of the impact of severe alcohol-use disorder upon social cognition  
*Sharon Cox (London South Bank University, UK)*
- Self-evaluative emotions in severe alcohol use disorders  
*Delphine Grynberg (University of Lille, France)*
- Clinical impact of social cognition in treatment seeking patients with severe alcohol-use disorders  
*Claudia Rupp (University of Innsbruck, Austria)*
- Dehumanizing experiences of patients with severe alcohol-use: links with fundamental needs and important clinical outcomes  
*Sullivan Fontesse (University of Louvain, Belgium)*

**Sharon Cox** (London South Bank University, UK)

“It’s complicated: Different tasks lead to different conclusions as to the severity of the impact of severe alcohol-use disorder upon social cognition”

Severe alcohol-use disorder (SAUD) is associated with deficits in social cognition, frequently evidenced by errors in emotional facial recognition and a lesser ability to theorise about another’s state of mind. To date, little evidence exists on the clinical impact of these deficits, including how they influence treatment outcomes and are perceived by others. Findings from two studies will be presented; the first study (N=123) highlights the impact of poor social cognition on treatment outcomes (completion versus drop out), and how different tasks (binary versus non-binary responses) generate varying conclusions as to the extent of the deficits caused by SAUD. Results suggest that non-binary tasks which are non-time defined allow adults with SAUD scope to explore their answers and self-correct, leading to lower error rates. In the second study (N=89), how apparent these problems are to professionals who support these individuals is explored through the correlation of estimates of deficits to experimental data. Professionals’ ability to recognise these deficits is contained to those who clients presenting with SAUD and other clinical symptoms (e.g., anxiety, psychosis). Taken together, results suggest that the nature of the experimental tasks affects the degree to which we can estimate the severity of the impact of SAUD upon social cognition.

**Delphine Grynberg** (University of Lille, France)

“Self-evaluative emotions in severe alcohol use disorders”

Guilt and shame have been very little explored in severe alcohol-use disorder (SAUD). Although previous findings suggest that the experience of shame may play a major role in the maintenance of SAUD, they do not allow to determine (1) whether proneness to experience guilt and shame is associated with the experience of the emotions in the context of their consumption, (2) whether SAUD and healthy controls (HC) differ in terms of guilt and shame concerning their alcohol consumption, and (3) whether these later are associated with alcohol use severity. The present study examined these hypotheses in 40 patients diagnosed with SAUD according to DSM-V criteria and 54 HC (AUDIT scores < 7). Participants were instructed to complete the Test of Self-Conscious Affect-3, the Personal Feelings Questionnaire-2 (PFQ-2), the Hospital Anxiety and Depression Scale, the Alcohol Use Disorders Identification Test (AUDIT) and a questionnaire that has been developed for the present study, the “Substance Shame and Guilt” (SSG). Main results show (1) PFQ-2-shame and PFQ-2-guilt are moderately associated with SSG-guilt and SSG-shame, (2) SAUD report higher SSG-guilt and SSG-shame than HC and (3) SSG-guilt is positively associated with the AUDIT in SAUD only. These results suggest that patients with SAUD experience greater shame and guilt associated with their consumption and that the consumption severity is associated with greater guilt in SAUD. This study thus emphasized the importance to consider shame and guilt in the maintenance of SAUD.

**Claudia Rupp** (University of Innsbruck, Austria)

“Clinical impact of social cognition in treatment seeking patients with severe alcohol-use disorders”

Past years research witnessed growing evidence that cognitive deficits in patients with severe alcohol-use disorder (SAUD) include social cognition, most prominently deficits in (facial) emotion recognition. Until now, less is known about the clinical relevance of these impairments in SAUD. In our prospective research studies, we were interested whether deficits in social cognition (1) contribute to less successful treatment outcome (relapse/dropout), and (2) recover “naturally” (with abstinence) during (about 8-week) SAUD treatment, or are persistent cognitive problems. Main results of our studies are that (1) patients with SAUD presenting poorer (facial) emotion recognition showed less successful treatment outcome with respect to relapse and/or early dropout. In addition, (2) patients with SAUD showed no recovery with controlled abstinence during treatment in social cognition deficits, including impaired (facial) emotion recognition ability, compared with healthy controls. Our findings evidence the clinical impact of social cognition deficits, particularly emotion recognition deficits on treatment success in SAUD. Moreover, our findings indicate that clinically relevant social cognitive deficits are rather persistent cognitive problems in SAUD that would need special (e.g., neurocognitive rehabilitation) treatments. New research focusing on the improvement of these deficits in SAUD seems warranted.

**Sullivan Fontesse** (UCLouvain, Belgium)

“Dehumanizing experiences of patients with severe alcohol-use: links with fundamental needs and important clinical outcomes”

Dehumanization, the denial of one's humanness, has important negative consequences for social interactions. Dehumanization from the dehumanizer's perspective has been widely studied in social psychology. However, victims' perspective has been neglected. Moreover, despite dehumanization being described as endemic in medicine, no study has investigated dehumanizing experiences (i.e the feeling of being dehumanized by others) in psychiatric populations. To address these gaps, dehumanizing experiences of 120 patients with severe alcohol-use disorder (SAUD) were investigated. We argue that because patients with SAUD are rejected and stigmatized against, two known antecedents of dehumanization, they might feel dehumanized by others. Our model proposed that dehumanizing experiences would be associated with fundamental needs thwarting. These needs are the psychological equivalent of thirst or hunger: shared by all and causing important negative health consequences when thwarted. Additionally, it was hypothesized that dehumanizing experiences and fundamental needs thwarting would together be linked to patients' emotions, cognitions, and behaviors. Results supported that dehumanizing experiences were associated with increased fundamental needs thwarting. Dehumanizing experiences were also associated with negative emotions, negative self-esteem, and dysfunctional coping strategies, such as alcohol use. Our results suggest that dehumanizing experiences might play a crucial role in the vicious circle leading to SAUD.

## INVITED TALK

- [Applying Precision Medicine to Alcohol and Drug Use Disorders](#)

*KRANZLER Henry (Benjamin Rush Professor of Psychiatry and the Director of the Center for Studies of Addiction. Center for Studies of Addiction, Perelman School of Medicine, University of Pennsylvania)*

Applying Precision Medicine to Alcohol and Drug Use Disorders  
Henry R. Kranzler, M.D.

Although most medications approved to treat alcohol use disorder (AUD) and opioid use disorder (OUD) have been shown to be efficacious in placebo-controlled trials, effect sizes vary among them. Even medications with the largest effect sizes, however, are not efficacious for all (or perhaps even most) patients treated with them. Recent efforts to enhance the therapeutic effects of these medications have used a precision medicine approach, including the use of pharmacogenetics (PGx) to identify genetic predictors of treatment response. This lecture will discuss recent developments in the PGx of AUD and OUD. Specific topics to be covered are: 1) a variant in the mu-opioid receptor gene (*OPRM1*) and response to naltrexone treatment of AUD, 2) a variant in the kainate receptor gene and response to topiramate treatment of AUD, 3) a variant near *OPRM1* and usual methadone dose for treating OUD, and 4) a variant in the delta-opioid receptor gene (*OPRD1*) and response to buprenorphine treatment of OUD. Findings from these studies underscore the potential utility of a precision medicine approach to treating alcohol and drug use disorders and some of the obstacles to be overcome in advancing the field.

## **SYMPOSIUM**

### **Novel concepts in alcoholic liver disease**

*MUELLER Sebastian (Center for Alcohol Research and Salem Medical Center, University of Heidelberg)*

- **Systemic inactivation of hypoxia-inducible factor prolyl 4-hydroxylase 2 in mice protects from alcohol-induced fatty liver disease**  
*Anna Laitakari, Oulu, Finland*
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*Tomomi Kogiso, Tokyo, Japan*
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*Ryuta Kitagawa, Tokyo, Japan*

## Systemic inactivation of hypoxia-inducible factor prolyl 4-hydroxylase 2 in mice protects from alcohol-induced fatty liver disease

Anna Laitakari<sup>1</sup>, Teemu Ollonen<sup>1</sup>, Thomas Kietzmann<sup>2</sup>, Gail Walkinshaw<sup>3</sup>, Daniela Mennerich<sup>2</sup>, Valerio Izzi<sup>1</sup>, Kirsi-Maria Haapasaari<sup>4</sup>, Johanna Myllyharju<sup>1</sup>, Raisa Serpi<sup>1</sup>, Elitsa Y. Dimova<sup>1</sup>, Peppi Koivunen<sup>1,\*</sup>

<sup>1</sup>Biocenter Oulu, Faculty of Biochemistry and Molecular Medicine, Oulu Center for Cell-Matrix Research, University of Oulu, Oulu, Finland. <sup>2</sup>Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland. <sup>3</sup>FibroGen Inc., San Francisco, CA, USA. <sup>4</sup>Department of Pathology, Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland.

\*Corresponding author

Alcoholic fatty liver disease (AFLD) is a growing health problem for which no targeted therapy is available. We set out to study whether systemic inactivation of the main hypoxia-inducible factor prolyl 4-hydroxylase, HIF-P4H-2 (PHD2/Egln1), whose inactivation has been associated with protection against metabolic dysfunction, could ameliorate it. HIF-P4H-2-deficient and wild-type (WT) mice or HIF-P4H inhibitor-treated WT mice were subjected to an ethanol diet for 3-4 weeks and their metabolic health, liver and white adipose tissue (WAT) were analyzed. Primary hepatocytes from the mice were used to study cellular ethanol metabolism.

The HIF-P4H-2-deficient mice retained a healthier metabolic profile, including less adiposity, better lipoprotein profile and restored insulin sensitivity, while on the ethanol diet than the WT. They also demonstrated protection from alcohol-induced steatosis and liver damage and had less WAT inflammation. In liver and WAT the expression of the key lipogenic and adipocytokine mRNAs, such as *Fas* and *Ccl2*, were downregulated, respectively. The upregulation of metabolic and antioxidant hypoxia-inducible factor (HIF) target genes, such as *Slcs 16a1* and *16a3* and *Gclc*, respectively, and a higher catalytic activity of ALDH2 in the HIF-P4H-2-deficient hepatocytes improved handling of the toxic ethanol metabolites and oxidative stress.

Pharmacological HIF-P4H inhibition in the WT mice phenocopied the protection against AFLD. Our data show that global genetic inactivation of HIF-P4H-2 and pharmacological HIF-P4H inhibition can protect mice from alcohol-induced steatosis and liver injury, suggesting that HIF-P4H inhibitors, now in clinical trials for renal anemia, could also be studied in randomized clinical trials for treatment of AFLD.

## **Modeling of fibrosis pattern formation: From mouse models to human patients of chronic liver diseases**

Seddik Hammad<sup>1,2</sup>, Jieling Zhao<sup>3,4</sup>, Jan G. Hengstler<sup>3</sup>, Sebastian Müller<sup>5</sup>, Dirk Drasdo<sup>3,4</sup>, Steven Dooley<sup>1</sup>

<sup>1</sup>Molecular Hepatology Section, Department of Medicine II, Medical Faculty Mannheim, Heidelberg University, 68167-Mannheim, Germany, <sup>2</sup>Department of Forensic Medicine and Veterinary Toxicology, Faculty of Veterinary Medicine, South Valley University, 83523-Qena, Egypt. <sup>3</sup>Leibniz Research Centre for Working Environment and Human Factors at TU Dortmund (IfADo), 44139, Dortmund, Germany; <sup>4</sup>INRIA de Paris, 2 Rue Simone IFF, 75012, Paris, France, <sup>5</sup>Department of Medicine and Center for Alcohol Research, Salem Medical Center, University of Heidelberg, Zeppelinstr, 69121 Heidelberg, Germany

Fibrosis is a consequence of repetitive liver injuries, e.g. alcohol consumption. Liver fibrosis develops in different patterns according to the etiological factor i.e. pericellular to septal pattern in alcoholic hepatitis. The mechanism behind the generation of the different “scar” patterns is still elusive. Furthermore, this scar pattern is considered an early stage of cirrhotic nodules in the advanced disease stage. We aim to define (a) molecular driver(s) of fibrosis pattern formation. We exposed mice to acute or chronically repeated doses (twice/ week for 6 weeks) of CCl<sub>4</sub>, which like alcohol is metabolized by CYP2E1, but presents with more severe liver damage in a shorter time frame. Spatial distribution of fibrosis formation and metabolic enzyme expression, namely CYP2E1, were analyzed in liver tissues. We found that recovery of CYP2E1<sup>+</sup> hepatocytes after acute CCl<sub>4</sub> form exactly the same septal pattern of collagen scar walls that form upon chronic injury, suggesting that CYP2E1<sup>+</sup> hepatocytes are the trigger of the fibrotic pattern. To study this hypothesis, we assumed a dynamic activator/inhibitor CYP2E1 mathematical model. The current model already partially captures the aforementioned CYP2E1/ECM pattern. We are currently challenging the model by mechanistic studies. These include i) crosstalk between activated/reverted HSCs and LSECs differentiation; ii) communication between ECs lining the CV and metabolically zoned hepatocytes; iii) testing a potential diffusible inhibitor in the portal compartment, i.e. the bile acid concentration. We are targeting the WNT/ $\beta$ -Catenin pathway (CYP2E1 regulator) by monoclonal antibodies against R-spondin1/2/3 in the fibrosed liver, and iv) test the mechanical role of ECM deposited by HSCs. Clinical application of the suggested model will be iteratively investigated in F1, F2, F3 and F4 cohorts of patients with alcoholic liver disease.

## The characteristics of alcoholic liver disease in Japan

KOGISO Tomomi, Tokyo Women's Medical University (kogiso.tomomi@twmu.ac.jp)

**Aim)** The clinical features of alcoholic liver disease (ALD) including the genetic background were not fully identified in Japan. Here, we investigated that the clinical characteristics, hepatocellular carcinoma (HCC), and the single nucleotide polymorphisms (SNPs) associated with alcohol, glucose, and fat metabolism in patients with ALD compared to non-alcoholic fatty liver disease (NAFLD).

**Methods)** 1) ALD (n = 118; male, 86%; median age, 62 years; liver cirrhosis, 58%; HCC, 31%) and NAFLD (n = 200; male, 55%; age, 61 years; cirrhosis, 19%; HCC, 12%) patients were evaluated. 2) The survival and recurrence rates of HCC were examined in the patients of multi-center in Japan (532 ALD-HCC and 209 NAFLD-HCC).

**Results:** 1) Comparing with NAFLD, ALD were predominantly male and lower body mass index and the complication of lifestyle-related diseases. As the genetic background, the ADH1B genotype GG and ALDH2 genotype GG were observed more frequently and the MTP genotype GG was decreased in ALD (ALD vs. NAFLD, ADH1B, 16% vs. 4%; ALDH2 84% vs. 44%; MTP 62% vs. 72%, respectively; all  $p < 0.01$ ). Comparing with and without HCC, the KCNJ15 genotype GG were identified as the risk factors of ALD-HCC. 2) The patients showed 5-year survival rates of ALD-HCC 43.7% vs. NAFLD-HCC 49.1%; 5-year recurrence rates of 65.4% vs. 69.6 %, respectively.

**Conclusion:** The SNPs for the enzymes of alcohol metabolism were associated with ALD and the risk of diabetes were co-related to ALD-HCC. The survival and recurrence rates of HCC were equally shown in ALD and NAFLD.

## **The role of intestinal microbiota in alcoholic liver disease**

Ryuta Kitagawa, Kazuyoshi Kon, Maiko Suzuki, Kenichi Ikejima

Department of Gastroenterology, Juntendo University Graduate School of Medicine

Emerging attention has been paid for gut microbiota in the human health and disease. Indeed, gut microbiota is dynamically altered by dietary factors, lifestyle, and alcohol intake. Gut microbiota-dependent activation of hepatic innate immunity is important in the pathogenesis of steatohepatitis caused by both alcohol and metabolic syndrome. Chronic alcohol exposure, as well as dietary overload, compromises gut barrier function causing increases in intestinal permeability, thereby aggravating translocation of bacterial products into the portal blood. Pathogen-associated molecular patterns (PAMPs) derived from gut microbiota elicit production and release of inflammatory cytokines through multiple innate immune signaling pathways, resulting in the exacerbation of steatohepatitis. The comorbidity of alcoholic liver disease and metabolic syndrome has become an emerging clinical problem worldwide. We have recently applied the mouse model of chronic-binge EtOH liver injury (NIAAA model) for obese KK-A<sup>y</sup> mice, mimicking alcoholic liver injury comorbid metabolic syndrome. For therapeutic approach, we investigated the effect of rifaximin (RFX), an oral non-absorbed antibiotic, in this model. EtOH-feeding/binge caused more severe hepatic steatosis, oxidative stress, and induction of inflammatory cytokines in KK-A<sup>y</sup> mice as compared to B1/6 controls, which were markedly prevented by RFX treatment. RFX dramatically modified the small intestinal microbiota following chronic EtOH feeding, decreasing the relative abundance of the order Erysipelotrichales and increasing the order Bacteroidales, without affecting EtOH-induced increase of net amount of viable bacteria. It is postulated that the modulation of small intestinal microbiota is critical for the prevention of alcoholic liver injury comorbid metabolic syndrome.

## **SYMPOSIUM**

Natural history of alcoholic liver disease: Risk factors, early disease detection and prognostic markers  
*THIELE Thiele (Dpt. Gastroenterology & Hepatology, Odense University Hospital)*

- Epidemiology and natural history of alcohol-related liver disease  
*Gro Askgaard, postdoc, MD, PhD (Copenhagen, Denmark)*
- Current state-of-the-art prognostic and diagnostic markers including genetic traits  
*Maja Thiele, associate professor, MD, PhD (Odense, Denmark)*
- Pharmacological decrease of liver stiffness in alcohol-related liver disease predicts long-term clinical outcome  
*Felix Piecha (Hamburg, Germany)*
- Spleen stiffness/length to liver stiffness ratio significantly differs between ALD and HCV and predicts disease-specific complications  
*Elsharaawy Omar, (Egypt and Heidelberg, Germany)*

## *Epidemiology and natural history of alcohol-related liver disease*

Gro Askgaard, Zealand University Hospital, Copenhagen, Denmark  
National Institute of Public Health, University of Southern Denmark

End-stage alcohol-related liver disease is a rare disease in the general population (0.06% per year or lower in men > 45 years) and knowledge of risk factors is needed to target screening for early liver disease in individuals of high risk of the disease. In this session we will focus on risk factors for alcohol-related liver disease. We will discuss the influence of alcohol amount (recent and earlier in life), alcohol drinking patterns (binge drinking/daily drinking, wine/beer/liquor, meal-related alcohol consumption) and age, smoking, and obesity as risk factors of alcohol-related disease. Will cutting down on alcohol amount decrease the risk of further progression in liver disease? Individuals at particular high risk for end-stage liver disease may be found at specialized alcohol treatment centers or as hospital patients given an alcohol problem diagnosis. These populations have a risk for end-stage alcohol-related liver disease of 7 to 16% after about 10 years. Moreover, some studies suggest half of patients with end-stage alcohol-related liver disease had healthcare contacts in general practice or at the hospital before their diagnosis with obvious alcohol problems. This indicates that there may be opportunities to reach about half of patients with end-stage alcohol-related liver disease with preventive interventions before diagnosis.

Current state-of-the-art prognostic and diagnostic markers including genetic traits

Maja Thiele, Center for Liver Research, Department of Gastroenterology and Hepatology, Odense University Hospital and University of Southern Denmark, Odense, Denmark

This talk will include an overview of the current best-in-class non-invasive markers for diagnosis of fibrosis and prognostication in alcohol-related liver disease patients. These markers include ultrasound elastography techniques and serum markers that reflect extracellular matrix formation, so called direct fibrosis markers. Beyond the marker's diagnostic role in an outpatient hospital setting, they may be used for case-finding or screening at a population level. However, due to the lower prevalence of advanced fibrosis in at-risk populations, compared to patients referred to secondary healthcare, preselection of patients using risk scores may be used to increase true-positive rate and decrease false-positives, thereby minimizing risk of overdiagnosis. Such risk scores are likely to include genetic traits, co-occurrence of metabolic risk factors, alcohol drinking history and drinking pattern.

## FREE ORAL COMMUNICATIONS 1

- **In vivo longitudinal study of risky alcohol consumption effects on the brain**  
*LANQUETIN Anastasia (Physiopathology and Imaging of Neurological Disorders, Normandie University, Caen, France)*
- **BEARNI as screening tool for neuropsychological impairments and brain shrinkage in alcohol use disorder and Korsakoff's patients**  
*RITZ Ludivine (Normandie University, Caen, France)*
- **Is impulsivity related to executive deficits in patients with Alcohol Use Disorder?**  
*CABé Nicolas (Normandie University, Caen, France)*
- **A Smartphone App to Assess Alcohol Consumption Behaviours: Development, Validity, Compliance, and Reactivity**  
*POULTON Antoinette (Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Australia)*
- **Affect regulation training (ART) program in alcohol abstinence consolidation**  
*CLAISSE Caroline (SCALab Laboratory UMR CNRS 9193)*

# *In vivo* longitudinal study of risky alcohol consumption effects on the brain

A Lanquetin<sup>1</sup>, A Drieu<sup>1</sup>, C Freyssainge<sup>1</sup>, D Vivien<sup>1,2</sup>, AL Pitel<sup>3</sup>, M Rubio<sup>1</sup>

1. *Physiopathology and Imaging of Neurological Disorders (PhIND), Normandie University, Caen, France*

2. *Department of Clinical Research, CHU Côte de Nacre, Caen, France*

3. *Normandy Univ, UNICAEN, PSL Université, EPHE, INSERM, U1077, CHU de Caen, GIP Cyceron, Neuropsychologie et Imagerie de la Mémoire Humaine, Caen, France.*

**Introduction:** Neuroimaging and neuropsychological studies revealed structural and functional brain alterations associated with Alcohol Use Disorder (AUD). In 50 to 80 % of AUD patients, these brain alterations result in cognitive and/or motor impairments. However, the consequences of risky alcohol consumption (not reaching AUD but superior to the recommendations) have been less studied in both humans and rodents. In this longitudinal study, we aimed at studying the effects of risky alcohol consumption in mice at different time points.

**Mat & met:** Mice were divided into 3 groups: control (drinking water), risky alcohol consumption (10% ethanol solution *ad libitum*) and risky alcohol consumption with repeated periods of abstinence (ethanol replaced by water before and during the test week). From 6 weeks to 12 months alcohol exposure, every 3 months, and we conducted i) a battery of behavioral tests to measure motor abilities (balance beam), anxiety (open field) and memory (Y-maze, fear conditioning); ii) MRI examinations to study regional brain volumes. Beverage intake and body weight were measured all along the experiment and did not show any difference between groups (~6 ml/mouse/day).

**Results:** Behavioral alterations were significant after 6 months of risky alcohol consumption, as revealed by persistent memory impairments. After 9 and 12 months of alcohol exposure, balance abilities were gradually altered in the two groups with risky alcohol consumption. Anxiety levels did not differ between groups at any time. Brain volumes in various regions classically affected by alcohol consumption did not show any between-group difference after 6, 9 or 12 months of alcohol exposure.

**Conclusion:** Our results show that risky alcohol consumption, even when not reaching AUD, drives a series of behavioral alterations which are less severe but compatible with the deficits described in AUD patients. Interestingly, these deficits do not seem to be related to macrostructural brain alterations. Microscopic analyses to study neuronal density, microgliosis and astrogliosis that could explain the behavioral deficits observed are ongoing.

**Key words:** risky alcohol consumption, longitudinal, behavioral study, MRI

## **BEARNI as screening tool for neuropsychological impairments and brain shrinkage in alcohol use disorder and Korsakoff's patients**

Ludivine Ritz<sup>1,3</sup>; Shailendra Segobin<sup>1</sup> ; Coralie Lannuzel<sup>1,2</sup>; Céline Boudehent<sup>1,2</sup>; François Vabret<sup>1,2</sup>; Anne Lise Pitel<sup>1</sup> and Hélène Beaunieux<sup>1,3</sup>

<sup>1</sup>Normandie Univ, UNICAEN, PSL Research University, EPHE, INSERM, U1077, CHU de Caen, Neuropsychologie et Imagerie de la Mémoire Humaine, 14000 Caen, France

<sup>2</sup>Service d'Addictologie, Centre Hospitalier Universitaire de Caen, 14000 Caen, France

<sup>3</sup>Normandie Univ, UNICAEN, Laboratoire de Psychologie Caen Normandie (LPCN, EA 4649), Pôle Santé, Maladies, Handicaps – MRSH (USR 3486, CNRS-UNICAEN), 14000

Caen, France

Chronic and excessive alcohol consumption results in Alcohol Use Disorder (AUD) without neurological complications and Korsakoff's syndrome (KS) when combined with thiamine deficiency. These two clinical forms are accompanied by widespread structural brain damage in both the fronto-cerebellar and Papez circuits as well as in the parietal cortex. Grey matter (GM) shrinkage in these brain regions is in agreement with the neuropsychological impairments observed in AUD patients early in abstinence including notably executive and motor deficits as well as episodic memory disorders. AUD and KS can be distinguished on the severity of the brain damage and cognitive deficits. The main specificity of KS is a disproportionately encoding deficit in episodic memory, whose severity allows distinguishing KS from AUD. BEARNI is a screening tool especially designed to detect neuropsychological impairments in AUD. But the relevance of BEARNI for the detection of KS patients and its relationships with brain damage remain unknown.

Ten KS patients, 26 AUD patients and 16 healthy controls (HC) underwent the BEARNI and a 3T-MRI examination. On BEARNI, KS had lower performance than AUD patients (who did not differ from HC) for the episodic memory and fluency scores. The specificity of KS deficits on the memory subtests suggests that BEARNI is sensitive to amnesia. Statistical cluster analysis revealed that several AUD patients were classified within the same cluster as KS patients based on the BEARNI episodic memory results. Thus, a low score on this subtest (inferior or equal to 1.5 points /6) would enable the detection of patients at risk for developing

KS. Multiple regression analyses conducted between GM volume and performance on each BEARNI subtest revealed correlations with the FCC, the PC and parietal cortices. The comparison between KS and AUD regarding the GM volume in the FCC and parietal cortices revealed that they were atrophied to the same extent, suggesting that BEARNI is sensible to the severity of alcohol-related GM abnormalities. Within the PC, the volume of the parahippocampal gyrus correlated with the fluency score and was the only region to be specifically atrophied in KS, suggesting that BEARNI is sensible to specific brain abnormalities occurring in KS.

It is worthwhile noting that BEARNI remains a screening test and should not be considered as a sufficient tool to diagnose KS. An extensive neuropsychological assessment associated with a follow-up examination (in order to confirm the persistence of the neuropsychological impairments) is required for a confirmed KS diagnosis.

## Is impulsivity related to executive deficits in patients with Alcohol Use Disorder?

Cabé N<sup>1,2</sup>; Laniepce A<sup>1</sup>; Boudehent C<sup>1,2</sup>; Eustache F<sup>1</sup>; Vabret F<sup>1,2</sup>; Beaunieux H<sup>1,3</sup> et Pitel AL<sup>1</sup>

- (1) Normandie Univ, UNICAEN, PSL Research University, EPHE, INSERM, U1077, CHU de Caen, Neuropsychologie et Imagerie de la Mémoire Humaine, 14000 Caen, France
- (2) Service d'Addictologie, Centre Hospitalier Universitaire de Caen, 14000 Caen, France
- (3) Normandie Univ, UNICAEN, Laboratoire de Psychologie Caen Normandie (LPCN, EA 4649), Pôle Santé, Maladies, Handicaps – MRS (USR 3486, CNRS-UNICAEN), 14000 Caen, France

### **Introduction:**

Impulsivity, strongly associated with Alcohol Use Disorder (AUD), is a multidimensional construct encompassing various different cognitive and behavioral components. Impulsivity is involved in the induction of the first alcohol consumption, reactivity to alcohol stimuli, loss of control over alcohol consumption, development of dependence, risk of relapse, and craving (1,2). Anxiety and depression are also associated with craving and risk of relapse and may be related to impulsivity (3,4). In clinical practice, impulsivity is assessed by self-questionnaires such as the Barratt Impulsiveness Scale (BIS), which has been designed to measure trait impulsiveness and their dimensions. Some authors rather consider impulsivity as a result of an executive dysfunction and more particularly as an inhibition failure. Chronic and excessive alcohol consumption is indeed known to be associated with cognitive impairments and especially with executive deficits such as altered planning, flexibility or inhibition (5). Impulsivity observed in AUD patients may thus reflect the behavioral consequences of impaired executive abilities related to a history of excessive and chronic alcohol consumption. A better understanding of the cognitive substrates of impulsivity is crucial to offer appropriate care when impulsivity is considered as a therapeutic target.

Our aim was to investigate the relationships between impulsivity and executive functions in AUD patients, taking patients' alcohol history, as well as anxiety and depression, into account. Considering that impulsivity is a consequence of the dysexecutive syndrome, we would expect the impulsivity score to be related to executive abilities and alcohol exposure. If we rather consider impulsivity as a matter of personality or emotions, we would expect it to be related to thymic variables such as anxiety and depression, as well as early alcohol consumption.

### **Method:**

Eighty-five recently detoxified AUD patients and sixty-three healthy controls (HC) matched for age, education, and sex were included. Sociodemographic data and information about patients' alcohol consumption history were collected. Anxiety was measured by the State-Trait Anxiety Inventory (STAI), and depression by the Beck Depression Inventory (BDI).

Executive functions were assessed with an extensive neuropsychological battery, which explored flexibility, inhibition, manipulation of information stored in working memory, organization and strategy.

Impulsivity was measured by the Barratt Impulsiveness Scale 10 (BIS10) which is the latest validated version of this questionnaire in French.

Parametric statistical analyses (Student's T-tests) and chi-square tests when appropriate were used to compare AUD and HC groups. We then conducted correlational analyses (Bravais-Pearson) to investigate the relationships between BIS 10 total score, cognitive abilities including executive functions, anxiety and depression, and alcohol history or clinical variables.

## Results:

Compared to HC, AUD patients were significantly more anxious and depressed and presented a more frequent alcohol family history. Moreover, AUD patients were significantly more impulsive than HC as indicated by their BIS 10 total score.

AUD patients were impaired on all the executive tests used in the study except on the verbal fluency task. Their speed processing was significantly lower than in HC. Correlations revealed that in AUD patients, the BIS 10 total score correlated with none of the executive and alcohol history measures. By contrast, impulsivity correlated with higher anxiety and depression.

## Discussion:

AUD patients presented a high level of impulsivity, which was related neither to their executive deficits nor to their history of alcohol consumption. Our findings indirectly indicate that impulsivity, as evaluated by the BIS 10, does not seem to be a consequence of an alcohol-related dysexecutive syndrome. Impulsivity could rather be a vulnerability factor, linked to affective parameters (anxiety, depression) and potentially favoring the development of excessive and chronic drinking, which in turn would result in altered executive functions. These unexpected results could be interpreted within the theoretical framework of the neurocognitive dual-process model, which proposes that addictions may be the product of an imbalance between 1) a “reflective system,” involved in the cognitive evaluation of the stimuli by means of memory and executive functions, responsible for controlled-deliberate responses and 2) an “affective- automatic system,” involved in the emotional evaluation of the stimuli, initiating automatic-appetitive responses. The imbalance between these two systems would account for rapid decision making, prioritizing short-term reward irrespective of the long-term consequences (6). Our findings suggest that impulsivity would correspond to the hyperactivation of the affective system rather than to the alteration of the reflective system. Another explanation could be that AUD patients have difficulty to self-evaluate their level of impulsivity when asked through questionnaires (7). Further studies are required to explore whether our findings could be replicated using other impulsivity questionnaires (like UPPS or BIS 11) and executive tasks targeting other functions such as decision-making or planning. Clinically, these results suggest that impulsivity and executive abilities should be evaluated and managed separately and in complementary perspective to prevent or reduce the relapse risk in AUD patients.

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## **A Smartphone App to Assess Alcohol Consumption Behaviours: Development, Validity, Compliance, and Reactivity**

Antoinette Poulton<sup>1</sup>, Jason Pan<sup>1</sup>, Loren Richard Bruns<sup>2</sup>, Richard O. Sinnott<sup>2</sup>, Robert Hester<sup>1</sup>

<sup>1</sup>Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Australia

<sup>2</sup>Computing and Information Systems, University of Melbourne, Parkville, Australia

**Background:** Although research into problem drinking often relies on retrospective measures to assess alcohol intake, such methods have been found to distort actual consumption levels/patterns. Real-time electronic protocols in the form of smartphone apps are consequently advocated. There is limited research pertaining to the development, validity, compliance, and reactivity of using such apps in the experimental arena.

**Methods:** An iterative process guided the development of the CNLab-A app. Healthy individuals ( $N = 671$ ) completed demographic questions and a 21-day Timeline Followback before using CNLab-A for 21 days. We considered the size and diversity of the sample; compared data reported via retrospective measures with that captured using CNLab-A; and, assessed the data for evidence of compliance and reactivity as a function of hazard versus non-hazard drinker status.

**Results:** CNLab-A yielded a large and diverse sample. On average, participants submitted data on 20.27 out of 21 days. Compared to Timeline Followback, a significantly greater percentage of drinking days (24.79% vs. 26.44%) and significantly higher total intake (20.30 vs. 24.26 standard drinks) was recorded via the app. Both hazard and non-hazard drinkers were highly compliant with app protocols. Linear growth analyses revealed hazardous drinkers decreased their alcohol intake by 0.80 standard drinks over the 21-day experimental period. There was no change to the drinking of non-hazard individuals. Both hazard and non-hazard drinkers showed a slight decrease in responding (“yes”) to drinking behavior over the same period.

**Conclusions:** Smartphone apps appear an effective and methodologically sound means of obtaining alcohol consumption information.

# Affect regulation training (ART) program in alcohol abstinence consolidation

Caroline Claisse, Marie-Charlotte Gandolphe, Lydie Defrance, Mickael Naassila & Jean-Louis Nandrino

**Background.** This study aimed at exploring the evolution of emotion regulation strategies and abilities with training remediation program in abstinent patients suffering from severe alcohol use disorders (AUD). The main objective of this project is the consolidation of abstinence with the acquisition or reinforcement of new emotion regulation strategies. In the Adaptive Coping with Emotions Model (ACE Model, Berking & Whitley, 2014), adaptive emotion regulation is conceptualized as a situation-dependent interaction between emotion regulation skills. To this end, the Affect Regulation Training (ART, Berking & Whitley, 2014) was conceptualized in six training modules (psycho-education, muscle and breathing relaxation, non judgmental awareness, acceptance and tolerance, compassionate self-support, analyzing emotions and modifying emotions) related to these emotion regulation skills. Each unit focuses on one emotion regulation skill.

**Method.** 117 alcohol-abstinent individuals abstinent from two weeks to several years were recruited in day hospitals and groups of Alcoholics Anonymous association. 87 individuals participated in the ART program and were compared to 30 alcohol-abstinent individuals who do not have the program. The ART program consisted of six weekly 3-hour therapy sessions in a group of 5 to 8 persons. In control group (n=30), evaluations were proposed within therapy session. For all individuals, the evaluation consisted of two parts: a clinical questionnaire (concerning their current situation, alcohol use and context) and a cognitive and emotional assessment. Drinking history and emotion regulation processes were assessed using the Difficulties in Emotion Regulation Scale (DERS-F) and the Cognitive Emotion Regulation Questionnaire (CERQ). Anxiety and depression scores were assessed using Hospital Anxiety Depression Scale (HADS). Finally, mindfulness levels were assessed with Five Facets Mindfulness Questionnaire (FFMQ). For the experimental group, the same evaluation was performed before ART program (T0 ART), after the ART program (T1 ART) and six months later (T2 ART). In control group, the evaluation was performed twice, one at (T0 control) and six weeks later (T1 control).

**Result:** Differences in emotion regulation strategies were found according to the participation in ART remediation program. The results showed less difficulties in emotion regulation (DERS-F) for all individuals immediately after (T1 ART) or six months after the end of therapy (T2 ART) than before ART session (T0 ART). Compared to control group, individuals with emotion training partially recovered on DERS-F subscales especially for strategies and emotional clarity. In addition, individuals had higher levels of non-adaptive strategies for emotion regulation (CERQ) before (T0 ART) than after therapy session (T1 ART, T2 ART). In comparison to control group (T0 control), they had less non-adaptive strategies after ART session (T1 ART). Finally, mindfulness total score was higher after therapy session in ART group (T1 ART) than in control group (T0 control, T1 control).

**Conclusion:** These results demonstrated a recovery of emotion regulation abilities after emotional training in a sample of abstinent alcohol individuals. Compared to Control group, the ART group presented less difficulties in emotional abilities and regulation strategies, and higher levels of mindfulness. The ART program allows greater flexibility in the emotion regulation strategies involved in consolidating abstinence and should be used both initially at the beginning of withdrawal but also in individuals in the process of maintaining their abstinence.

## **SYMPOSIUM**

Non-invasive screening for alcoholic liver fibrosis based on liver stiffness: algorithms and challenges

*MUELLER Sebastian (Center for Alcohol Research and Salem Medical Center, University of Heidelberg)*

- Open questions in the assessment of alcoholic cirrhosis based on liver stiffness  
*S Mueller*
- Computer-based digital algorithms to assess fibrosis stage in ALD  
*M Thiele*
- Screening for ALD in an addiction care setting using simple bio clinical items  
*J Trabut*
- Examination conditions for liver stiffness measurement: not so easy!?  
*J Boursier*

## SYMPOSIUM

### Pathophysiology of alcoholic liver disease

*IKEJIMA Kenichi (Juntendo University)*

- **Alcoholic liver disease: Impact of the type of alcoholic beverage**  
*BERGHEIM Ina (Department of Nutritional Sciences, RF Molecular Nutritional Science, Althanstr. 14, UZA2, 1090 Vienna, Austria)*
- **Vinyl chloride-induced interaction of nonalcoholic and toxicant-associated steatohepatitis: protection by the ALDH2 activator Alda-1.**  
*BEIER Juliane (University of Pittsburgh)*
- **The liver matrixome and inflammatory liver injury in ALD**  
*ARTEEL Gavin (Department of Medicine and Pittsburgh Liver Research Center,)*
- **Endoplasmic reticulum stress and oxidative stress in alcoholic liver injury comorbid metabolic syndrome**  
*KON Kazuyoshi (Juntendo University Graduate School of Medicine)*

## **Alcoholic liver disease: Impact of the type of alcoholic beverage**

Prof. Dr. Ina Bergheim

Department of Nutritional Sciences, RF Molecular Nutritional Science, Althanstr. 14, UZA2, 1090 Vienna, Austria

Alcohol intake is still among the leading causes of chronic liver diseases world-wide. Despite marked efforts made in many countries around the world to increase the awareness in the general population regarding the negative effects on health associated with chronic and especially elevated chronic intake of alcohol, the global proportion of the alcohol consumers has not markedly dropped throughout the last decades. Indeed, alcohol consumption still accounts for nearly 10% of global deaths among populations aged 15-49. Epidemiological studies also suggest that per capita consumption of alcohol from various alcohol beverages e.g. the beer, wine, spirits and other alcohol containing beverages including palm wine, or fermented beverages made of banana, sorghum or maize differs markedly between different areas of the world. Epidemiological but also clinical and animal studies further suggest that different alcoholic beverages may impact the development of alcoholic liver disease differentially. These findings along with possible molecular mechanism involved will be reviewed and discussed.

## **Vinyl chloride-induced interaction of nonalcoholic and toxicant-associated steatohepatitis: protection by the ALDH2 activator Alda-1.**

Juliane I Beier

Department of Medicine; Division of Gastroenterology, Hepatology and Nutrition; University of Pittsburgh; Pittsburgh, PA 15213, USA

The abundant environmental toxicant vinyl chloride (VC) shares similar metabolic pathways in the liver to alcohol. Specifically, VC is metabolized via CYP2E1 and aldehyde dehydrogenase dependent pathways to produce the corresponding alcohol (chloroethanol, CE) and aldehyde (chloroacetaldehyde, CAA). VC causes steatohepatitis at high levels, but is considered safe at lower (i.e., sub-OSHA) levels. However, we have previously shown that even lower VC levels exacerbate experimental fatty liver disease caused by high-fat diet (HFD). Mitochondrial oxidative injury and subsequent metabolic dysfunction appeared to play key roles in mediating this interaction. Mitochondrial aldehyde dehydrogenase 2 (ALDH2) serves as a key line of defense against endogenous and exogenous reactive aldehydes. The current study therefore tests the hypothesis that allosteric activation of ALDH2 with Alda-1 will protect against VC-enhanced fatty liver disease. Mice were exposed to low VC concentrations (<1 ppm), or room air for 6 hours/day, 5 days/week for 12 weeks, while on HFD or low-fat control diet (LFD). Some mice received Alda-1 (20 mg/kg i.p., 3×/week) for the last 3 weeks of diet/VC exposure. Indices of liver injury, oxidative stress, metabolic and mitochondrial (dys)function were measured. As observed previously, low-dose VC did not cause liver injury in control mice; while liver injury caused by HFD was enhanced by VC. VC decreased hepatic ALDH2 activity of mice fed HFD. Alda-1 attenuated oxidative stress, liver injury, and dysmetabolism in mice exposed to HFD+VC under these conditions. Importantly, alterations in mitochondrial function caused by VC and HFD were diminished by Alda-1. Previous studies have indicated that liver injury caused by HFD is mediated, at least in part, by enhanced mitochondrial autophagy (mitophagy). Here, Alda-1 suppressed PINK1/PARKIN-mediated mitophagy. Taken together, these results support the hypothesis that ALDH2 is a critical defense against mitochondrial injury caused by VC in experimental fatty liver disease. The ALDH2 activator Alda-1 conferred protection against liver damage under these conditions, most likely via increasing clearance of aldehydes and preserving mitochondrial respiratory function.

## The liver matrisome and inflammatory liver injury in ALD

Gavin E. Arteel<sup>1,2</sup>.

<sup>1</sup>Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, and <sup>2</sup>Pittsburgh Liver Research Center. University of Pittsburgh.

The strategic location of the liver between the intestinal tract and the rest of the body makes it a critical organ for clearance of xenobiotics and toxins that enter the portal blood. As the main detoxifying organ in the body, the liver has a high likelihood of toxic injury. It is therefore not surprising that the liver has tremendous ability to heal and regenerate from injury. The complex and synchronized regenerative response in the liver can be perturbed and thereby can impact normal tissue recovery from injury or damage, leading to progressive injury, and potentially liver failure. The extracellular matrix (ECM) consists of a diverse range of components that work bi-directionally with surrounding cells to create a dynamic and responsive microenvironment that regulates cell signaling, recruitment, and tissue function. The basic definition of the ECM comprises fibrillar proteins (e.g., collagens, glycoproteins and proteoglycans). More recently, groups have extended the definition to include ECM affiliated proteins (e.g., collagen-related proteins), ECM regulator/modifier proteins (e.g., lysyl oxidases and proteases) and secreted factors that bind to the ECM (e.g., TGF $\beta$  and other cytokines); this broader definition has been coined the 'matrisome' (1). The ECM not only provides structure and support for the cells in a tissue, but also acts as a reservoir for growth factors and cytokines and as a signaling mechanism by which cells can communicate with their environment and vice-versa (2). Quantitative and qualitative changes to the ECM structure and superstructure can impact overall health of the organ and organism. Remodeling of the hepatic ECM/matrisome in response to injury is well understood in some contexts. For example, changes to the extracellular matrix associated with fibroproliferative diseases (i.e., fibrosis/cirrhosis) are considered almost synonymous with hepatic ECM changes. Proteomic-based studies in other organs have demonstrated that the matrisome responds dynamically in composition after insult well before fibrotic changes to the organ (3, 4). These changes to the ECM may not alter overall ECM architecture and are therefore histologically undetectable. Nevertheless, these changes have potential to alter hepatic phenotype and function (5). These acute responses can be viewed as an arm of the wound healing response and facilitate recovery from damage, which resolves once the damage is repaired. However, under conditions of chronic injury, these changes likely contribute to activation of a significant remodeling response that leads to scar formation (i.e., fibrosis). This presentation will discuss some of the salient processes and players involved in the acute phase response of the ECM to liver injury after alcohol and other insults.

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## **Endoplasmic reticulum stress and oxidative stress in alcoholic liver injury comorbid metabolic syndrome**

Kazuyoshi Kon, Maiko Suzuki, Kenichi Ikejima

Department of Gastroenterology, Juntendo University Graduate School of Medicine

The endoplasmic reticulum (ER) is a multifunctional organelle required for the regulation of calcium homeostasis, lipid metabolism, and protein synthesis. A number of cellular stress conditions lead to the accumulation of unfolded or misfolded proteins in the ER and disruption of the ER homeostasis, which can trigger ER stress. ER stress activates the unfolded protein response (UPR). The UPR pathway includes induction of several molecular chaperones that restore cellular homeostasis by promoting the folding or degradation of unfolded proteins; however, if ER stress is prolonged or too severe, the signaling switches from pro-survival to pro-death, leading to ER stress-induced apoptosis. Several studies have shown that ER stress contributes to the development of alcoholic liver disease. Here we investigated the role of ER stress in chronic-binge ethanol (EtOH) model using obese KK-A<sup>y</sup> mice. Chronic-plus-binge EtOH intake induced massive hepatic steatosis along with hepatocyte apoptosis and inflammation, and increased ER stress markers including binding immunoglobulin protein (Bip), unspliced and spliced forms of X-box-binding protein-1 (uXBP1 and sXBP1, respectively), inositol trisphosphate receptor (IP3R), and C/EBP homologous protein (CHOP), and also enhanced oxidative stress markers heme oxygenase-1 and 4-hydroxynonenal. Administration of 4-phenylbutyric acid, the chemical chaperone, during chronic EtOH exposure ameliorated steatohepatitis after chronic-binge EtOH, and completely inhibited both ER and oxidative stress markers. These findings indicated that binge EtOH intake after chronic consumption induces massive ER stress-related oxidative stress followed by liver injury, and inhibition of ER stress by chemical chaperone is a potential preventive therapy for alcoholic liver injury especially in obese subjects.

## SYMPOSIUM

### Genetics of alcohol dependence

*GELERNTER Joel (Yale Univ School of Medicine)*

- The Genetics of Antisocial Personality Disorder in the Context of Alcohol Dependence  
*MCQUILLIN Andrew (University College London)*
- Polygenic contributions to alcohol use and alcohol use disorders across population-based and clinically ascertained samples  
*JOHNSON Emma (Washington University School of Medicine, St. Louis)*
- Genetics of alcohol dependence in a family sample  
*EDEMBERG Howard (Indiana University School of Medicine)*
- Alcohol GWAS results in different populations – AA, EA, Asian – and attendant implications  
*GELERNTER Joel (Yale University School of Medicine and VA CT Healthcare Center)*

# The Genetics of Antisocial Personality Disorder in the Context of Alcohol Dependence

MCQUILLIN Andrew, University College London

Antisocial personality disorder (ASPD) is characterised by impulsive, irresponsible and criminal behaviour. These personality traits begin in childhood or early adolescence and continue into adulthood. The prevalence of ASPD in the general population is 2-3%, with estimates of 3% in men and 1% in women. The rates are higher in certain populations with ASPD rates of 47% amongst male prisoners. ASPD is highly comorbid with substance use disorders (SUD) and studies have reported that 80–85% of individuals with ASPD also meet criteria for a substance use disorder. Alcohol use disorder in particular is highly comorbid with ASPD and in one study 71% of ASPD patients abused alcohol.

We have conducted a GWAS of ASPD symptom scores in two alcohol dependence cohorts from the UK and the US (n=3,223). This analysis produced a genome wide significant finding for a SNP located close to the *SLCO3A1* gene on chromosome 15 ( $p=3.77 \times 10^{-08}$ ). *SLCO3A1* is a member of a family of organic anion transporter genes. Previous studies have reported association of this gene with AUD comorbid with bipolar disorder, with smoking behaviour and with inattentive symptoms in ADHD. Polygenic risk score analyses provided evidence for shared risk of ASPD in AUD subject with smoking behaviour, educational attainment and reproductive traits.

We report the first genome-wide significant finding for ASPD and evidence for shared genetics risk for ASPD in AUD across a range of behavioural traits.

# Polygenic contributions to alcohol use and alcohol use disorders across population-based and clinically ascertained samples

## Authors:

Emma Johnson<sup>1</sup>, Sandra Sanchez-Roige<sup>2</sup>, Arpana Agrawal<sup>1</sup>, Toni-Kim Clarke<sup>3</sup>, Alexis C. Edwards<sup>4</sup>, The Collaborative Study on the Genetics of Alcoholism, The Avon Longitudinal Study of Parents and Children

## Affiliations:

<sup>1</sup> Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, US

<sup>2</sup> Department of Psychiatry, University of California San Diego, La Jolla, CA, USA

<sup>3</sup> Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK

<sup>4</sup> Department of Psychiatry, Virginia Commonwealth University School of Medicine, Virginia Institute for Psychiatric and Behavioral Genetics, Richmond, VA, US

Recent studies suggest that alcohol consumption and alcohol use disorders (AUD) have distinct genetic architecture. We examined polygenic risk scores (PRS) from a genome-wide association study of the consumption and problem subscales of the Alcohol Use Disorders Identification Test (AUDIT-C and AUDIT-P) in four independent samples: an ascertained cohort, the Collaborative Study on the Genetics of Alcoholism (COGA); and three population-based cohorts, the Avon Longitudinal Study of Parents and Children (ALSPAC), Generation Scotland (GS), and a subset of the UK Biobank (UKB). Regression models examined the correlation between the AUDIT-C and AUDIT-P PRS and a variety of alcohol-related phenotypes. Cox proportional hazards models determined whether the PRS increased risk of onset of hazardous drinking and dependence. In COGA, the AUDIT-P PRS was strongly associated with alcohol dependence, symptom count, and maximum drinks, while AUDIT-C PRS was not an independent predictor of any phenotype. In ALSPAC, both PRS were strongly associated with alcohol dependence, whereas AUDIT-P PRS was a superior predictor of maximum drinks. In GS, AUDIT-C PRS was a better predictor of weekly alcohol use, whereas AUDIT-P PRS was strongly associated with CAGE scores. AUDIT-P PRS was also associated with ICD-based alcohol-related disorders in the UK Biobank sample. Lastly, AUDIT-P PRS was associated with increased risk of onset of alcohol dependence in COGA, whereas AUDIT-C PRS was associated with increased risk of onset of hazardous drinking in ALSPAC. Our findings demonstrate that AUDIT PRS could dissect genetic influences across alcohol use to misuse in both population-based and ascertained cohorts.

## Genetics of alcohol dependence in a family sample

Howard J. Edenberg

Indiana University School of Medicine (edenberg@iu.edu)

There is clear evidence that genetic variants affect the risk for alcohol dependence (AD), but few specific variants have been identified to date. The Collaborative Study on the Genetics of Alcoholism (COGA) conducted genome-wide association studies (GWAS) on AD, with secondary analyses looking at DSM-IV criteria endorsed. The primary analyses were among European Americans (EA), followed by trans-ancestral meta-analysis with an African American (AA) sample.

In the GWAS of the EA sample, the functional SNP in *ADH1B*, rs1229984, was genome wide significant (GWS) for DSM-IV criterion count and 2 of the 7 criteria. Trans-ancestral analysis strengthened the signal for criterion count ( $p = 2.6e-13$ ) and 2 criteria, and elevated the signal for DSM-IV AD to GWS. Other GWA findings from the trans-ancestral GWAS were rs61826952 on chromosome 1 for AD, and rs7595960 on chromosome 2 for 'time spent drinking'. Adding in data from several independent GWAS supported most of these findings. A polygenic risk score (PRS) derived from the EA discovery GWAS significantly predicted a small amount of variance in other EA datasets (SAGE-EA, OZALC-EA).

A promising endophenotype related to the risk for alcoholism is the sensitivity to alcohol (SRE). Individuals who need to consume more alcohol to feel its effects are at higher risk for heavy drinking and problems. GWAS and meta-analysis of SRE showed some novel associations on chromosomes 6, 11, and 13. A PRS derived from the EA SRE significantly predicted alcohol dependence and criterion count in the independent SAGE-EA subset, and one from the AA SRE did in the SAGE-AA subset.

Further progress will require substantially larger samples, including non-European populations, and will benefit from detailed characterization of AD and its symptomology.

## **Alcohol GWAS results in different populations – AA, EA, Asian – and attendant implications**

Joel Gelernter (a), Hang Zhou (a), Daniel Levey(a), Henry Kranzler(b), Murray Stein(c)

(a) Yale Univ. School of Medicine and VA CT Healthcare Center, West Haven, USA

(b) Univ. Pennsylvania School of Medicine, Philadelphia, USA

(c) University of Californian San Diego School of Medicine, San Diego, USA

Alcohol-related phenotypes – alcohol use disorder, quantity-frequency measures such as AUDIT-C, and maximum habitual alcohol use – are moderately heritable; as is the case for other complex traits, the specific risk variants and overall genetic architecture differ somewhat between populations. In some cases, as for the trait of “maximum habitual alcohol use,” the interpopulation differences are very useful in a practical sense for fine mapping of risk variants, because of differences in linkage disequilibrium between populations. At least one high odds ratio protective variant is almost entirely specific to certain Asian populations (*ALDH2* rs671). Notwithstanding these differences and the general recognition of the importance of studying major world populations, the available characterized samples for European-ancestry subjects are much larger than those for African- and Asian-ancestry. The US Million Veteran Program (MVP) presently includes samples of European-ancestry subjects in the hundreds of thousands, and African-ancestry, in the tens of thousands. The largest reported studies of Asian ancestry barely reach the thousands. This presentation will review available results in these three populations; emphasize knowledge that has been gained only through the study of multiple populations; and prospects for the future.

## SYMPOSIUM

Epigenetic effects of alcohol exposure in brain and in blood: an implication of methylation biomarker for alcohol use disorder

*XU Ke (Yale School of Medicine)*

- Fetal alcohol exposures promote the development of aggressive tumors in the endocrine glands

*SARKAR Dipak (Endocrinology Program and Department of Animal Sciences, Rutgers University, USA)*

- GENOME-WIDE DNA METHYLATION IN PFC OF AUD SUBJECTS: INSIGHTS ON THE EPIGENETIC REGULATION OF THE GLUCOCORTICOID RECEPTOR

*GATTA Eleonora (SCHOOL OF PUBLIC HEALTH / PSYCHIATRIC INSTITUTE (SPHPI), UNIVERSITY OF CHICAGO)*

- DNA-methylation abundantly associates with fetal alcohol spectrum disorder and its sub-phenotypes

*KRZYZEWSKA I.M. (Amsterdam University Medical Centers)*

- A Rapid Methylation Sensitive Digital PCR Test That Can Sensitive and Specifically Assess Heavy Alcohol Consumption and Monitor Alcohol Treatment using DNA from Blood or Saliva

*PHILIPERT Robert (University of Iowa, Iowa City)*

## **Fetal alcohol exposures promote the development of aggressive tumors in the endocrine glands**

Dipak K Sarkar, PhD, D.Phil

Endocrinology Program and Department of Animal Sciences, Rutgers University, New Brunswick, NJ 08901, USA

There have been several studies demonstrating that alcohol abuse promotes development of aggressive tumors in breast, prostate, pancreas, and colon tissues in human patients. Whether fetal alcohol exposures promote development of aggressive tumors in the offspring during adult period are not well studied. Using rat animal model of fetal alcohol exposure, we studied the susceptibility of the growth of aggressive tumors in the mammary, prostate and the pituitary glands during the adult period. Pregnant laboratory rats were fed between gestational days 7 and 21 with a liquid diet containing alcohol, pair-fed with isocaloric liquid diet, or fed *ad libitum* with rat chow. Between 50 to 90 days of age, fetal alcohol-exposed and control rats were given a dose of N-Nitroso-N-methylurea (NMU) to induce mammary cancer growth in female offspring, NMU and testosterone to induce prostate tumor in male offspring, or ovariectomized and implanted with an estrogen capsule to induce pituitary tumors in female offspring. Mammary glands, prostate glands or pituitary tissues were processed for determination of biochemical changes and histopathologies for tumor characterization. In the case of mammary tumor development, overall tumor multiplicity was greater in the offspring from the alcohol-fed group compared to the control groups, indicating a decrease in tumor latency. Alcohol-exposed animals developed more malignant tumors and more estrogen receptor- $\alpha$ -negative tumors relative to the control groups. In the case of prostate tumorigenesis, prenatal alcohol-exposed rats showed histological evidence for high-grade prostatic intraepithelial neoplasia (PIN) primarily in the ventral prostate, whereas control animals showed only low-grade PIN. Prenatally ethanol-exposed rats treated with carcinogen and testosterone also showed increased number of proliferative cells and androgen receptor with concomitant decreased levels of tumor suppressor proteins in the ventral prostate. Our results also show that pituitaries of fetal alcohol-exposed rats upon estrogen challenge developed prolactin-secreting tumors (prolactinomas) that were hemorrhagic and often penetrated into the surrounding tissue. Pituitary tumors of fetal alcohol-exposed rats produced higher levels of hemorrhage-associated genes and proteins and multipotency genes and proteins. Cells of pituitary tumor of fetal alcohol exposed rat grew into tumor spheres in ultra-low attachment plate, expressed multipotency genes, formed an increased number of colonies, showed enhanced cell migration, and induced solid tumors following inoculation in immunodeficient mice. These data suggest that fetal alcohol exposure programs some of the endocrine tissue to develop aggressive tumors. Although the exact mechanism for the tumor promotion effect of fetal alcohol is not clearly established, but our preliminary studies suggest the possibility that fetal alcohol programs some of these endocrine cells acquire stemness that enhances neoplastic properties for developing aggressive tumors.

## **GENOME-WIDE DNA METHYLATION IN PFC OF AUD SUBJECTS: INSIGHTS ON THE EPIGENETIC REGULATION OF THE GLUCOCORTICOID RECEPTOR**

Eleonora Gatta, Dennis R. Grayson, James Auta, Vikram Saudagar, Erbo Dong, Ying Chen, Harish R. Krishnan, Jenny Drnevich, Subhash C. Pandey, Alessandro Guidotti

SCHOOL OF PUBLIC HEALTH / PSYCHIATRIC INSTITUTE (SPHPI)  
UNIVERSITY OF CHICAGO  
1601 W. TAYLOR ST.  
SPHPI MC 912  
CHICAGO IL 60612

Individual vulnerability to develop psychiatric disorders depends on an intricate interplay between the genetic background and the environment. Environmental factors, including substance abuse and stress, cause long-lasting changes in the regulation of gene expression in the brain *via* epigenetic mechanisms, such as DNA methylation. Similar to stress, alcohol stimulates glucocorticoids release that bind to specific receptors, i.e., the glucocorticoid receptor (encoded by NR3C1). The human NR3C1 gene is comprised of nine untranslated alternative first exons (1A-J) and eight translated exons (2 to 9). Seven of the exons 1 variants are embedded within a CpG island known to be susceptible to epigenetic regulation *via* DNA methylation. These epigenetic changes have been associated with psychopathological conditions in adulthood. However, little is known on the role of DNA methylation mechanisms in the expression of stress-responsive genes in the brain of alcohol use disorders (AUD) subjects.

Using a genome-wide DNA methylation approach (Infinium<sup>®</sup> MethylationEPICBeadChip, Illumina) followed by the identification of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) by specific immunoassay, we identified a differential pattern of DNA methylation in AUD. Post-mortem brain samples were obtained from 25 controls and 25 AUD subjects from the New South Wales Tissue Resource Centre (University of Sydney, Australia). Bioinformatic analyses of differentially methylated genes highlight biological processes containing genes related to stress adaptation, including NR3C1. We validated some of these data and observed that chronic alcohol drinking results in a significant increased methylation of the NR3C1 exon variant 1<sub>H</sub>, with a particular increase in the levels of 5hmC over 5mC. These changes in DNA methylation were associated with reduced NR3C1 mRNA and protein expression in PFC as well as other cortico-limbic regions of AUD subjects when compared to controls. Furthermore, we show that the expression of several stress-responsive genes (e.g., CRF, POMC, FKBP5) is altered in the PFC and hippocampus of AUD subjects. These data suggest that alcohol-dependent aberrant DNA methylation of NR3C1 and consequent changes in other stress-related genes might be fundamental in the pathophysiology of AUD and lay the groundwork for treatments targeting the altered epigenetic regulation of NR3C1 in AUD (supported by the P50AA022538 NIAAA-NIH grant to SCP and AG).

## **DNA-methylation abundantly associates with fetal alcohol spectrum disorder and its sub-phenotypes**

I.M.Krzyzewska<sup>2</sup>, J.M.Cobben<sup>1</sup>, A.Venema<sup>2</sup>, A.N.Mul<sup>2</sup>, A.Polstra<sup>2</sup>, A.V.Postma<sup>2,3</sup>, R.Smigiel<sup>4</sup>, K. Pesz<sup>5</sup>, J.Niklinski<sup>6</sup>, M.A.Chomczyk<sup>6</sup>, P.Henneman<sup>2</sup> & M.M.A.M.Mannens<sup>2</sup>

1 Department of Pediatrics, Amsterdam University Medical Centers, Location AMC, Emma Children's Hospital, Amsterdam, The Netherlands

2 Department of Clinical Genetics, Genome Diagnostics Laboratory, Amsterdam University Medical Centers, Location AMC, Amsterdam, The Netherlands

3 Department of Anatomy, Embryology & Physiology, Amsterdam University Medical Centers, Location AMC, Amsterdam, The Netherlands

4 Department of Pediatrics & Rare Disorders, Medical University of Wroclaw, Poland

5 Department of Genetics, Medical University of Wroclaw, Poland

6 Department of Molecular Biology, Medical University of Bialystok, Poland

### **Abstract**

**Aim:** Fetal Alcohol Spectrum Disorder (FASD) involves prenatal growth delay, impaired facial and central nervous system development and causes severe clinical, social-economic burdens. Here we aim to detect DNA-methylation aberrations associated with FASD and potential FASD diagnostic and prognostic biomarkers.

**Patients & methods:** FASD diagnosis was established according to golden-standard protocols in a discovery and independent replication cohort. Genome-wide differential methylation association and replication analyses were performed.

**Results:** We identified several loci that were robustly associated with FASD or one of its sub-phenotypes. Our findings were evaluated using previously reported genome-wide surveys.

**Conclusions:** We have detected robust FASD associated DMPs and DMRs for FASD in general and for FASD sub-phenotypes, i.e. on growth delay, impaired facial, and CNS development.

## **A Rapid Methylation Sensitive Digital PCR Test That Can Sensitively and Specifically Assess Heavy Alcohol Consumption and Monitor Alcohol Treatment using DNA from Blood or Saliva**

Robert Philibert MD PhD, Meeshanthini Dogan PhD, Jeffrey Long PhD, James Mills MS,

University of Iowa, Iowa City, IA 52242, Behavioral Diagnostics, Coralville, IA 52241.

**Background:** Alcoholism is the third largest preventable cause of morbidity and mortality. Uniquely, in the absence of acute intoxication, there is no readily employable test for assessing unhealthy levels of alcohol consumption. In 2014, we published the first genome wide study of heavy alcohol consumption (HAC). In this presentation, we expand on those earlier findings using data and biomaterials (both saliva and whole blood DNA) from 143 participants with current HAC and 200 abstinent controls.

**Results:** Using DNA from whole blood, we show that a set of four methylation sensitive digital PCR assays have a Receiver Operating Characteristic (ROC) area under the curve (AUC) of 0.96 for detecting those with HAC using DNA from whole blood with similar findings being obtained. After a mean of 21 days of inpatient enforced abstinence, methylation status at one of these markers, cg04987734, began to revert to baseline values. Re-examination of methylation data from our 2014 study with respect to this locus demonstrated a similarly significant reversion pattern at cg04987734 in association with treatment enforced abstinence. When the saliva DNA is used in place of whole blood, similar findings with respect to AUC and methylation reversion are observed.

**Conclusions:** We conclude that clinically implementable dPCR tools using DNA from blood or saliva can sensitively detect the presence of HAC and monitor alcohol treatment results. These digital PCR tools will be useful to clinicians and researchers in monitoring those enrolled in substance use disorder treatment, employee wellness programs and insurance underwriting.

## SYMPOSIUM

### Novel Alcohol-induced Epigenetic Signaling: Neuroimmune Genes and miRNAs

*CREWS Fulton (University of North Carolina at Chapel Hill)*

- **Neuronal-Glial Signaling Through miRNA let7 and Toll-like Receptor 7 Induce Interferons and Negative Affect.**  
*COLEMAN Leon (UNC-School of Medicine, Chapel Hill, NC USA)*
- **Neuroimmune and Epigenetic Mechanisms Regulate Adolescent Binge Ethanol-induced Loss of Basal Forebrain Cholinergic Neurons and Hippocampal Neurogenesis**  
*VETRENO Ryan (Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA)*
- **Glial- to- Neuron Transfer of Inflammatory Proteins and miRNAs via Exosomes: A New Mechanism Underlying Ethanol-induced Neuroinflammation**  
*GUERRI Consuelo (Prince Felipe Research Center, Valencia, 46012, Spain)*
- **Role of microRNA 137 Mediated Changes in Histone Methylation in Adolescent Alcohol-induced Anxiety and Alcohol Drinking Behaviors in Adulthood**  
*PANDEY Subhash (University of Illinois at Chicago, Chicago and Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois)*

## Neuronal-Glial Signaling Through miRNA let7 and Toll-like Receptor 7 Induce Interferons and Negative Affect

**L.G. Coleman**, J. Zou, L. Qin, S.S. Moy, and F.T. Crews. *Dept. Pharmacology, Bowles Center for Alcohol Studies, UNC-School of Medicine, Chapel Hill, NC USA.*

Neuroimmune activation is a prominent feature of alcoholic neuropathology. This includes activation of neuroimmune Toll-like Receptors (TLRs) with release of their endogenous agonists. Neuronal-glia interactions likely mediate neuroimmune responses to alcohol, however specific intercellular signaling mechanisms associated with alcoholic behavioral pathology need to be identified. We found a novel glial to neuronal signaling pathway, involving TLR7 and its endogenous agonist, miRNA let-7, which contributes to neuronal cell death, interferon (IFN) induction, and negative affect. Ethanol causes the secretion of let-7 in media microvesicles from microglia and astrocytes, which can activate TLR7 in neurons. In postmortem human alcoholic cortex, TLR7 and its downstream signaling components IRF7 and IFNs were induced. In mice, chronic binge ethanol induced let-7, TLR7, and pIRF7, and sensitized to immune responses and degeneration due to the TLR7 agonist Imiquimod (IMQ). In primary ex-vivo slice culture, ethanol caused secretion of miRNA let-7 in media microvesicles, and inhibition of TLR7 with siRNA or a novel small molecule antagonist prevented ethanol-induced cell death. The TLR7 antagonist also blocked neurodegeneration to chronic binge ethanol in mice. Using immortalized neuronal (SH-SY5Y), microglial (BV2), and astrocyte (U373MG) cell lines, and conditioned media transfers, we found ethanol induced glial secretion of miRNA let-7 and IFN induction in neurons. Microglial depletion *ex-vivo* blunted ethanol or IMQ-TLR7 induction of TNF $\alpha$  and IL-1 $\beta$ , but not IFNs suggesting neuronal or astrocytic responses. In a 5-week binge model, ethanol caused persistent negative affect (anxiety-like behavior, and conditioned fear memory), with IFN $\beta$  expression correlating with persistent conditioned fear memory.

## **Neuroimmune and Epigenetic Mechanisms Regulate Adolescent Binge Ethanol-induced Loss of Basal Forebrain Cholinergic Neurons and Hippocampal Neurogenesis**

Ryan Vetreno, PhD

Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA

Adolescence is a critical period of neurotransmitter system refinement and neuroplasticity that coincides with development of adult cognition. Binge drinking and alcohol abuse are common during adolescence causing lasting pathology. We find adolescent intermittent ethanol (AIE), which models human binge drinking, persistently decreases basal forebrain cholinergic neuron (BFCN) populations and hippocampal neurogenesis. Reciprocal connections between the basal forebrain and hippocampus are critical for maintenance of BFCNs and neurogenesis. We linked proinflammatory signaling to loss of BFCNs and neurogenesis as treatment with lipopolysaccharide mimics and anti-inflammatory treatments prevent AIE pathology. An imbalance between neuroimmune/neurotrophic signaling might contribute to persistent AIE-induced loss of BFCNs and neurogenesis through epigenetic mechanisms. In the basal forebrain, AIE increases histone 3 lysine 9 dimethylation (H3K9me2) occupancy at Chat and Trka gene promoters, which is associated with silencing of gene transcription. Exercise and acetylcholinesterase inhibitor (AChEI) treatment post-AIE reversed the loss of BFCNs, and restored the neuroimmune/hippocampal NGF balance and increased histone methylation. These data suggest AIE-induced loss of BFCNs might involve silencing of cholinergic phenotype and not cell death. Similarly, we find AIE increases H3K9me2 and reduces H3K9 acetylation in the adult hippocampus, the latter associated with diminished BDNF expression. Treatment with an AChEI and the histone deacetylase inhibitor TSA post-AIE reversed the AIE loss of hippocampal neurogenesis, and restored the neuroimmune/BDNF neurotrophic balance and epigenetic modifications. Together, these data suggest that an imbalance of neuroimmune/neurotrophic signaling might contribute to persistent AIE-induced neuropathology through epigenetic mechanisms that can be reversed in adulthood.

## **Glia-to-neuron transfer of inflammatory proteins and miRNAs via extracellular vesicles: A new mechanism underlying ethanol-induced neuroinflammation.**

F. Ibáñez<sup>1</sup>, M. Pascual<sup>1,2</sup>, J. Ureña<sup>1</sup>, C. Guerri<sup>1</sup>.

<sup>1</sup> Cellular Pathology Department, Príncipe Felipe Research Center, Valencia (Spain).

<sup>2</sup> Physiology Department, University of Valencia, Valencia (Spain).

Extracellular vesicles (EVs) participate in intercellular signaling, and in the regulation and amplification of neuroinflammation. We have shown that ethanol activates glial cells through Toll-like receptor 4 (TLR4), triggering neuroinflammation. We evaluate if ethanol and the TLR4 response change the release and the inflammatory content of astrocytes-derived EVs, and whether these vesicles are internalized by neurons, spreading neuroinflammation. Cultures of cortical neurons and astrocytes were used. EVs were isolated from the extracellular medium of WT and TLR4-KO astrocytes, treated or untreated with ethanol (40 mM) for 24 h. The EVs content in inflammatory proteins, mRNA and miRNAs was analyzed by Western blot and RT-PCR in astrocyte-derived EVs and in neurons incubated or not with these EVs. A functional analysis of the miRNAs was also performed. We show that ethanol increases the number of secreted nanovesicles and alters their content by raising the levels of both inflammatory-related proteins (TLR4, p65, IL-1R, caspase-1, NLRP3) and miRNA (mir-146a, mir-182 and mir-200b) in the EVs from WT-astrocytes compared with those from the untreated WT cells. Ethanol did not change the number and the content of TLR4-KO astrocytes-EVs. We further showed that astrocytes' EVs were internalized by naïve neurons, changing their physiological functions and increasing inflammatory markers and miRNAs (e.g. mir-146a) levels, along with miRNAs-target genes (Traf6, Mapk14, Stat1 and Foxo3) in neurons treated with ethanol-treated WT astrocyte-derived EVs. These results suggest that astrocyte-derived EVs could act as cellular transmitters of inflammation signaling by spreading and amplifying the neuroinflammatory response induced by ethanol through TLR4 activation.

## **Role of microRNA 137 Mediated Changes in Histone Methylation in Adolescent Alcohol-induced Anxiety and Alcohol Drinking Behaviors in Adulthood**

**Subhash C. Pandey**, Evan J. Kyzar, J. Peyton Bohnsack, Huaibo Zhang  
Center for Alcohol Research in Epigenetics, Department of Psychiatry, University of Illinois at Chicago, Chicago and Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois, 60612 USA

Binge drinking during adolescence increases risk for psychiatric disorders later in life. Epigenetic mechanisms such as microRNAs (miRNAs) may contribute to this increased risk via molecular changes in the amygdala. Here, we investigated the role of miR-137 and its targeting of the epigenetic enzyme lysine-specific demethylase 1 (LSD1) in the adult amygdala after adolescent intermittent ethanol (AIE) exposure. Rats were exposed to 2g/kg ethanol (2 days on/off; AIE) or intermittent n-saline (AIS) during postnatal days (PND) 28-41 and allowed to grow to adulthood for analysis of behavior and biochemical measures. Some adult rats were cannulated in the central nucleus of amygdala (CeA) and infused with miR-137 antagomir with or without concurrent *Lsd1* siRNA infusion prior to analysis. AIE increases miR-137, decreases *Lsd1* expression, and decreases LSD1 occupancy at the brain-derived neurotrophic factor exon IV (*Bdnf IV*) promoter in adult amygdala. Infusion of miR-137 antagomir into the CeA rescues AIE-induced alcohol drinking and anxiety-like behaviors. miR-137 antagomir infusion in the CeA also normalizes the decreased *Lsd1* expression, decreased LSD1 occupancy, and decreased *Bdnf IV* expression due to increased H3K9me2 occupancy seen in the amygdala of AIE adult rats. Finally, co-infusion of *Lsd1* siRNA into CeA prevents the miR-137 antagomir-induced rescue of molecular changes and anxiety-like behaviors. These results suggest that increased miR-137 in the CeA play an important role in chromatin remodeling and adult psychopathology caused by adolescent alcohol exposure (Supported by the NIAAA-NIH U01AA-019971, U24AA-024605 & P50AA022538 grants and senior VA career scientist award to SCP and F30AA024948 to EJK).

## **SYMPOSIUM**

Alcohol-related cognitive impairment (ARCI) : Korsakoff, Alzheimer, and friends  
*VORSPAN Florence (APHP, Université Paris Diderot, INSERM UMRS 1144)*

- **Epidemiology** : Early-onset dementia is mostly related to alcohol use disorders  
*Michaël Schwarzingler, Translational Health Economics Network (THEN), Paris*
- **Clinical care** : diagnosis and prognosis of Wernicke-Korsakoff disease and severe Alcohol-Related Cognitive Impairment: Alcomemo cohort  
*Julien Azuar, APHP, Département de Psychiatrie et de Médecine Addictologique, Hôpital Fernand Widal, Paris*
- **Neuropsychological Impairments and brain alterations** in « uncomplicated » patients with Alcohol Use Disorder  
*Anne-Lise Pitel, Université de Caen-Normandie, CYCERON, INSERM UMRS 1077, Caen, France*
- **Biomarkers in ARCI** : what can we learn from the past 20 years of Alzheimer's disease research and care ?  
*Emmanuel Cognat, APHP, Centre de Neurologie Cognitive, , INSERM UMRS 1144, Paris*

## **Epidemiology : Early-onset dementia is mostly related to alcohol use disorders**

Dr Michaël Schwarzinger, Translational Health Economics Network (THEN), Paris

Abstract: We conducted a nationwide retrospective cohort of all adult ( $\geq 20$  years) patients admitted to hospital in metropolitan France between 2008 and 2013. Of all 81,958 cases of early-onset dementia ( $< 65$  years), 39,206 (47.8%) were related to alcohol use disorders. Early-onset dementia was more frequently recorded in men (51,339 [62.6%]) than women (30,619 [37.4%]), with stronger association with alcohol use disorders (29,944 [58.3%] vs. 9,262 [30.2%]). This nationwide study suggests that the burden of early-onset dementia could be substantially alleviated by reinforcing alcohol policies.

**Clinical care : diagnosis and prognosis of Wernicke-Korsakoff disease and severe Alcohol-Related Cognitive Impairment: Alcomemo cohort**

Dr Julien Azuar, APHP, Département de Psychiatrie et de Médecine Addictologique, Hôpital Fernand Widal, Paris

*Azuar Julien, Questel Frank, Clergue-Duval Virgile, Barré Thomas, Vorspan Florence*

Abstract : Cognitive disorders are common in patients with alcohol-related disorders (AUD). They must be screened and characterized in order to obtain appropriate care. An observational cohort of patients with AUD and severe cognitive impairment is being constructed from 2013 in our Addictology Department, with the help of a multicenter network called Resalcog. This network helps to keep a patient off alcohol for several months. We will describe the characteristics of this cohort of 124 patients, including clinical description of comorbidities and evolution, circuit care, anatomic and functional brain imaging (IRM, 18-FDG-PET), nutritional status, CSF biomarkers (tau, beta-amyloid peptides, neurogranin, neurofilament light chain). We will insist on the prevalence of multifactorial origin of cognitive disease in this population. For the patients with strictly alcohol-related cognitive disorder, we will identify items correlated with a better prognosis of this disease.

## **Neuropsychological Impairments and brain alterations in « uncomplicated » patients with Alcohol Use Disorder**

Dr Anne-Lise Pitel, Université de Caen-Normandie, CYCERON, INSERM UMRS 1077, Caen, France

Anne-Lise Pitel, Alice Lanièce, Nicolas Cabé, Laurent Coulbault, Géraldine Rauchs, Shailendra Segobin

Alcohol Use Disorder (AUD) is associated with altered brain structure and function well before the development of neurological complications. Two brain circuits are mainly affected in “uncomplicated” AUD patients: the frontocerebellar circuit involved in motor abilities as well as working memory and executive functions; and the Papez circuit implicated in episodic memory. However, the pathophysiology of the brain dysfunction observed in AUD remains unclear since the nature and extent of cerebral damage and cognitive deficits do not seem to be directly related to the duration or severity of the alcohol history. Other factors including associated malnutrition potentially resulting in altered thiamin metabolism or deficiency, liver disease, or sleep disturbances may favor the development or exacerbate alcohol-related neuropsychological deficits and brain abnormalities. The role played by these factors must be further investigated since they are potential therapeutic targets that could prevent or reduce brain dysfunction in AUD.

## **Biomarkers in ARCI : what can we learn from the past 20 years of Alzheimer's disease research and care ?**

Dr Emmanuel Cognat, APHP, Centre de Neurologie Cognitive, , INSERM UMRS 1144, Paris

Cognitive neurodegenerative disorders are slowly progressive brain diseases with overlapping clinical features and limited access to brain pathology in living patients. Thus, important efforts have been made during the past decades to develop diagnostic biomarkers that reflect pathological processes and prognostic markers that could predict the course of the disease. Research in this field has been most active in Alzheimer's disease, the most frequent cause of cognitive disturbance in older patients but recent advances have been made in the past few years in other neurocognitive disorders such as frontotemporal lobar degeneration.

Alcohol-related cognitive impairment (ARCI) is a complex condition with frequent multifactorial origin and difficulties to predict prognosis. Co-existing neurodegenerative pathology does not seem rare. Thus there is a crucial need for both diagnostic and prognostic biomarkers useable in patients with ARCI. Development of such biomarkers may take advantage of the lessons learnt from past and current research and use in clinical practice of biomarkers in AD and other neurodegenerative disorders.

## SYMPOSIUM

Alcohol use disorders in context of dual diagnosis: Did DSM make us lose the MATCH?

*BROUSSE Georges (CHU Clermont-Ferrand)*

- **What are the challenges of dual disorders in management of AUD in France?**  
*Pr Amine Benyamina (Department of psychiatry and addictology, Paul Brousse hospital, Villejuif, Paris Sud university, INSERM U 1178, Paris, France)*
- **Are personality disorders the key to dual pathologies in AUD?**  
*Pr Alain Dervaux (Service de Psychiatrie et Addictologie de liaison, CHU Sud, Université de Picardie Jules Verne, INSERM U1247, Amiens, France)*
- **What tools are available to screen psychiatric disorders in patients with AUD?**  
*Pr Georges Brousse (Université Clermont Auvergne EA7280, CHU Clermont-Ferrand, France)*
- **Dimensional perspectives for a pragmatic therapeutic approach**  
*Pr Maurice Dematteis (CHU Grenoble-Alpes, service de pharmaco-addictologie, 38043 Grenoble, France)*

## **Dimensional perspectives for a pragmatic therapeutic approach**

Maurice DEMATTEIS. University Hospital of Grenoble. France.

Dual disorders are common and polydrug use is the norm. In patients combining both, the clinical presentation results from a mixture that makes a categorical diagnosis as well as a specific and stepped care difficult to establish. However complex the clinical picture, its deconstruction by elementary functional dimensions and endophenotypes (e.g. impulsivity) allows for pragmatic, gradual and integrative holistic treatment suited to the patient's needs, resources and ecology. There are currently no validated strategies in the literature. According to our experience and the Research Domain Criteria approach, we propose a framework that provides functional understanding (drug's functions in the psychic economy) and treatment of addictions which are considered to be dysfunctional adaptive strategies. Motivation- and education-based treatment aims at restoring functional autonomy and quality of life in accordance with the patient's needs, and combines dimensional pharmaco-psychotherapy, including:

1. substitutive strategies of harm reduction: substitution of consumption modalities and/or substances and/or behaviours (e.g. how to cope differently, starting from strategies applied by the patients and reinforcing what intuitively better works for them);
2. an integrative psychotherapy based on psychosocial rehabilitation modalities and introduced gradually, to first address the most basic functions (life rhythms, negative emotions, etc) then the more and more complex issues (social processes, cognitions);
3. and a behavioral pharmacology according to medication's mechanism of action (neuroscience-based nomenclature) allowing for treatment of elementary dimensions and endophenotypes, complemented by specific treatments when categorical diagnosis is possible.

In our experience, such an integrated and integrative approach allows for efficient treatment of the most complex patients in an outpatient setting.

## **SYMPOSIUM**

### **Mechanisms underlying binge drinking and compulsive alcohol use**

*LESSCHER Heidi (Department of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University)*

- **BEHAVIOURAL TRAITS AND NEUROBIOLOGICAL MECHANISMS UNDERLYING LOSS OF CONTROL OVER ALCOHOL USE IN RATS**  
*LESSCHER Heidi (Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, The Netherlands)*
- **BRAIN-WIDE FUNCTIONAL ARCHITECTURE REMODELING BY ALCOHOL DEPENDENCE AND ABSTINENCE PROVIDES EVIDENCE FOR THE THREE-STAGE HYPOTHESIS**  
*GEORGE Olivier (Department of Psychiatry, University of California San Diego, La Jolla, California 92093)*
- **FACE AND PREDICTIVE VALIDITIES OF A NEW PRECLINICAL MODEL OF OPERANT BINGE DRINKING**  
*JEANBLANC Jerome (INSERM UMR1247, Research Group on Alcohol and pharmacodependences)*

## **BEHAVIOURAL TRAITS AND NEUROBIOLOGICAL MECHANISMS UNDERLYING LOSS OF CONTROL OVER ALCOHOL USE IN RATS**

Johanna A.S. Smeets<sup>1</sup>, A. Maryse Minnaard<sup>1</sup>, Geert M.J. Ramakers<sup>2</sup>, Roger A.H. Adan<sup>2</sup>, Louk J.M.J. Vanderschuren<sup>1</sup>, Heidi M.B. Lesscher<sup>1</sup>

*<sup>1</sup>Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*

*<sup>2</sup>Department of Translational Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands*

Alcohol use disorder (AUD) is characterized by loss of control over alcohol use, but only a minority of individuals who drink alcohol develop AUD. Importantly, the mechanisms underlying loss of control over alcohol use and an individual's risk to develop AUD remain incompletely understood. Age of onset of alcohol use and certain personality traits are thought to play an important role in the risk for AUD. Neurobiologically, exaggerated involvement of the dorsolateral striatum (DLS) has been proposed to contribute to habitual alcohol seeking and loss of control over alcohol use. We assessed the contribution of these factors to the propensity to lose control over alcohol seeking in rats. Our studies showed that conditioned suppression of alcohol seeking, as a measure for alcohol use in the face of adversity, was less pronounced in rats with adolescent-onset alcohol consumption, compared to adult-onset animals. Further, rats that show high levels of social play behaviour as juveniles consumed more alcohol but showed intact conditioned suppression of alcohol seeking, unlike rats that displayed low levels of social play, reflecting a lack of control over alcohol seeking. Furthermore, preliminary analysis of optically-induced excitatory neurotransmission in cortical-DLS projection neurons showed increased facilitation of paired-pulse responses in the DLS in rats with a high alcohol drinking phenotype. These data suggest a complex relationship between age of onset, social development and DLS plasticity the development of AUD-like behaviour in rats.

**Brain-wide functional architecture remodeling by alcohol dependence and abstinence provides evidence for the three-stage hypothesis.** Olivier George, Ph.D.<sup>1</sup>, Daniel J. Lurie,<sup>2</sup> Andres Collazo, Ph.D.,<sup>3</sup> Max Kreifeldt,<sup>1</sup> Harpreet Sidhu, Ph.D.,<sup>1</sup> Mark D'Esposito, M.D.,<sup>2</sup> Candice Contet, Ph.D.,<sup>1</sup> Adam Kimbrough, Ph.D.,<sup>1</sup>. Department of Psychiatry, University of California San Diego, La Jolla, California 92093. <sup>2</sup>Helen Wills Neuroscience Institute, University of California, Berkeley, California, 94720. <sup>3</sup>Beckman Institute, Cal-Tech, MC 139-74, Pasadena, California, 91125

The identification of the psychological constructs and neurobiological mechanisms underlying the transition to addiction remains one of the most critical steps to better understand and treat alcohol and drug addiction. Converging lines of evidence suggest that multiple neurobiological modules processing reward, incentive salience, habits, stress, pain, and executive function may explain the vulnerability to alcohol addiction, and three major theories - incentive salience, hedonic allostasis and habit - have been proposed to contribute to addiction. However, because of technical limitations, we have been unable to directly test these hypotheses and visualize changes throughout the whole brain at single-cell resolution in subjects that are dependent on alcohol to validate the existence of these modules. The present study used an unbiased single-cell whole-brain imaging approach to map neuronal activity in alcohol-dependent mice and found that alcohol abstinence resulted in whole-brain reorganization of functional architecture and a pronounced decrease in modularity not observed in moderate drinkers. Structuring of the alcohol abstinence network revealed that addiction is driven by three major brain modules, reminiscent of the three-stage theory of addiction. Many hub brain regions controlling this pathological network were identified, including several that have been typically overlooked in addiction research. Further, a handful of the hub regions identified were predictive of addiction-like behavior. These results provide a single-cell resolution map for addiction and demonstrate that alcohol dependence remodels brain-wide functional architecture to decrease modularity similarly to other brain disorders. Such neuroadaptations strongly reinforce the brain disease model and may explain why addiction is such a pervasive disease and why relapse is so common.

## **FACE AND PREDICTIVE VALIDITIES OF A NEW PRECLINICAL MODEL OF OPERANT BINGE DRINKING**

Jérôme Jeanblanc<sup>1</sup>, Pierre Sauton<sup>1</sup>, Maria del Carmen Gonzalez-Marin<sup>1</sup>, Alessia D'Ippolito<sup>1</sup>, Virginie Jeanblanc<sup>2</sup>, Mickaël Naassila<sup>1</sup>

<sup>1</sup> INSERM UMR1247, Research Group on Alcohol and pharmacodependences, <sup>2</sup> Animal Facility PlatAnN  
Université de Picardie Jules Verne, 80000 Amiens, France

Binge drinking has multiple definitions in Humans (OMS, NIAAA, binge score for research...) making it difficult to define it accurately in animal models. Here we developed an animal model in outbred rats (Long Evans males and females) based on an operant ethanol self-administration paradigm. Rats were trained to self-administer a 20% ethanol solution under a FR1 (fixed ratio 1) then FR3 schedule for 1 hour. Slowly, the duration of the session was reduced first to 30 minutes then to 15 minutes and the levels of consumption reached on average 1.2g/kg within 15 minutes sessions. We found that such high consumption is associated with a higher motivation for the drug and for highly concentrated alcohol solutions (30% vs. 10%). Moreover, we showed that the speed of consumption is the factor that can differentiate heavy drinkers compared to binge drinkers. We also found that prolonged binge drinking leads to a decrease in decision making in a gambling task and that poor decision making is associated to lower dopamine release in the nucleus accumbens. We then tested drugs used in the treatment of alcohol use disorders (Acamprosate, (R)-Baclofen, GHB, Nalmefene and Naltrexone) and we found that all of them reduced binge drinking and all of them but acamprosate decreased both the motivation to consume and the relapse after prolonged abstinence. We thus demonstrated the face and predictive validities of our model and further neurobiological and behavioral studies are in progress to better characterize this damaging behavior.

## **SYMPOSIUM**

Targeting GABAB receptors to treat AUD: recent advances in clinical and preclinical models  
*AUGIER Eric (Linköping University)*

- The use of baclofen for patients with alcohol use disorder: where do we stand?  
*LEGGIO Lorenzo (NIH/NIAAA)*
- Biobehavioral mechanisms underlying baclofen's effects on alcohol seeking and consumption: Lessons learned from a human laboratory study  
*FAROKHNIA Mehdi (NIH/NIAAA)*
- France and the recent approval for baclofen in AUD  
*ROLLAND Benjamin (Neuroscience Research Center (CRNL))*
- Effect of the novel GABAB PAM ADX74441 on preclinical models of AUD and pathological alcohol choice  
*AUGIER Eric (Linköping University, Institute for Clinical and Experimental Medicine)*

## **The use of baclofen for patients with alcohol use disorder: where do we stand?**

Lorenzo Leggio, Mehdi Farokhnia, Roberta Agabio

Baclofen, a selective gamma-aminobutyric acid-B (GABA-B) receptor agonist, has emerged as a promising drug for Alcohol use disorder (AUD). This talk will provide an overview of the clinical work done with this medication in patients with AUD. Baclofen may be particularly advantageous in those with liver disease, due to its limited hepatic metabolism and safe profile in this population. Baclofen is mostly used off-label in some European countries and Australia, and in particular, for patients who have not benefitted from the currently used and approved medications for AUD. In France, baclofen has been extensively studied and was recently approved at the dose of up to 80 mg per day, by the French authority that regulates drugs approval and marketing (see Dr. Benjamin Rolland's talk). However, the use of this drug remains controversial, in part due to uncertainty regarding dosing and efficacy, alongside concerns about safety. A recent Consensus Statement among 26 international experts in the field was developed and published (Agabio et al. *Lancet Psychiatry* 2018) where the current state-of-the-art was briefly summarized and the need for future research was emphasized. On the latter point, human laboratory studies may shed light on the biobehavioral and other mechanisms how baclofen may work in some individuals with AUD (see Dr. Mehdi Farokhnia's talk). Finally, beyond baclofen, positive allosteric modulation of the GABA-B receptor may represent a better pharmacological approach towards the development of novel treatments for patients with alcohol and substance use disorders (see Dr. Eric Augier's talk).

**Biobehavioral mechanisms underlying baclofen's effects on alcohol seeking and consumption:  
Lessons learned from a human laboratory study**

Mehdi Farokhnia, Sara Deschaine, Armin Sadighi, Melanie Schwandt, Lisa Farinelli, Mary Lee,  
Fatemeh Akhlaghi, Lorenzo Leggio

The GABA-B receptor agonist baclofen has been broadly studied and used as a pharmacotherapy for alcohol use disorder. The biobehavioral mechanisms underlying baclofen's effects are, however, not well understood. Human laboratory studies provide an informative platform to shed light on this domain. In the present randomized, double-blind, placebo-controlled study, thirty-four alcohol-dependent individuals received baclofen (30 mg/d) or placebo for a week, and then participated in a laboratory experiment consisting of three procedures: alcohol cue-reactivity, priming, and self-administration. Repeated blood samples were also collected for pharmacokinetic measurements. Group analyses showed that baclofen, compared to placebo, did not significantly attenuate cue-elicited craving or the amount of alcohol self-administration. However, baclofen disrupted the link between alcohol priming and self-administration, as indicated by significant interaction effects between drug condition (baclofen vs. placebo) and some of the priming variables (alcohol craving:  $F_{3,9} = 6.03$ ,  $p = 0.01$ ; alcohol sedation:  $F_{3,6} = 7.16$ ,  $p = 0.01$ ; breath alcohol concentration:  $F_{1,25} = 5.22$ ,  $p = 0.03$ ) on the total amount of alcohol self-administered. Considerable interindividual variability in baclofen pharmacokinetic parameters was observed. Maximum plasma concentrations of baclofen negatively correlated with cue-induced alcohol craving ( $r = -0.57$ ,  $p = 0.03$ ) and priming-induced ratings of 'like more' ( $r = -0.59$ ,  $p = 0.02$ ). These data suggest that baclofen may work by dissociating the link between an initial drink and subsequent alcohol consumption. Considerable pharmacokinetic variability is an important factor to take into account when employing baclofen as a treatment for alcohol use disorder.

## **France and the recent approval for baclofen in AUD**

Benjamin Rolland

In Oct 2018, the French Drug Agency granted an approval to the GABA-B receptor agonist baclofen for Alcohol Use Disorder (AUD). Baclofen is thus now labeled for “supporting drinking reduction in AUD”, up to the dose of 80 mg per day, and after failure of other drugs approved for AUD. This regulatory decision results from a long story of off-label use, sometimes at doses exceeding 300 mg per day. The French practice consists of using baclofen in patients who are still displaying heavy drinking. As baclofen is a sedative drug, interaction with alcohol can raise safety concerns. In 2019, many uncertainties remain with respect to the efficacy and tolerability features of baclofen in AUD.

Despite this, France is now the first country in which baclofen is officially labeled for AUD. The French drug agency explained that this decision was not based only on scientific considerations, but also on the pragmatic statement that more than 60,000 patients were still treated with baclofen for AUD in France. As such the country will thus constitute an interesting real-life laboratory regarding the public health impact of this medication in AUD patients.

## **Effect of the novel GABA<sub>B</sub> PAM ADX74441 on preclinical models of AUD and pathological alcohol choice.**

Eric Augier, Russell Dulman, Gaëlle Augier, Markus Heilig

Alcohol effects on gamma-aminobutyric acid (GABA) transmission are key for the development and maintenance of alcohol addiction. Previous research indicate that GABA<sub>B</sub> receptor agonists such as baclofen can affect addiction-related behaviors in preclinical models of alcoholism. More importantly, baclofen has also shown promise in clinical studies, in particular in severely alcohol-dependent patients. However, despite promising results in both clinical and preclinical models, baclofen itself has inherent limitations as a therapeutic for alcohol addiction, and failed to obtain an approval for this indication.

An attractive alternative approach to targeting the same mechanism is offered by positive allosteric modulators (PAM:s) of the GABA<sub>B</sub> receptor, which have the potential to achieve mechanistic and therapeutic effects similar to GABA<sub>B</sub> agonists, while avoiding tolerance and overdose toxicity. In this symposium, I will present recent data obtained with ADX71441, a novel GABA<sub>B</sub> PAM that has entered Phase 1 clinical testing, on several alcohol-related behaviors in rats that model important aspects of human alcoholism. In particular, ADX71441 dose-dependently decreased alcohol self-administration, with a higher efficacy in animals with a history of dependence. Furthermore, both cue- and stress-induced alcohol seeking were blocked by the GABA<sub>B</sub> receptor PAM. Importantly, these effects are observed in the absence of significant sedative side effects. Finally, I will show new data evaluating the potential of positive allosteric modulation of GABA<sub>B</sub> receptors to rescue pathological alcohol choice over high value alternative rewards.

## FREE ORAL COMMUNICATION 2

- **Effect of inflammatory pain on alcohol induced dopamine release in the NAc and alcohol relapse in rats**  
*ZORNOZA Teodoro (UNIVERSIDAD DE VALENCIA)*
- **Emotional memory in young binge drinkers**  
*CARBIA Carina (APC Microbiome Ireland, Biosciences Building, University College Cork, Cork, Ireland)*
- **Methylation profiles during acute alcohol withdrawal in a clinical sample**  
*SIRIGNANO Lea (Department of Genetic Epidemiology in Psychiatry, CIMH Mannheim/Heidelberg University, Mannheim, Germany)*
- **Alcohol consumption during pregnancy: preliminary data on the effects of environment enrichment on transcriptional regulation of relevant key genes in mothers and offspring**  
*BELLIA Fabio (Faculty of Bioscience, University of Teramo, Italy)*
- **Dual ligands for the treatment of alcohol use disorders: a preclinical approach** *ECHEVERRY-ALZATE Victor (Instituto IBIMA, Universidad de Málaga, School of Psychology, Complutense University of Madrid, Spain)*

## **Effect of inflammatory pain on alcohol induced dopamine release in the NAc and alcohol relapse in rats**

Yolanda Campos-Jurado, Jose Luís González-Romero, Jesús Lorente, Ana Polache, Luis Granero, Teodoro Zornoza, Lucía Hipólito

Epidemiologic data have shown a relationship between pain and addiction especially to opioids and alcohol. Indeed, a recent clinical study uncovered that the correct management of pain in patients with a previous history of alcohol use disorder decreases the risk of relapse in alcohol drinking, suggesting that in this prone population, pain may increase the vulnerability to relapse in alcohol consumption. Previous data in rats revealed that inflammatory pain desensitizes mu opioid receptors (MORs) in the ventral tegmental area (VTA) and increases intake of high doses of heroine. Due to the relevant role of MORs in alcohol effects, we hypothesize that this desensitization may also alter the pattern of activation of the mesocorticolimbic system exerted by alcohol and therefore have an effect on alcohol relapse. In our study, we evaluated the effect of inflammatory pain on accumbal increase of dopamine release elicited by 1.5 g/kg of ethanol (s.c.). This microdialysis study showed that the presence of inflammatory pain blunted the increase of extracellular dopamine levels in the Nucleus Accumbens induced by ethanol. Later on, we evaluated the effect of inflammatory pain on the alcohol deprivation effect (ADE) in long-term ethanol-experienced rats. After four cycles of free ethanol intake and abstinence periods, inflammatory pain did not affect to the magnitude of the ADE. These data further support the impact of pain on the neurochemical events on the dopaminergic mesocorticolimbic system following alcohol administration and also underscore the necessity of finding an appropriate paradigm to determine the behavioral consequences.

## Emotional memory in young binge drinkers

Carina Carbia <sup>a</sup>, Montserrat Corral <sup>b</sup>, Francisco Caamaño-Isorna<sup>c</sup>, Fernando Cadaveira<sup>b</sup>

<sup>a</sup> APC Microbiome Ireland, Biosciences Building, University College Cork, Cork, Ireland

<sup>b</sup> Department of Clinical Psychology and Psychobiology, Universidade de Santiago de Compostela, Santiago de Compostela, Galicia, Spain

<sup>c</sup> Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP). Department of Preventive Medicine, University of Santiago de Compostela, Galicia, Spain

**Background:** College binge drinking (BD) has been linked to persistent cognitive difficulties, especially in episodic memory. However, despite impairments in emotional functioning have been associated with the development of alcohol use disorders, the emotional sphere has been relatively unexplored in BDs. The purpose of this study is to examine the effects of BD in emotional episodic memory.

**Methods:** A cohort of 180 (96♀) healthy college students was followed during two years (18-20 years old) and their alcohol use was recorded. In the last assessment, participants completed an adaptation of the Emotional Verbal Learning Test (EVLTL). Generalized linear mixed models were applied. The models were adjusted by psychopathological symptoms (BSI-18). The neuropsychological analyses were carried out separately for males and females, in accordance with sex differences in the development of emotion circuitry in adolescents.

**Results:** In females, BD was associated with poor performance in the emotional memory task, in particular lower recall of neutral words and greater recall of negative versus neutral words. Whereas in males, no alcohol-related effects were found.

**Conclusions:** Females binge drinkers present difficulties in emotional episodic memory linked to the interference of negative content. This is in line both with sex-related differences in the recall of emotional memory and an alcohol-related aberrant processing for emotionally salient stimuli, which might result in greater vulnerability to affective disturbances among women. Further research is needed to understand the role of emotional functioning in the escalation of alcohol abuse, from a gender perspective.

## **Methylation profiles during acute alcohol withdrawal in a clinical sample**

Lea Sirignano<sup>1</sup>, Stephanie H Witt<sup>1</sup>, Josef Frank<sup>1</sup>, Jens Treutlein<sup>1</sup>, Fabian Streit<sup>1</sup>, Ulrich Frischknecht<sup>2</sup>, Jerome C Foo<sup>1</sup>, Franziska Degenhardt<sup>3,4</sup>, Gabi Koller<sup>5</sup>, Ulrich Preuss<sup>6</sup>, Peter Zill<sup>5</sup>, Kristina Adorjan<sup>5</sup>, Markus Nöthen<sup>3,4</sup>, Rainer Spanagel<sup>7</sup>, Falk Kiefer<sup>2</sup> & Marcella Rietschel<sup>1</sup>

<sup>1</sup>Department of Genetic Epidemiology in Psychiatry, CIMH Mannheim/Heidelberg University, Mannheim, Germany;

<sup>2</sup>Department of Addictive Behaviour and Addiction Medicine, CIMH Mannheim/Heidelberg University, Mannheim,

Germany; <sup>3</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany; <sup>4</sup>Department of Genomics, Life and

Brain Center, University of Bonn, Bonn, Germany; <sup>5</sup>Institute of Psychiatric Phenomics and Genomics, Ludwig-

Maximilians-University (LMU), Munich, Germany; <sup>6</sup> Department of Psychiatry and Psychotherapy, Vitos Hospital

Herborn, Herborn Department of Psychiatry, Psychotherapy and Psychosomatics, Martin-Luther University, Halle-

Wittenberg; <sup>7</sup>Institute of Psychopharmacology, CIMH Mannheim/Heidelberg University, Mannheim, Germany

Withdrawal is a serious and sometimes life threatening event in alcohol-dependent individuals. It has been suggested, that epigenetic processes may play a role in this context. Identification of genes involved in such processes may hint to relevant mechanisms underlying withdrawal.

In the present study we sought to longitudinally investigate epigenome-wide methylation patterns in 100 severely alcohol-dependent patients during alcohol withdrawal and after 2 weeks of recovery, and also in 100 matched controls. More than 850,000 methylation sites were assessed using Illumina EPIC bead chips. Reflecting the high quality of our methylation data, we found – consistent with earlier reports – that correlation of methylation age with biological age of assessed individuals was very high ( $r=0.9$ ).

We found pronounced genome-wide significant differences between patients in withdrawal and after 2 weeks, among them in genes which have been reported to play a role in withdrawal symptomatology in previous studies (*SLC29A1*, *FYN*).

As expected, methylation between patients and controls differed considerably, also in genes implicated in withdrawal (*FKBP5*, *BDNF*, *EFNA5*).

Search for differentially methylated regions and gene ontology based gene set analysis revealed involvement of apoptotic processes in acute withdrawal. This has been also shown in other assessments with alcoholic patients.

This epigenome-wide longitudinal methylation study conducted in the so far largest sample of severely alcohol-dependent individuals suffering from withdrawal symptoms replicates known and suggests novel genes, which may play a crucial role in alcohol withdrawal.

## **Alcohol consumption during pregnancy: preliminary data on the effects of environment enrichment on transcriptional regulation of relevant key genes in mothers and offspring**

Bellia F<sup>1</sup>, Wille-Bille A<sup>2</sup>, Pucci M<sup>1</sup>, Miranda-Morales RS<sup>2</sup>, Pautassi RM<sup>2,3</sup>, D'Addario C<sup>1,4</sup>

<sup>1</sup>*Faculty of Bioscience, University of Teramo, Italy;* <sup>2</sup>*Instituto de Investigación Médica M. y M. Ferreyra (INIMEC–CONICET-Universidad Nacional de Córdoba), Córdoba, C.P, Argentina;* <sup>3</sup>*Facultad de Psicología, Universidad Nacional de Córdoba, Córdoba, Argentina;* <sup>4</sup>*Karolinska Institutet, Department of Clinical Neuroscience, Center for Molecular Medicine, Stockholm, Sweden*

### Introduction:

The consumption of alcohol by mothers during pregnancy may lead to mental or physical issues for the newborn, as well as be potentially dangerous to themselves after delivery. possibly increasing the risk of alcohol use due to heightened stress. The different phenotypes occurring in both mothers and offspring might involve the epigenetic regulation of genes transcription. Using an animal model of prenatal ethanol exposure, we here studied in mothers postpartum and in their offspring the effects of brain transcriptional regulation of target genes and how environmental enrichment might modulate possible alterations.

### Methods:

The dams were given one daily intragastrically administration of 0.015 ml/g of a 16.8% v/v ethanol solution or a similar volume of vehicle (gestational days 17-20). After delivery litters were divided in two groups to distinguish infancy from adolescence. Starting from PD14 in both infants and adolescents were evaluated anxiety-like behavior and exploratory activity together with risk-taking behavior in the light-dark box and in the concentric square field test respectively. Mothers were sacrificed 21 days after delivery by decapitation, offspring were sacrificed one day after the behavioral tests (PD32), brain dissected and nucleic acids extracted for genes expression studies and genes promoter evaluation of DNA methylation/hydroxymethylation.

### Results and Discussion:

Our findings so far show selective altered expression for BDNF and prodynorhin genes in the VTA of adolescent rats prenatally exposed to alcohol and of dams exposed to alcohol during pregnancy, whereas environmental enrichment partially reverted these changes at least in adolescent offspring. Moreover, altered methylation at specific CpG sites at both gene promoters was observed consistently with the changes in genes transcription. These data, even if preliminary, might be promising in order to understand the protective role of environmental switch on the effects evoked by alcohol also suggesting molecular mechanisms accounting for it.

## INVITED TALK

- The role of cytochrome P4502E1 in alcoholic liver disease and alcohol mediated cancer  
*SEITZ Helmut (Center of Alcohol Research, University of Heidelberg, Germany)*

## **The role of CYP2E1 in alcoholic liver disease and alcohol mediated carcinogenesis**

Helmut K. Seitz und Sebastian Mueller

Alkoholforschungszentrum, Universität Heidelberg und Medizinische Klinik, Krankenhaus  
Salem, Heidelberg

Various factors are involved in the pathogenesis of alcoholic liver disease (ALD) and ethanol mediated carcinogenesis. In addition to genetic, epigenetic and immunologic mechanisms, acetaldehyde associated toxicity, oxidative stress as well as cytokine mediated inflammation are of major importance. Oxidative stress with the generation of reactive oxygen species (ROS) develops either in inflammation (alcoholic hepatitis) or during oxidation of ethanol via cytochrome P4502E1 (CYP2E1). CYP2E1 is induced by ethanol, oxidizes ethanol to acetaldehyde and generates ROS during this process. ROS results in protein damage, enhanced fibrogenesis and DNA lesions. Furthermore, CYP2E1 induction results in an enhanced activation of various procarcinogens and an increased degradation of retinol and retinoic acid (RA), a compound responsible for cell differentiation and proliferation. An inhibition of CYP2E1 results in an improvement of ALD and chemically induced carcinogenesis in animal experiments. In man, CYP2E1 is induced following the consumption of 40 grams of ethanol per day already after one week. However, the induction varies interindividually. The mechanism for this is still unclear. Patients with ALD show a significant correlation between CYP2E1, the occurrence of highly carcinogenic etheno DNA-adducts and the severity of fibrosis. First results of the effect of CYP2E1 inhibition by chlormethiazole, a specific CYP2E1 inhibitor on ALD can be expected soon.

## **SYMPOSIUM**

### Neuroimaging in Addiction: Recent Advances in the Monitoring and Prediction of Pharmacological Effects

*BACH Patrick (Clinical Department of Addictive Behaviour and Addiction Medicine, Heidelberg University)*

- Effects of High-dose Baclofen on Neural and Behavioural Cue-Reactivity in Alcohol Dependence  
*Anne Beck – Charité, Berlin*
- The ICCAM platform: using fMRI to characterize brain responses in addiction and their pharmacological modulation  
*Anne Lingford-Hughes, Imperial College, London*
- Comparison of the effects of naltrexone on cue reactivity across different substance use disorders  
*Joar Guterstam, Karolinska Institute, Stockholm*
- Identifying neurobiological predictors for pharmacological treatment response in addiction: Results from the recent TRANSALC study  
*Patrick Bach, Central Institute of Mental Health, Mannheim*

## **Effects of High-dose Baclofen on Neural and Behavioural Cue-Reactivity in Alcohol Dependence**

Speaker: Dr. Anne Beck – Charité, Berlin, email: [anne.beck@charite.de](mailto:anne.beck@charite.de)

Increased functional brain response towards alcohol-associated stimuli (“cue reactivity”) is a neural hallmark of alcohol dependence and a promising target for pharmacotherapy. In this study, we assessed the effects of individually titrated high-dose baclofen on cue-induced brain activation in alcohol-dependent (AD) patients in a randomized controlled trial (RCT).

Patients receiving baclofen showed a significant stronger decrease in cue-elicited brain activation in left orbitofrontal cortex (OFC), bilateral amygdala and left VTA than patients receiving placebo and had significantly reduced relapse rates. Thus, our data suggest the modulatory capacity of high-dose baclofen on alcohol-associated cue reactivity on a neuronal level, thereby potentially contributing to the relapse preventive effects of this compound in alcohol dependence.

## **The ICCAM platform: using fMRI to characterize brain responses in addiction and their pharmacological modulation**

Speaker: Prof. Anne Lingford-Hughes, Imperial College, London, email: [anne.lingford-hughes@imperial.ac.uk](mailto:anne.lingford-hughes@imperial.ac.uk)

This talk will describe the ICCAM platform which uses 3 fMRI tasks to characterise brain responses in alcoholism and polydrug (alcohol, opiate, cocaine) addiction. The study explored how any dysregulation is modulated by a range of pharmacological probes, e.g. a DRD3 antagonist, a NK1 antagonist, opiate antagonists and if this is consistent with likely therapeutic benefit.

## **Comparison of the effects of naltrexone on cue reactivity across different substance use disorders**

Speaker: Dr. Joar Guterstam, Karolinska Institute, Stockholm, email: [joar.guterstam@ki.se](mailto:joar.guterstam@ki.se)

The opioid antagonist naltrexone is often used in the treatment of alcohol and opioid use disorders, and some clinical trials have also shown that it might reduce the risk of relapse in amphetamine dependence. In recent years, a number of fMRI studies have investigated the effects of naltrexone on drug cue reactivity in individuals with these different substance use disorders. Several studies have reported that naltrexone attenuates neural responses to alcohol cues in alcohol dependent patients, and there is also preliminary evidence of a similar effect in patients with opioid use disorder. Recent studies of amphetamine users have found that they often exhibit strong neural and behavioral cue reactivity, but these reactions do not seem to be significantly affected by naltrexone pre-treatment. These divergent findings might point to differences in the pathophysiology of craving in alcohol, opioid and stimulant use disorders.

## Identifying neurobiological predictors for pharmacological treatment response in addiction: Results from the recent TRANSALC study

Speaker: Patrick Bach, Central Institute of Mental Health, Mannheim, email: [patrick.bach@zi-mannheim.de](mailto:patrick.bach@zi-mannheim.de)

Despite the high prevalence of alcohol use disorder (AUD), only a few medications are approved for its treatment and meta-analyses point towards a modest overall effect size of available medications, such as Naltrexone. Understanding the neural and behavioral mechanisms underlying the highly variable treatment response to anti-relapse medications therefore seem to be a key factor for improving individual treatment success and enhancing impact on clinical practice based on the principles of precision medicine. We will present data of a recent longitudinal open-label trial, investigating whether Naltrexone (NTX) could block increases in alcohol craving and neural alcohol cue-reactivity (CR) in patients with alcohol use disorder, compared to standard treatment using longitudinal combined neuroimaging and psychometric assessments. At baseline (before treatment initiation), all participants underwent baseline psychometric testing and fMRI assessment of mesolimbic alcohol CR. Following this, patients participated in a standard treatment program with the option of adjuvant NTX. After 2 weeks of treatment, AUD patients underwent a second combined neuropsychological and fMRI assessment of alcohol craving and mesolimbic CR. Results show higher mesolimbic CR in AUD patients vs. healthy controls at baseline. Over the treatment episode of 2 weeks, mesolimbic CR significantly increased in the standard treatment group ( $n=13$ ), but not in the NTX group ( $n=22$ ,  $F_{(1,12)} = 23.526$ ,  $p = 0.001$ ). Only NTX treated patients showed significant attenuation of CR in the left putamen over time (interaction time x medication:  $F_{(1,33)} = 6.823$ ,  $p = 0.013$ ) that was associated with a reduced relapse risk to heavy-drinking within three months of treatment (interaction treatment x time: Hazard Ratio = 0.255, 95%CI = 0.084 – 0.775,  $p = 0.016$ ). Further, NTX treated patients compared to patients receiving standard treatment reported a significantly higher proportion of abstinent days during follow-up ( $t_{(33)} = 1.834$ ,  $p = 0.042$ ).

In conclusion, NTX blocked increased in mesolimbic CR that was observed in the standard treatment group. NTX was most effective in the patients with high baseline CR in the left putamen, reflecting in a number needed to treat of 1.8 [95%CI 1.3 – 6.2] to prevent one heavy relapse. While the results from our naturalistic study await further confirmation from prospective randomized trials, they support the role of neural CR as a biomarker in the development of precision medicine approaches with NTX.

## SYMPOSIUM

From Fetal alcohol syndrome to Korsakoff syndrome through binge drinking: a neuroscientist/neuropsychological perspective

*PITEL Anne-Lise (Inserm UMRS-U1077, Université de Caen-Normandie)*

- **COGNITIVE, EMOTIONAL AND INTERPERSONAL CORRELATES OF PRENATAL ALCOHOL EXPOSURE IN ADULTHOOD**

*D'HONDT Fabien (Univ. Lille, CNRS, UMR 9193 - SCALab - Sciences Cognitives et Sciences Affectives, Lille, France)*

- **EVALUATING AND TRAINING EXECUTIVE FUNCTIONS IN BINGE DRINKING: A COMBINED NEUROSCIENCE APPROACH**

*Pierre Maurage, University of Louvain la Neuve, Belgium*

- **WHY WE SHOULD ASK BINGERS IF THEY SMOKE CANNABIS?**

*BEAUNIEUX Hélène (Université de Caen Normandie, EA 7452, Caen, France)*

- **DECORTICATING THE PATHOPHYSIOLOGICAL MECHANISMS UNDERLYING ALCOHOL USE DISORDER WITH AND WITHOUT KORSAKOFF'S SYNDROME: A NEUROIMAGING REVIEW AND FUTURE DIRECTIONS**

*SEGOBIN Shailendra (Normandie Univ, UNICAEN, PSL Research University, EPHE, INSERM, U1077, CHU de Caen, Cyceron, Neuropsychologie et Imagerie de la Mémoire Humaine, France)*

## **EVALUATING AND TRAINING EXECUTIVE FUNCTIONS IN BINGE DRINKING: A COMBINED NEUROSCIENCE APPROACH.**

Pierre Maurage<sup>1</sup>, Valérie Dormal<sup>1</sup>, Séverine Lannoy<sup>1,2</sup>

1 UCLouvain, Laboratory for Experimental Psychopathology, Louvain-la-Neuve, Belgium

2 Stanford University, School of Medicine, Stanford, USA

Binge drinking, constituting the most frequent alcohol consumption pattern among adolescents and young adults, is characterized by a repeated alternation between intense consumption episodes and abstinence periods. The neurocognitive deficits related to this drinking pattern have been widely explored during the last decade. Executive functions deficits have been specifically identified as key factors in the emergence and maintenance of such habit. This talk will present new neuropsychological, electrophysiological and neuromodulation data allowing to better understand the specific executive impairments associated with binge drinking. We will centrally underline that binge drinkers (1) present a differential impairment across executive functions, with a preserved performance for shifting and updating abilities, but impaired inhibition processes; (2) show a dissociation, observed at the electrophysiological level, between impaired error-related processing and preserved feedback processing; (3) have a sufficiently preserved brain plasticity to benefit from neurostimulation-based rehabilitation of executive functions. The theoretical, experimental and clinical impact of these new insights will then be discussed, notably to underline the potential usefulness of joint neuropsychological/neuromodulation interventions among people presenting binge drinking habits.

## WHY WE SHOULD ASK BINGERS IF THEY SMOKE CANNABIS?

Hélène Beaunieux<sup>1,2</sup>, Virginie Bagneux<sup>1,2</sup>, Ludivine Ritz<sup>1,2</sup>, Ingrid Banovic<sup>3</sup>, Anaelle Bazire<sup>1,2</sup>, Nicolas Cabé<sup>4</sup>, Caroline Cheam-Bernière<sup>1,2</sup>, Laure Marine Houel<sup>4</sup>, Denis Jacquet<sup>1,2</sup>, Reynald Le Boisselier<sup>4</sup>, Pascale Leconte<sup>2,5</sup>, Jean-Baptiste Marchand<sup>1,2</sup>, Nicolas Margas<sup>2,6</sup>, Maxime Mauduy<sup>1,2</sup>, Fabrizio Scrima<sup>3</sup>, Cécile Sénémeaud<sup>1,2</sup>, Jessica Mange<sup>1,2</sup>.

1.Université de Caen Normandie, EA 7452, Caen, France

2.Université de Caen Normandie, USR 3486, CNRS-UNICAEN, Caen, France

3.Université de Rouen Normandie, EA 7475, Rouen, France

4.CHU Côte de Nacre, Caen, France

5.Université de Caen Normandie, UMR-S 1075 INSERM-UNICAEN, Caen, France

6.Université de Caen Normandie, EA 4260, Caen, France

Studies focusing on college students' consumptions of psychoactive substance and their consequences have mainly focused on alcohol use and more recently on binge drinking (BD). Distinct BD patterns associated with specific psychological profiles have been identified (Lannoy et al., 2017; Gierski et al., 2018). Based on its effects on various psychological parameters, cannabis use, which frequently co-occurs with BD, may modulate these differentiated psychological profiles. Further, in contrast to the wide evidence of neuropsychological deficits associated with the use of cannabis or alcohol separately, few studies have investigated the risk of alcohol use disorder and neuropsychological deficits related to the combined consumption of alcohol and cannabis. The aims of the present study were to examine the effect of cannabis on (1) the risk for alcohol use disorder and (2) the neuropsychological deficits observed in bingers. First, students of the University of Caen consuming alcohol and/or cannabis were screened through an internet survey-based study focusing on alcohol and cannabis experiences. Results showed that compared to bingers, students who both binged and smoked cannabis had an earlier onset of alcohol consumption, drank more and were more at risk of alcohol use disorder. Motivation and socio-normative parameters associated with alcohol consumption were significantly higher in this group than in binge drinkers who did not smoke cannabis. The neuropsychological evaluation revealed that compared to bingers, students who binged and smoked cannabis had more severe neuropsychological impairments, especially for episodic memory. These results suggest that cannabis consumption associated with BD is a risk factor for alcohol use disorder and episodic memory deficits. Those findings reinforce the idea that BD prevention programs may gain efficacy if considering its frequent combination with cannabis.

**Key-words:** cannabis, binge-drinking, alcohol, college students, psychological & neuropsychological assessment.

# **DECORTICATING THE PATHOPHYSIOLOGICAL MECHANISMS UNDERLYING ALCOHOL USE DISORDER WITH AND WITHOUT KORSAKOFF'S SYNDROME: A NEUROIMAGING REVIEW AND FUTURE DIRECTIONS**

Shailendra Segobin<sup>1</sup>, Alice Laniepce<sup>1</sup>, Nicolas Cabé<sup>1,2</sup>, François Vabret<sup>1,2</sup>, Francis Eustache<sup>1</sup>, Anne-Lise Pitel<sup>1</sup>.

1 Normandie Univ, UNICAEN, PSL Research University, EPHE, INSERM, U1077, CHU de Caen, Cyceron, Neuropsychologie et Imagerie de la Mémoire Humaine, 14000 Caen, France

2 Service d'Addictologie, Centre Hospitalier Universitaire de Caen, 14000 Caen, France.

Alcohol Use Disorder (AUD) exists in two main clinical forms. The first one, often referred to as “uncomplicated AUD”, is associated with mild-to-moderate deficits of episodic memory, executive functions, working memory and motor abilities. Some, but not all, uncomplicated AUD patients develop the second clinical form that is Korsakoff's syndrome, characterized by severe and irreversible amnesia, resulting from long-term excessive alcohol consumption and thiamine deficiency. Clinically, it is extremely relevant to identify uncomplicated AUD patients at risk of developing Korsakoff's syndrome. In this talk, the contribution of neuroimaging biomarkers towards understanding the pathophysiological mechanisms underlying these two clinical forms will be reviewed. More precisely, the brain circuits predominantly affected in both clinical forms, namely the frontocerebellar and Papez circuits will be discussed in terms of alterations to their macrostructural and microstructural integrity. The thalamus, a key region consisting of several nuclei is shared by these two brain circuits. Consolidating evidence showing that the loci and nature of structural alterations occurring within the thalamus potentially defines the specificity of Korsakoff's syndrome will be presented. To conclude this talk, current and prospective biomarkers in the field of cognition, neuroimaging and biology will be discussed based on how they bring incremental value towards the global understanding of the pathological mechanisms underlying Korsakoff's syndrome. Such a better understanding will ultimately enable clinicians to identify patients at risk for such a severe and debilitating neurological disease in order to provide appropriate prevention treatment.

## SYMPOSIUM

### Genetics/genomics in alcohol induced liver injury - what next?

*RAUSCH Vanessa (RPA Hospital and Centenary Institute, The University of Sydney)*

- **Genetics of hepatic steatosis and fibrosis**  
*Marcin Krawczyk, MD. Saarland University Hospital, Homburg, Germany*
- **An exploratory genome-wide analysis of patients with alcoholic hepatitis**  
*Suthat Liangpunsakul, MD. Indiana University, USA*
- **Contribution of common SNPs in explaining genetic variability in alcoholic cirrhosis**  
*Devanshi Seth, PhD. RPA Hospital and Centenary Institute, University of Sydney, Australia*
- **The role of PNPLA3 and MBOAT7 polymorphisms during alcohol detoxification–  
Identification of different mechanisms for fibrosis development**  
*Vanessa Rausch, PhD. University Hospital Heidelberg and Salem Medical Center, Heidelberg,  
Germany*
- **Alcoholic liver cirrhosis, beyond single variant analysis**  
*Tae-Hwi Linus Schwantes-An, PhD. Indiana University School of Medicine, Indiana, USA*

**Marcin Krawczyk, MD.** Saarland University Hospital, Homburg, Germany  
*Genetics of hepatic steatosis and fibrosis*

Fatty liver disease (FLD) belongs to the most frequent conditions in hepatology. Indeed, more than 20% of adult Europeans suffer from fatty liver and the incidence of this condition is predicted to increase even further. A subgroup of patients with FLD will also develop liver fibrosis, which is a common hallmark of chronic liver diseases. Both hepatic lipid accumulation as well as liver scarring have for long been expected to be modulated by the inherited predisposition. In the recent years genetic variants in several genes, for example PNPLA3, TM6SF2 and MBOAT7, have been linked to the progression of chronic liver diseases. Prosteatotic and/or profibrotic variants in these genes were first detected in large genome wide association studies (GWAS) and afterwards these associations were replicated in the following candidate gene analyses. In particular carriers of the PNPLA3 p.I148M variant have been proven to be at risk of severe liver steatosis, fibrosis, cirrhosis and hepatocellular carcinoma (HCC) rendering variant PNPLA3 a common genetic risk factor for progressive liver injury. Interestingly, the same variant also seems to modulate the response to the FLDh therapies. The most recently detected splice variant rs72613567 in hydroxysteroid 17 $\beta$  dehydrogenase 13 (HSD17B13) seems to, in turn, reduce the risk of FLD. Here we will summarize the current knowledge on the genetic background of hepatic steatosis and fibrosis, discuss the effects of the known variants on the disease progression as well address the potential use of genetic analyses in the clinical workh up of patients with FLD.

**Suthat Liangpunsakul, MD.** Indiana University, USA

*An exploratory genome-wide analysis of patients with alcoholic hepatitis*

An exploratory genome-wide association study (GWAS) was conducted comparing patients with alcoholic hepatitis (AH) and heavy drinking matched controls without liver disease in order to identify variants or genes associated with risk for AH. Individuals were genotyped using the multi-ethnic genotyping array, after which the data underwent conventional quality control. Using bioinformatics tools, pathways associated with AH were explored on the basis of individual variants, and based on genes with a higher 'burden' of functional variation.

Although no single variant reached genome-wide significance, an association signal was observed for PNPLA3 rs738409 ( $p = .01$ , OR 1.9, 95% CI 1.1–3.1), a common single nucleotide polymorphism that has been associated with a variety of liver-related pathologies including alcoholic cirrhosis. Using the improved gene set enrichment analysis for GWAS tool, it was shown that, based on the single variants' trait association  $p$  values, multiple pathways were associated with risk for AH with high confidence (false discovery rate [FDR] < 0.05), including several pathways involved in lymphocyte activation and chemokine signaling, which coincides with findings from other research groups. Several Tox Functions and Canonical Pathways were highlighted using Ingenuity Pathway Analysis, with an especially conspicuous role for pathways related to ethanol degradation, which is not surprising considering the phenotype of the genotyped individuals. This preliminary analysis suggests a role for PNPLA3 variation and several gene sets/pathways that may influence risk for AH among heavy drinkers.

**Devanshi Seth**, PhD. RPA Hospital and Centenary Institute, The University of Sydney, Australia *Contribution of common SNPs in explaining genetic variability in alcoholic cirrhosis*

Genetic pathways contributing to the pathophysiology of liver cirrhosis in drinkers are fundamentally important to understand this disease. There is limited comprehension of the genetic basis of variation in Alcoholic Liver Cirrhosis (ALC) susceptibility as only up to 20% of heavy chronic drinkers progress to cirrhosis. Cirrhosis is the major medical consequence and health problem of excessive alcohol abuse with high morbidity and mortality. Genetics of ALC is poorly understood despite several candidate gene studies and a single GWAS reporting a strong association with PNPLA3. Other reported SNPs, e.g. TM6SF2, MBOAT7 and HSD17B13, only show modest GWAS level association with ALC. Our multinational GenomALC Consortium also performed GWAS in the world's largest collection of drinkers in a case-control study design. Age, gender and ethnicity matched Cases (drinkers with cirrhosis) and Controls (drinkers with no liver disease) were subjected to GWAS (Infinium GSA Array). Our data showed increased risk of alcoholic cirrhosis in offspring of parents with alcohol problems who died of liver disease, underscoring the heritability of this disease. We confirmed PNPLA3 (rs739409) and HSD17B13 (rs4607179) that strongly associated with alcoholic cirrhosis. However, all these common variants identified from GWAS approach only account for about 20% of the overall genetic variance leaving much of the genetic contribution to ALC unexplained. Due to typical GWAS array design, which focuses on common variants, protein coding exonic and rare variants, as well as other non-genetic factors are yet to be for ALC.

**Vanessa Rausch**, PhD. University Hospital Heidelberg and Salem Medical Center, Heidelberg, Germany.  
*The role of PNPLA3 and MBOAT7 polymorphisms during alcohol detoxification – Identification of different mechanisms for fibrosis development*

The PNPLA3 rs738409 and MBOAT7 rs626283 polymorphisms are genetic risk factors for ALD progression; however, their molecular mechanisms are still poorly understood. We investigate the impact of these variants on important clinical parameters in response to alcohol withdrawal. Therefore, we prospectively enrolled 516 ALD patients for alcohol detoxification. Patients were genotyped and CAP, LS and ultrasound as well as laboratory markers were assessed before and after detoxification. In 105 patients, liver biopsy was also obtained and histologically analyzed.

Carriers of MBOAT7 CC and PNPLA3 GG showed a strong and combined effect on fibrosis development ( $P < 0.05$ ), however display striking differences with regard to inflammation, fibrosis and steatosis in response to alcohol withdrawal. Inflammation was not different and resolved equally in all MBOAT7 genotypes during detox (AST,  $P < 0.001$ ), whereas PNPLA3 GG carriers presented with significantly enhanced liver injury after detoxification and with delayed resolution of inflammation (M30 and AST,  $P < 0.001$ ). Finally, steatosis resolved equally in both polymorphisms and genotypes to the same extent and no differences prior and after detoxification have been observed. In the histology cohort, PNPLA3 GG was significantly associated with inflammation (steatohepatitis, ballooning and lobular inflammation) and steatosis and MBOAT7 CC with fibrosis. In summary, both variants are associated with fibrosis progression, but the response of steatosis and liver injury to alcohol withdrawal is remarkably different. While PNPLA3 is associated with liver injury and steatosis, MBOAT7 is not affecting liver injury. Interestingly, both variants did not alter the resolution of steatosis during alcohol withdrawal.

**Tae-Hwi Linus Schwantes-An**, PhD. Indiana University School of Medicine, Indiana, USA *Alcoholic liver cirrhosis, beyond single variant analysis*

In the posth GWAS era, much of the genetic underpinnings of complex diseases such as alcoholic liver cirrhosis (ALC) remain unexplained. Missing heritability, the large unexplained portion of genetic heritability after discoveries from GWAS studies, spurred adaptation of next gen sequencing to identify genetic variations that are not captured by GWAS arrays. In parallel, statistical genetic methods have evolved from simple single variant analysis (e.g. assessing effect of each genetic variant one at a time) to polygenic risk scores that include more than several tens of thousands of common variants to stratify risk for disease. In this talk, using examples from GenomAlc Consortium analyses, examples of recent statistical genetic methods will be highlighted.

## **SYMPOSIUM**

The role of Cytochrome P450 2E1 in alcoholic liver disease and cancer

*SEITZ Helmut K. (Center of Alcohol Research, University of Heidelberg)*

- **Ethanol metabolism in the liver: the role of CYP2E1**  
*ZAKHARI S. (SVP, Distilled Spirits Council, USA)*
- **Alcohol and drug interaction: the role of CYP 2E1**  
*TESCHKE Rolf (Academic Teaching Hospital of the Goethe University of Frankfurt)*
- **Role of CYP2E1 in Carcinogenesis**  
*MURRAY Gary (NIAAA, NIH)*
- **The role of CYP2E1 in alcoholic liver disease and alcohol-mediated carcinogenesis**  
*SEITZ Helmut Karl (Center of Alcohol Research, University of Heidelberg, Germany)*

## **Ethanol metabolism in the liver: the role of CYP2E1**

S. Zakhari, Ph.D., SVP, Distilled Spirits Council, USA

About 95-98% of ingested alcohol is metabolized in the liver in a two-stage, enzymatically-catalyzed oxidation process; the remainder is excreted in breath, urine and sweat. A small proportion of alcohol metabolism occurs via non-oxidative metabolic pathways resulting in the formation of fatty acid ethyl esters and phosphatidyl ethanol. A smaller portion undergoes conjugation with glucuronic acid or sulphate, and these conjugates are excreted in urine.

The major pathway of oxidative metabolism of ethanol in the liver involves cytosolic alcohol dehydrogenase (ADH) to produce acetaldehyde. The cytochrome P450 isozymes, including CYP2E1, 1A2 and 3A4, also contribute to ethanol oxidation to acetaldehyde in the liver, particularly at elevated alcohol concentrations. CYP2E1 is induced by chronic ethanol consumption. It also metabolizes numerous medications such as acetaminophen, and other xenobiotics. The catalase enzyme can also metabolize alcohol to acetaldehyde; however, this pathway appears to play a minor role in alcohol oxidation by the liver.

This presentation focuses only on CYP2E1 and will address its role in: health and disease; ethanol-mediated oxidative stress; drugs, xenobiotics and procarcinogens metabolism; fatty acid metabolism; and ethanol-induced hepatotoxicity and carcinogenesis. Discussion will also focus on hepatic CYP2E1 in pathological conditions such as obesity, diabetes and chemical inducers, as well as on drugs that inhibit ethanol-induced CYP2E1 and their role as protective agents against ethanol-mediated liver injury.

# Alcohol and drug interaction: the role of CYP 2E1

Rolf Teschke

Department of Internal Medicine II, Section of Gastroenterology and Hepatology, Klinikum Hanau, Academic Teaching Hospital of the Goethe University of Frankfurt/Main

Following the discovery of the hepatic microsomal ethanol-oxidizing system (MEOS) by Charles S. Lieber and Leonore M. DeCarli in 1968 and its subsequent purification and isolation from alcohol dehydrogenase and catalase through column chromatography in 1972, additional studies identified the microsomal cytochrome P450 (CYP) with its isoenzyme CYP 2E1 as its major constituent. CYP 2E1 metabolizes not only ethanol but also other short chain alcohols and various drugs and chemicals. Among these are paracetamol, halothane, and carbon tetrachloride. Consequently, the broad substrate specificity explains molecular interactions at the level of CYP 2E1. In particular, a few substrates such as disulfiram, diallylsulfide, and clomethiazole are known for their inhibitory effect on CYP 2E1, whereas the use of many other chemicals and drugs including acetone and isoniazid upregulate CYP 2E1 gene expression. Most importantly, prolonged ethanol consumption upregulates CYP 2E1 and thereby induces MEOS activity through a process involving reduced degradation of CYP 2E1 by inhibition of hepatic proteasome peptidase activities. This induction of MEOS activity explains the adaptive increase of alcohol metabolism in individuals with prolonged alcohol abuse. In addition, upregulation of CYP 2E1 and associated production of toxic intermediates is responsible for increased acute liver injury by paracetamol or carbon tetrachloride in patients with a past history of alcohol abuse. Ethanol-related upregulation of CYP 2E1 is also observed in the intestinal tract, modifying thereby the intestinal microbiome, considered as mechanistic contributor to alcoholic liver injury, and facilitating the activation of carcinogens and potential tumor development in the gastrointestinal tract of patients with an alcohol problem. In essence, microsomal CYP 2E1 plays an essential role in drug-alcohol interactions, increased risk of toxicity in the liver, and potential tumor development in the gastrointestinal tract. Current literature: Teschke, R. *Biomedicines* 2018; 6, 106; *Alcoholism, Clinical and Experimental Research* 2019; 43: 386-400.

# CYP2E1, Ethanol and Carcinogenesis

Gary J. Murray

*gary.murray@nih.gov*

Division of Metabolism and Health Effects,  
National Institute on Alcohol Abuse and Alcoholism  
National Institutes of Health

Although there is a strong association between chronic and excessive consumption of alcohol and increased risk of various cancers, the causative role and the specific mechanisms involved remain a subject of ongoing research. Cytochrome P4502E1 (CYP2E1) is induced in the liver after chronic alcohol consumption. As a consequence, in addition to alcohol, there is increased oxidation of many toxic and carcinogenic xenobiotics, including a variety of drugs, steroids, and other compounds. The oxidation and reduction reactions catalyzed by most P450 class enzymes are important mechanisms for detoxification of these xenobiotics but these same processes may lead to the creation of damaging products including the direct activation of carcinogens by oxidation of less toxic precursors. Another important pathway involves the generation of reactive oxygen species (ROS) including lipid peroxidation products and 4-hydroxynonenal that react with DNA resulting in the formation of exocyclic etheno DNA-adducts. These highly carcinogenic adducts contribute to the development of more serious pathology in liver and other tissues. There remains some controversy on the specific role of CYP2E1 in the production of ROS and of oxidative damage, and the significance of the observations that microsomes and purified P450s generate ROS, has been questioned. This is countered by observations in liver biopsies from patients with alcoholic liver disease (ALD) that the generation of these adducts were correlated with the induction of CYP2E1 in the liver after chronic alcohol consumption. Newer data has also emerged to implicate mitochondrial CYP2E1 in the production of ROS. The causative role of increased CYP2E1 activity in the development of alcohol-related cancers will be discussed.

# **The role of CYP2E1 in alcoholic liver disease and alcohol mediated carcinogenesis**

Helmut K. Seitz und Sebastian Mueller

Alkoholforschungszentrum, Universität Heidelberg und Medizinische  
Klinik, Krankenhaus Salem, Heidelberg

Various factors are involved in the pathogenesis of alcoholic liver disease (ALD) and ethanol mediated carcinogenesis. In addition to genetic, epigenetic and immunologic mechanisms, acetaldehyde associated toxicity, oxidative stress as well as cytokine mediated inflammation are of major importance. Oxidative stress with the generation of reactive oxygen species (ROS) develops either in inflammation (alcoholic hepatitis) or during oxidation of ethanol via cytochrome P4502E1 (CYP2E1). CYP2E1 is induced by ethanol, oxidizes ethanol to acetaldehyde and generates ROS during this process. ROS results in protein damage, enhanced fibrogenesis and DNA lesions. Furthermore, CYP2E1 induction results in an enhanced activation of various procarcinogens and an increased degradation of retinol and retinoic acid (RA), a compound responsible for cell differentiation and proliferation. An inhibition of CYP2E1 results in an improvement of ALD and chemically induced carcinogenesis in animal experiments. In man, CYP2E1 is induced following the consumption of 40 grams of ethanol per day already after one week. However, the induction varies interindividually. The mechanism for this is still unclear. Patients with ALD show a significant correlation between CYP2E1, the occurrence of highly carcinogenic etheno DNA-adducts and the severity of fibrosis. First results of the effect of CYP2E1 inhibition by chlormethiazole, a specific CYP2E1 inhibitor on ALD can be expected soon.

## SYMPOSIUM

### Drugs with Novel Mechanisms for the Treatment of Alcohol Dependence

*SWIFT Robert (Brown University Center for Alcohol and Addiction Studies)*

- Effect of the mGluR5 Modulator GET 73 on Alcohol Pharmacokinetics and Pharmacodynamics and Alcohol Craving and Consumption in a Human Laboratory Model  
*Robert Swift (Brown University Center for Alcohol and Addiction Studies, Providence RI USA)*
- Probenecid Reduces Alcohol Drinking in Rats: is Pannexin1 a Novel Therapeutic Target for Alcoholism?  
*Pietro Paolo Sanna (The Scripps Research Institute, La Jolla, CA, USA)*
- ANS-6637, a Selective Reversible Inhibitor of ALDH2, suppresses Alcohol Consumption and Cue-Induced Alcohol Self Administration in the Absence of Alcohol and Acetaldehyde  
*Ivan Diamond, MD, PhD (Amygdala Neurosciences)*
- An Evaluation of the Peroxisome Proliferator Receptor-Alpha (PPAR- $\alpha$ ) Agonist, Fenofibrate, in a Human Laboratory Model of Alcohol Use Disorder  
*Barbara J. Mason, Ph.D. (Scripps Research, La Jolla, CA)*

## **Effect of the mGluR5 Modulator GET 73 on Alcohol Pharmacokinetics and Pharmacodynamics and Alcohol Craving and Consumption in a Human Laboratory Model**

Robert Swift<sup>1</sup>, Carolina Haas-Koffler<sup>1,2</sup>, Lorenzo Leggio<sup>2</sup>, Roberto Cacciaglia<sup>3</sup>

<sup>1</sup>Brown University Center for Alcohol and Addiction Studies, Providence RI USA; <sup>2</sup>Laboratory of Neuropsychopharmacology and Neuroendocrinology, NIH, Bethesda, MD, USA; <sup>3</sup>Laboratorio Farmaceutico CT, San Remo, Italy

GET 73 is a novel, small molecule compound that reduces alcohol consumption and has anxiolytic and anti-stress activity in animals. GET 73 acts as a negative allosteric modulator (NAM) at the mGluR5 receptor and could reduce the high glutamatergic allostatic state associated with alcohol use disorders.

To establish safety/tolerability and to determine whether GET 73 reduces alcohol craving and alcohol drinking, we conducted a placebo-controlled, within-subjects crossover study with GET 73 in twenty non-treatment seeking alcohol dependent persons. After screening for medical and psychiatric suitability, eligible subjects were randomized to the 14-day inpatient study, receiving 3 days of treatment with GET 73 or placebo, followed by a 7-day outpatient washout, followed by 3 days of the alternate medication. Under each condition (GET 73 or placebo), on Day 2 and Day 12, participants received an oral dose of alcohol to bring BAC to 0.12 g/L. Alcohol and GET 73 pharmacokinetics and pharmacodynamics (intoxication, impairment, mood, etc) were monitored and compared between drug and placebo conditions. On Day 3 and Day 13, participants received an alcohol cue-reactivity (craving) session, followed by alcohol self-administration. The results showed that GET 73, was safe and did not affect alcohol pharmacokinetics. GET 73, compared to placebo, increased alcohol sedation on the BAES but did not affect performance. GET73 did not affect subjective craving or alcohol self-administration in the laboratory. However, GET73 appeared to reduce naturalistic alcohol consumption during the outpatient washout period.

## **Probenecid Reduces Alcohol Drinking in Rats: is Pannexin1 a Novel Therapeutic Target for Alcoholism?**

Pietro Paolo Sanna<sup>1</sup>, Brendan J. Tunstall<sup>2</sup>, Sam A. McConnell<sup>2</sup>, Katrina L. Gazo<sup>2</sup>, Lia J. Zallar<sup>2,3</sup>, Carolina Haass-Koffler<sup>4</sup>, Vez Repunte-Canonigo<sup>1</sup>, George F. Koob<sup>2</sup>, Leandro F. Vendruscolo<sup>2</sup>

<sup>1</sup>The Scripps Research Institute, La Jolla, CA, USA; <sup>2</sup>IRP, NIDA, Baltimore, MD; <sup>3</sup>IRP, NIAAA, Bethesda, MD; <sup>4</sup>Brown University, Providence, RI.

The development of novel and more effective medications for alcohol use disorder (AUD) is a pressing unmet medical need. Approved medications for AUD generally have limited efficacy and are prescribed for fewer than 10% of U.S. patients with AUD. Drug repositioning or repurposing is an appealing strategy to bring new therapies to the clinic because it greatly reduces the overall costs of drug development and expedites the availability of treatments to those who need them. We recently found that the glycyrrhetic acid derivative carbenoxolone (CBX; 3 $\beta$ -hydroxy-11-oxoolean-12-en-30-oic acid 3-hemisuccinate), a medication that was previously approved for the treatment of gastritis and peptic ulcer, reduces both dependent and nondependent alcohol intake in rodents, suggesting that it is a candidate for drug repositioning for the treatment of AUD. Carbenoxolone is a multi-target drug that shapes cellular responses to glucocorticoids by inhibiting 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) isozymes. It also inhibits pannexin1 channels, which contribute to adenosine triphosphate release, in the extracellular space. Probenecid is a medication that is used clinically primarily to increase uric acid excretion in the urine in hyperuricemic conditions, such as gout, through its activity as a competitive substrate for anion-transporting polypeptides in the kidney. Probenecid was also shown to inhibit pannexin1 channels. Therefore, we tested its effects on alcohol intake in rats. Similar to CBX, probenecid reduced alcohol intake in both dependent and nondependent rats. These results raise the possibility that pannexin1 may be a novel therapeutic target for the treatment of AUD. The clinical use of probenecid has been found to be generally safe, suggesting that it may be a candidate for drug repositioning for the treatment of AUD.

**ANS-6637, a Selective Reversible Inhibitor of ALDH2, suppresses Alcohol Consumption and Cue-Induced Alcohol Self Administration in the Absence of Alcohol and Acetaldehyde.**

Ivan Diamond, MD, PhD\*, Speaker; Stephanie O'Malley, PhD\*\*, Maria Arolfo, PhD\*\*\*, Lina Yao, MD, PhD^, Peidong Fan, PhD^ and Brent Blackburn, PhD\*.

Amygdala Neurosciences\*, Yale University\*\*, Teon Therapeutics^

According to prevailing clinical concepts Disulfiram discourages drinking because of adverse symptoms caused by increased acetaldehyde as a consequence of irreversible inhibition of hepatic ALDH2. However, Daidzin, a known ALDH2 inhibitor derived from Kudzu extracts, suppresses Golden Syrian hamster drinking without increasing acetaldehyde. This suggested that inhibiting ALDH2 in brain might reduce urges to drink alcohol. We used highly selective reversible inhibitors of ALDH2 to prevent excessive self-administration of alcohol. Paradoxically, these inhibitors also inhibited cue-induced reinstatement of drinking in the absence of alcohol and acetaldehyde. We soon discovered that ALDH2 inhibition in VTA prevents dopamine surges underlying craving/drug-seeking for alcohol, cocaine and other addictive drugs. Here we demonstrate that expression of viral ALDH2 antisense in the VTA also suppresses drinking, independently confirming ALDH2 as a CNS target. Transient ALDH2 antisense expression correlates directly with transient reduction of alcohol drinking. We then developed ANS-6637, a safe highly selective reversible inhibitor of ALDH2. We studied the dose-response of ANS-6637 interaction with alcohol in subjects consuming 5 standard drinks in 2.5 hours. There were virtually no adverse effects from ANS-6637 except an insensitive flushing reaction. We know ANS-6637 only targets ALDH2. In contrast, Disulfiram has recognized broad toxicity. The adverse alcohol-dependent adverse effects of Disulfiram are likely due to strong inhibition of hepatic ALDH1, not merely ALDH2. Our findings suggest that ANS-6627 holds great promise for treating alcoholism and preventing relapse by attenuating craving/alcohol seeking behavior without immediate adverse effects.

## **An Evaluation of the Peroxisome Proliferator Receptor-Alpha (PPAR- $\alpha$ ) Agonist, Fenofibrate, in a Human Laboratory Model of Alcohol Use Disorder**

Barbara J. Mason, Ph.D. Scripps Research, La Jolla, CA

The PPAR- $\alpha$  agonist, fenofibrate, has been shown to decrease alcohol consumption and preference in rats and mice, and a human genome-wide association study showed an association of a single nucleotide polymorphism in PPAR- $\alpha$  with alcohol withdrawal. Taken together, these data provide a compelling rationale for testing fenofibrate for therapeutic potential in a human laboratory model of AUD. We hypothesized that fenofibrate would significantly attenuate craving in response to in vivo alcohol cue exposure in the laboratory and reduce drinking under natural conditions during treatment and post-treatment follow-up. Fifty non-treatment-seeking, cue-reactive volunteers with AUD (39 males, 11 females; aged  $37.6 \pm 11.8$  years) were randomized to 9 days of treatment with fenofibrate (145mg/d) or matched placebo, and were followed for 1-week post-treatment. Subjects were required to be abstinent for 3 consecutive days prior to testing on Day 9; abstinence was verified by alcohol glucuronide testing. On Day 9, subjects were exposed to standardized mood induction procedures and in vivo beverage cues (alcohol or water). Subjects consistently showed significantly greater craving in response to alcohol cues relative to water cues, but no differences in craving between fenofibrate and placebo were observed. Similarly, no pre-post treatment differences were found for drinking. Fenofibrate concentration in plasma did not correlate significantly with alcohol cue reactivity or drinking measures. These data do not show an advantage in therapeutic potential for fenofibrate over placebo in individuals with AUD. This research was supported by U01AA025476 and P60AA006420.

## SYMPOSIUM

Recent advances in probing the link between alcohol and neuroendocrine pathways

*HAASS-KOFFLER Carolina (Brown University)*

- The effects of oral and intravenous alcohol administration on appetitive and stress-related hormones: Results from human laboratory experiments  
*FAROKHNIYA Mehdi (Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, NIAAA Division of Intramural Clinical and Biological Research and NIDA Intramural Research Program, National Institutes of Health. Center on Compulsive Behaviors, National Institutes of Health, Bethesda.)*
- Dysregulations of glucocorticoid and mineralocorticoid receptor systems in alcohol dependence: Converging evidence from rats and humans  
*VENDRUSCOLO Leandro F. (Neurobiology of Addiction Section NIH/NIDA – IRP/INRB Baltimore)*
- Effect of GLP-1 analogue and desacyl ghrelin administration on alcohol cue reactivity in humans: the Gut Hormone in Addiction Study  
*GOLDSTONE Tony, PsychoNeuroEndocrinology Research Group, Neuropsychopharmacology Unit, Centre for Psychiatry, Division of Brain Sciences, Imperial College London, UK*
- Intravenous administration of ghrelin increases serum cortisol and aldosterone concentrations in heavy-drinking alcohol-dependent individuals  
*HAASS-KOFFLER Carolina (Brown University)*

## **The effects of oral and intravenous alcohol administration on appetitive and stress-related hormones: Results from human laboratory experiments**

FAROKHNIYA Mehdi (Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, NIAAA Division of Intramural Clinical and Biological Research and NIDA Intramural Research Program, National Institutes of Health. Center on Compulsive Behaviors, National Institutes of Health, Bethesda.)

A growing body of evidence from preclinical and clinical research suggest that endocrine pathways play important roles in the pathophysiology of addictive behaviors, including alcohol use disorder (AUD). A number of these pathways, especially appetitive and stress-related hormones, are currently under investigation as potential targets to develop novel treatments for AUD. To this end, it is also important to understand whether and how alcohol consumption and dependence may affect endogenous concentrations of endocrine markers. In a series of human laboratory experiments, we examined the effect of alcohol administration on blood concentrations of different endocrine markers in heavy-drinking alcohol-dependent individuals. Four separate sessions were conducted across these studies: oral self-administered (variable dose) alcohol, oral fixed dose alcohol, intravenous self-administered (variable dose) alcohol, and intravenous fixed-dose alcohol. Repeated blood samples were obtained during each session and the following hormones were measured: total ghrelin, acyl-ghrelin, leptin, glucagon-like peptide 1, GLP-1, insulin, amylin, pancreatic polypeptide (PP), peptide YY, gastric inhibitory peptide (GIP), insulin-like growth factor 1 (IGF1), growth hormone (GH), prolactin, Adrenocorticotrophic hormone (ACTH), cortisol, and aldosterone. The results of alcohol administration, via different routes and with different dosing schedules, on each endocrine marker will be discussed.

**Dysregulations of glucocorticoid and mineralocorticoid receptor systems in alcohol dependence:  
Converging evidence from rats and humans**

Leandro F. Vendruscolo, Pharm.D., Ph.D.  
Staff Scientist, Neurobiology of Addiction Section  
NIH/NIDA – IRP/INRB  
251 Bayview Blvd, BRC Room 08A727  
Baltimore, MD 21224

Alcohol consumption and withdrawal in alcohol use disorder (AUD) activate the hypothalamic-pituitary-adrenal (HPA) axis to release corticosteroids (corticosterone in rats or cortisol in humans), which binds to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). We hypothesized that excessive activation of the HPA axis by alcohol intoxication and withdrawal dysregulates GR and MR systems and that these changes contribute to negative emotional states that drive compulsive alcohol drinking. We found that GR expression and function were altered in cortical and subcortical brain regions in dependent rats compared with nondependent rats. Systemic and intracerebral (central nucleus of the amygdala, ventral tegmental area, and nucleus accumbens) GR antagonism blocked the enhanced alcohol drinking and the motivation for alcohol in alcohol dependent rats. In a translational proof-of-concept human laboratory study, we found that GR antagonism decreased cue-induced alcohol craving and alcohol drinking in humans with alcohol use disorders. Additionally, we found that levels of MR in the CeA were negatively associated with anxiety-like behavior and compulsive-like alcohol drinking in dependent rats. In non-abstinent patients with AUD, the levels of aldosterone, an MR agonist, positively correlated with alcohol drinking, craving and anxiety scores, suggesting a potential functional role of MR in AUD. These findings provide converging evidence from rats and humans for dysregulations of GR and MR systems in AUD.

## **Effect of GLP-1 analogue and desacyl ghrelin administration on alcohol cue reactivity in humans: the Gut Hormone in Addiction Study**

*Tony Goldstone, PsychoNeuroEndocrinology Research Group, Neuropsychopharmacology Unit, Centre for Psychiatry, Division of Brain Sciences, Imperial College London, UK*

Common neurobiological mechanisms underlie addictive behaviours, including alcohol use disorder (AUD), drug dependence and overeating. Recent pre-clinical research has shown that gut-brain hormonal signals regulating food intake play an important role in non-food reward behaviours, and that the role for the appetitive hormones, glucagon like peptide-1 (GLP-1), and acyl ghrelin (AG), extends beyond food intake regulation to include reward behaviour and consumption of alcohol.

Although desacyl ghrelin, the precursor for AG (active at the GHSR1a receptor), is not an antagonist or inverse agonist at GHSR1a, it has opposite metabolic effects to AG in some pre-clinical and clinical studies, and reduced sugar intake in humans. Furthermore, in clinical studies a DAG analogue, AZP-531, reduces body weight and improves glycaemic control in adults with obesity and type 2 diabetes mellitus.

Dr. Goldstone will present novel results from his MRC-funded experimental medicine, Gut Hormone in Addiction study, examining the effects of acute infusion of the GLP-1 analogue, Exendin-4, and DAG on brain responses to evaluation of alcohol pictures using functional magnetic resonance imaging in 3 groups: adults with obesity, ex-smokers and abstinent alcohol dependence (<http://clinicaltrials.gov/ct2/show/NCT02690987>). This will reveal the possible benefits of targeting the GLP-1 and ghrelin systems for treatment of alcohol use disorder, and potential underlying mechanisms for reductions in alcohol consumption by attenuating alcohol cue reactivity.

# **Intravenous administration of ghrelin increases serum cortisol and aldosterone concentrations in heavy-drinking alcohol-dependent individuals**

Carolina L. Haass-Koffler

Center for Alcohol and Addiction Studies, Department of Psychiatry and Human Behavior, Brown University, Providence, RI; <sup>2</sup>Center for Alcohol and Addiction Studies, Department of Behavioral and Social Sciences, School of Public Health, Brown University, Providence, RI; Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, National Institute on Alcohol Abuse and Alcoholism Division of Intramural Clinical and Biological Research and National Institute on Drug Abuse Intramural Research Program, National Institutes of Health, Bethesda, MD.

## **Abstract**

Increasing evidence supports the role of appetite-regulating hormones, including ghrelin, in alcohol use disorder (AUD). Effects of ghrelin administration on cortisol and aldosterone concentrations have been observed in ghrelin-exposed tissues or cells, rodents and healthy volunteers, however whether these effects replicate in individuals with AUD is unknown. Here we tested the hypothesis that intravenous administration of ghrelin leads to increase in endogenous serum cortisol and aldosterone concentrations in alcohol-dependent heavy drinking individuals, and that these changes may predict ghrelin-induced alcohol craving. This was a double-blind, placebo-controlled human laboratory study in non-treatment-seeking, heavy-drinking alcohol-dependent individuals randomized to receive either placebo, 1 mcg/kg or 3 mcg/kg of intravenous ghrelin. Then, participants underwent a cue-reactivity procedure in a bar-like setting, which included exposure to both neutral (juice) and alcohol cues. Repeated blood samples were collected and used to measure endogenous cortisol and aldosterone serum concentrations in response to exogenous ghrelin administration. Furthermore, cortisol and aldosterone serum concentrations were used to develop a model to predict the effect of exogenous ghrelin administration on alcohol craving. Intravenous ghrelin administration increased endogenous cortisol and aldosterone serum concentrations. While the effects on cortisol were greater than those on aldosterone, only the ghrelin-induced changes in aldosterone serum concentrations predicted alcohol craving. These findings provide evidence of ghrelin effects on glucocorticoids and mineralocorticoids in individuals with AUD, thereby providing additional information on the potential mechanisms how the ghrelin system may play a role in alcohol craving and seeking in AUD.

## **Funding**

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## **Conflict of Interest**

The authors report no biomedical financial interests or potential conflicts of interest.

# POSTERS

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The abstracts are given in the alphabetic order of the main author.

Some abstracts are missing. A new version of this book of abstracts will be available after the conference.

## Posters

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*By alphabetic order of the main author*

**1 - Self-Estimation of Blood Alcohol Concentration in patients admitted in Emergency Department**

*ALAUX-CANTIN Stéphanie (Service de Psychiatrie et d'Addictologie de Liaison - GRAP INSERM UMR1247 - CHU Amiens)*

**2 - The role of the gut-brain axis in alcohol dependence: design of the Gut2Brain study**

*AMADIEU Camille (Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, Belgium)*

**3 - The Effects of Hangover on Inhibitory Control in Young Binge Drinkers: An Event-Related Potentials Study**

*ANTUNES Natália (University of Minho)*

**4 - GENE EXPRESSION CHANGES ASSOCIATED WITH STRESS-INDUCED ALCOHOL ESCALATION**

*BARBIER Estelle (Center for Social and Affective Neuroscience, IKE, University of Linköping, Linköping, Sweden)*

**5 - Investigating the role of neuroinflammation in Long-Term Depression impairment induced by two ethanol binge exposures (TEBE) in young rats**

*BERTRAND Cédric (UMR1247 - Groupe de Recherche sur l'Alcool et les Pharmacodépendances)*

**6 - Ketosis modulates alcohol consumption in adult male mice**

*BLANCO-GANDÍA M. Carmen (Departamento de Psicología y Sociología, Facultad de Ciencias Sociales y Humanas, Universidad de Zaragoza, Teruel, Spain)*

**7 - Time course and specificity of attentional bias in binge drinking: an eye-tracking**

*BOLLEN Zoé (UC Louvain)*

**8 - Fetal alcohol syndrome prevention in women: attitude to pregnancy**

*BURINA Ekaterina (Saint Petersburg State University)*

**9 - Genome-wide DNA methylation analysis of the human postmortem nucleus accumbens identifies differential methylation in AUD individuals**

*CERVERA-JUANES Rita (Genetics Division, Neuroscience Division. Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, USA)*

**10 - H<sub>2</sub>O<sub>2</sub>, a major reactive oxygen species of alcohol metabolism induces autophagy without involving mTOR**

*CHEN Cheng (Center for Alcohol Research and Salem Medical Center, University of Heidelberg)*

**11 - Prevalence and demographic characteristics of alcohol use disorder in Chinese Shandong provincial adult residents: a cross-section epidemiologic survey**

*CHEN Xu (Binzhou Medical University; Shandong Mental Health Center)*

**12 - Effect of trauma and family alcohol using situations in childhood on the occurrence of alcohol dependence in Chinese male patients**

*CHEN Xu (Binzhou Medical University; Shandong Mental Health Center)*

- 13 - Cross-modal processing of emotions in Severe Alcohol Use Disorders: impaired discrimination of anger and fear under dynamic audiovisual conditions  
*CREUPELANDT Coralie (Institute of research in Psychology & Institute of Neuroscience, UC Louvain)*
- 14 - A NEW ANIMAL MODEL OF PAIN-INDUCED ALCOHOL RELAPSE: INVOLVEMENT OF MU AND KAPPA OPIOID RECEPTORS IN THE MESOCORTICOLIMBIC SYSTEM  
*CUITAVI Javier (Department of Pharmacy and Pharmaceutical Technology and Parasitology, University of Valencia, Spain)*
- 15 - Adipokines (adipocytokines), selected clinical and nutritional variables in the patients with alcohol dependence  
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- 16 - INVOLVEMENT OF NEUROINFLAMMATION IN THE EFFECTS OF TWO ETHANOL BINGE EPISODES DURING ADOLESCENCE ON CA1 HIPPOCAMPAL SYNAPTIC PLASTICITY  
*DESCHAMPS Chloé (INSERM U1247 - GRAP)*
- 17 - The FASD Resource Center in Reunion Island: Back to 3 years of activity.  
*DORAY Bérénice (Centre Ressources ETCAF Fondation Père Favron – CHU de La Réunion - France)*
- 18 - SUPPRESSION of ETHANOL INDUCED NEUROINFLAMMATION BY PPAR- $\gamma$  AGONISTS IN AN ANIMAL MODEL OF FASD  
*DREW Paul (University of Arkansas for Medical Sciences)*
- 19 - Alcohol-related abnormalities in the early postnatal period can be corrected by multitarget low-affinity agonist of sigma-1, MT1 and MT3 receptors  
*DURNEV Andrei (Federal State Budgetary Institution Research Zakusov Institute of Pharmacology)*
- 20 - “Alcohol and you?” A two day assessment program with a multidisciplinary team  
*FABRY Lauriane (Centre Hospitalier Universitaire Brugmann, Université Libre de Bruxelles, Belgique)*
- 21 - The role of Alcohol on Iron metabolism and Erythropoiesis in Acute and Chronic Alcohol Mouse model  
*FARAZ Faiza (Center for Alcohol Research and Salem Medical Center, University of Heidelberg)*
- 22 - Alcohol use disorder and comorbid depression: a randomised controlled trial investigating the effectiveness of supportive text messages in aiding recovery  
*FARREN Conor (Trinity College Dublin)*
- 23 - HISTORICAL KEYPOINTS IN THE CONCEPT, DEFINITION and PATHOGENESIS OF ALCOHOLIC CARDIOMYOPATHY.  
*FERNANDEZ SOLA JOAQUIM (Alcohol Unit Hospital Clínic. IDIBAPS. University of Barcelona)*
- 24 - N-acetylcysteine alters GLT-1 and  $\gamma$ Fos B expression in the dorsolateral striatum of long-term ethanol-experienced rats during the abstinence period  
*FERNÁNDEZ-RODRÍGUEZ Sandra (Department of Pharmacy and Pharmaceutical Technology and Parasitology. University of Valencia)*
- 25 - HCC alcohol exposure inhibits the suppressor of tumor SLAMF3  
*FOUQUET Grégory (GRAP Inserm U1247)*
- 26 - Perceptions of alcohol use during pregnancy in France, Spain and Portugal – a cross-cultural qualitative study  
*FRANCO Renata (Department of Psychology, Institut Catholique de Toulouse)*

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GILDE David (*The Scripps Research Institute, Molecular and Cellular Neuroscience Department, La Jolla, USA*)

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## **Self-Estimation of Blood Alcohol Concentration in patients admitted in Emergency Department**

**Alaux-Cantin Stéphanie, Angerville Bernard, Naassila Mickaël, Dervaux Alain**

Studies conducted in general population reported that alcohol drinkers under-estimate their blood alcohol concentration (BAC). However, no study has investigated the accuracy of BAC self-estimation in alcohol intoxicated patients admitted in Emergency Department (ED).

To assess this question, all consecutive patients admitted in ED of the University Hospital of Amiens, France, with at least BAC of 0.6g/L were included in the study. Self-estimated BAC was assessed using a visual analogic scale, compared with objective measurements of BAC. We next performed comparisons between moderate, mild or severe AUD patients according to the DSM-5 criteria. We assessed the subjective effects of alcohol (SEA) using SEA scale.

Preliminary results showed that patients included in the present study under-estimated their BAC ( $-0.6 \pm 0.3$ g/L,  $n=20$ ). Interestingly, moderate AUD patients over-estimated BAC ( $0.5 \pm 0.5$ g/L,  $n=3$ ), mild AUD patients correctly estimated BAC ( $0.0 \pm 0.3$ g/L,  $n=5$ ), and severe AUD patients clearly under-estimated BAC ( $-1.1 \pm 0.3$ g/L,  $n=12$ ). The sensitivity to the positive sub-scale of the SEA (i.e. lively, funny, talkative) was important in moderate AUD, intermediate in mild AUD and low in severe AUD patients except regarding low arousal positive effects that remain relatively important (i.e. relaxed, calm, mellow). Both groups displayed a low sensitivity toward negative alcohol effects (i.e. aggressive, rude, woozy).

Taken together, our preliminary results showed that severe AUD patients under-estimated BAC and displayed an altered sensitivity to the SEA. Further investigations are necessary to assess whether such a brief intervention including the correction of under-estimated BAC could improve awareness in AUD patients and elicit motivational changes.

Title: The role of the gut-brain axis in alcohol dependence: design of the Gut2Brain study

Authors: Camille Amadiou<sup>1,2</sup>, Sophie Leclercq<sup>1,2</sup>, Peter Stärkel<sup>3</sup>, Philippe de Timary<sup>2</sup>, Nathalie M Delzenne<sup>1</sup>.

Affiliations:

1. Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, Belgium
2. Institute of Neuroscience, Université catholique de Louvain, Belgium.
3. Institute of Experimental and Clinical Research, Laboratory of Hepato-Gastroenterology, Université Catholique de Louvain, Belgium; Department of Hepato-Gastroenterology, Cliniques Universitaires Saint-Luc, Belgium.

**Rationale:** The gut microbiota, a huge community of microorganisms (comprising bacteria, viruses, fungi and yeast) living in our intestine, has been shown to regulate many important functions for human health including metabolism, immunity as well as brain functions and behavior. Our previous studies have shown that chronic alcohol abuse induced a leaky gut and alterations in the composition of the gut microbiota, which are correlated with psychological symptoms such as depression, anxiety and alcohol craving, suggesting the involvement of the gut-brain axis in the development of alcohol use disorders (AUD).

The Gut2Brain study aims at modulating the gut-brain axis of AUD patients by administering dietary fibers with prebiotic properties which are known to modify the composition of the gut microbiota.

**Methodology of the Gut2brain study:** This is a randomized, double-blind, placebo controlled study including 50 patients. Twenty-five patients are assigned to the prebiotics group and 25 patients are in the placebo group. AUD patients are hospitalized for a 3-week detoxification program in the alcohol withdrawal unit of St Luc academic hospital (Brussels, Belgium).

Biological (microbiota composition, bacterial metabolites, inflammatory markers) and psychological measurements (depression, anxiety, craving, sociability) have been performed twice, at the onset of alcohol withdrawal (T1 = before starting the prebiotic treatment) and at the end of the detoxification program (T2 = after 17 days of prebiotics supplementation). Because microbiota composition is heavily influenced by nutrition, diet anamnesis have been handled to evaluate the nutritional habits of AUD patients with a special focus on fiber intakes.

**Conclusion:** The Gut2Brain study will investigate for the first time the effects of prebiotics on gut microbiota composition and function, systemic inflammation and psychological symptoms of AUD patients. The results of this study will help to design new therapeutic and/or preventive targets for AUD patients.

# THE EFFECTS OF HANGOVER ON INHIBITORY CONTROL IN YOUNG BINGE DRINKERS: AN EVENT-RELATED POTENTIALS STUDY

Natália Antunes, Rui Rodrigues, Alberto Crego, Adriana Sampaio and Eduardo López-Caneda  
*Psychological Neuroscience Lab, Research Center in Psychology (CIPsi), School of Psychology, University of Minho,  
Campus Gualtar, 4710-057 Braga, Portugal*

During adolescence and at the time of entering university, young people seek new sensations and they are more likely to engage in high-risk behaviours, such as drug abuse. Alcohol besides being the most consumed drug in the world, it also represents the third higher risk factor for disease and largely contributes to the number of deaths worldwide. The excessive alcohol use can lead to a pattern known as binge drinking (BD), which is characterised by heavy alcohol intake over a short time, followed by periods of abstinence. This form of alcohol misuse has received special attention in the last decade mainly due to its high prevalence among youngsters and the negative consequences resulting from that.

One of the major consequences immediately after a BD episode is the hangover experience. Hangover, strictly related to BD, can be described as a series of unpleasant physical and mental symptoms, which follow the intake of large amounts of alcohol and are especially significant when the blood alcohol concentration reaches 0 g/dL. Some studies have demonstrated that alcohol hangover may affect cognitive functioning, namely memory, attention and psychomotor performance. Nevertheless, to the best of our knowledge, no study has been conducted with the aim of assessing the behavioural and electrophysiological consequences of alcohol hangover after a typical BD episode despite the important implications that might result from this research. Aiming to understand how inhibitory control may be affected the day after a single BD session, behavioural measurements and brain activity recorded from 64 electrodes were analysed while 10 college BDs (six females) performed a Go/NoGo task before and after a typical BD night. The reaction times; percentages of correct responses and correct inhibitions; the amplitude and latency of P2, N2, P3 and Late Positive Component (LPC) were assessed.

Despite having found no hangover effects at the behavioural level, electrophysiological abnormalities emerged the day after a heavy alcohol drinking episode. Specifically, decreased P2 amplitudes were observed after a BD night in comparison with a normal day without alcohol consumption, suggesting that a single BD episode may significantly compromise the allocation of attentional resources needed to perform the task in the following day. Additionally, after a night engaging in BD, students displayed a marginally significant decreased P3 and LPC amplitude. Although still tentatively, these results could indicate that a BD session may lead to impairments on attentional and working memory processes in young BDs.

*Keywords:* binge drinking, hangover, inhibitory control, event-related potentials, college students

## **GENE EXPRESSION CHANGES ASSOCIATED WITH STRESS-INDUCED ALCOHOL ESCALATION**

Estelle Barbier<sup>1</sup>, Riccardo Barchiesi<sup>1</sup>, Kanat Chanthongdee<sup>1</sup>, Markus Heilig<sup>1</sup>  
Center for Social and Affective Neuroscience, IKE, University of Linköping, Linköping,  
Sweden

Comorbidity of alcohol use and anxiety disorders is a major cause of disability and a challenge for mental health services. Both disorders are characterized by broad and persistent changes in gene expression within brain areas involved in regulation of negative affect including the prefrontal cortex and the amygdala. However, the shared underlying mechanisms are still not well known. In our study, we used a rat model of social defeat stress (SDS) to assess the impact on alcohol- and anxiety-like behaviors. In addition, a second group of rats were made to witness the SDS in order to unravel the psychological component from the combined physical and psychological stress in the defeated animals. In accordance with previous studies, we found individual variability in the behavioral outcomes following social stress. Stress induced by social defeat or by witnessing SDS, led to an increase in operant alcohol self-administration and anxiety-like behaviors only in a subset of animals. Behavioral studies were performed ten days after the last social defeat, suggesting a long lasting effect of the social stressors on both alcohol intake and anxiety-like behaviors. Gene expression changes observed on the subset of rats showing both alcohol and anxiety-related behaviors are assessed using our custom made NanoString panel. Our panel comprises about 400 genes involved in critical neuronal functions such as neurotransmitter release and synaptic plasticity. It also include epigenetic regulators. This is of particular interest as stress and heavy alcohol have been shown to reprogram the transcriptome, making interventions that target epigenetic mechanisms an attractive novel approach to develop therapeutics.

## **Investigating the role of neuroinflammation in Long-Term Depression impairment induced by two ethanol binge exposures (TEBE) in young rats**

Bertrand C, Vilpoux C, Naassila M, Pierrefiche O, Peineau S

GRAP UMR1247, INSERM, Centre Universitaire de Recherche en Santé, Université de Picardie Jules Verne, Amiens, France

Binge Drinking (BD), an alcohol consumption pattern described as a fast way to reach drunkenness, is associated to many cognitive impairments including hippocampus related memory issues and leads to chronic neuroinflammation with deleterious effect at the neuronal level. Hippocampus neuronal plasticity mechanisms, such as Long Term Depressions (LTD), are crucial in learning and memory processes and modulated by neuroinflammation factors.

We used young rat to model the early steps of BD consumption on cognitive deficits (hippocampus slices, plasticity recordings 48h after TEBE 3g ethanol/kg bodyweight, i.p. given 9h apart; Silvestre de Ferron et al., 2015) and found an abolition of population spike LTD in CA1 neurons, concomitant with learning impairment. We now investigated the impact of TEBE on synaptic LTD with patch-clamp techniques and investigated the role of neuroinflammation. We found a partial synaptic LTD inhibition, suggesting that population-spike LTD abolition could be due to an effect of TEBE at the synaptic level. We then hypothesized that synaptic LTD inhibition originates from an emerging neuroinflammation. We analyzed microglial cells morphology and inflammatory markers with MACS cell separation and RT-qPCR technique after TEBE. In parallel we treated TEBE rats with anti-inflammatory drugs. When applied before the first exposure, anti-inflammatory treatment enhances synaptic LTD impairment but not if rats are treated just before the second exposure. These results highlight a neuroprotective role of neuroinflammation in the early step of binge drinking episodes, in contrary to its deleterious role in multiple episodes of binge drinking.

## **Ketosis modulates alcohol consumption in adult male mice**

M.Carmen Blanco-Gandía<sup>1,2</sup>, Francisco Ródenas<sup>1</sup>, Marina D Reguilón<sup>1</sup>, José Miñarro<sup>1</sup>, Marta Rodríguez-Arias<sup>1</sup>

<sup>1</sup>Unidad de Investigación Psicobiología de las Drogodependencias, Departamento de Psicobiología, Facultad de Psicología, Universitat de Valencia, Valencia, Spain

<sup>2</sup>Departamento de Psicología y Sociología, Facultad de Ciencias Sociales y Humanas, Universidad de Zaragoza, Teruel, Spain.

In recent studies, metabolic or nutritional treatments for different disorders, such as epilepsy, Alzheimer's disease, cancer or autism, have proved to be successful. The ketogenic diet (KD) is a high-fat diet, low in carbohydrates and balanced in proteins, that induces changes in the body's main energy source, since it uses ketone bodies instead of glucose. The KD has been linked to the amelioration of all the above-mentioned conditions, but the mechanisms underlying its therapeutic effects are still unclear. On the other hand, several recent studies have suggested that the type of diet (for example palatable food or cafeteria diet) and the way it is consumed (continuous access or binge eating) modulate the development of drug addiction. For instance, high-fat and -sugar diets increase cocaine and ethanol consumption in mice, as well as their sensitivity to the conditioned rewarding effects of both drugs. The present work aimed to study if the KD can modulate the rewarding effects of alcohol and to assess its potential as a therapeutic target to decrease alcohol consumption.

A total of 30 adult male mice of the OF1 strain (PND 42) were assigned either a standard diet (n=14) or a Ketogenic Diet (KD) (n=16). When a ketosis state had been sustained for 7 days, the reinforcing and motivating effects of ethanol were measured by means of the oral self-administration paradigm, in which the number of reinforced responses (Fixed Ratio 1 and 3), ethanol consumption (g/kg) and the breaking point of the progressive ratio were analyzed.

Our results revealed that animals in a ketosis state exhibited, in general, a trend towards a decrease in ethanol consumption in terms of the FR1 and FR3, but did not show changes in their motivation to drink compared to animals fed a standard diet.

We propose that future investigations are necessary to clarify which neuroadaptations underlie the effects produced by the KD. Our results suggest that the nutritional state is a useful tool for the future treatment of alcohol use disorders.

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## **Time course and specificity of attentional bias in binge drinking : an eye-tracking**

**BOLLEN Zoé**  
*UCLouvain*

Attentional bias is a core characteristic of alcohol use disorders (AUD), playing a crucial role in their development and persistence. Many behavioural studies have showed an increased allocation of attention towards alcohol cues in patients with AUD and revealed a direct link between bias and craving, alcohol consumption or relapse risk. Nevertheless, its underlying mechanisms are still poorly understood. Eye-tracking measures, offering deeper insights regarding the timeline of the bias, constitutes an innovative way to renew its exploration. The present study used eye-tracking measures to: (a) investigate attentional bias in a subclinical AUD population (i.e. binge drinking), (b) determine the time course of the bias, by disentangling the early from late processing stages, (c) clarify the specificity of the bias towards alcohol or its generalization towards other appetitive stimulations (i.e. food stimuli), and (d) explore the relation between craving and attentional bias. Two groups of participants (42 binge drinkers, 43 controls) performed a visual probe task, which requires the detection of an arrow preceded by pictures from different conditions: (1) alcohol vs. soft, (2) alcohol vs. food, (3) salty or sugary food vs. healthy food. Eye-tracking measures highlights the presence of a bias towards soft and healthy food among control participants. Complementary analyses indicated that binge drinkers with high level of craving showed a bias towards alcohol and high-caloric food, unlike those with a low level of craving. The alcohol attentional bias is thus neither related to binge drinking, but rather to the association between this drinking pattern and craving.

# **Fetal alcohol syndrome prevention in women: attitude to pregnancy**

**Ulia O. Remenyuk, Ekaterina A. Burina**

**Saint Petersburg State University  
Saint Petersburg, Russia**

A number of studies addresses fetal alcohol syndrome (FAS) prevention, but almost none investigate FAS aspects in connection to pregnancy attitude. Awareness helps a woman to develop an adequate relation to pregnancy that leads to a healthy behavior (Health Belief Model, Rosenstock I., 1974). Therefore, a pilot study was conducted.

Sample: 35 non-pregnant women of childbearing age (M=27), never been pregnant and able to give birth.

Methods: screening; informed consent; interview (pregnancy attitude, FAS awareness, alcohol-exposed pregnancy (AEP) risk); personal inventory «Big 5»; personal time perspective measure; subjective control evaluation method. Primary preventive measures were realized with every participant. Analysis: statistical methods, «R Studio».

Study results revealed very poor FAS awareness – just 34% of women heard about the syndrome but only 14% of them gave correct answers. AEP-risk was observed in 26% with average 4 alcohol drinks a time, and contraception risk – in 31%.

Personal features of participants with AEP-risk showed average results with a tendency of women with low/normal alcohol use to have higher readiness for cooperation (U=62.5; p=0.0406). Subjective control was found on average level and was not related to at-risk behavior. Time perspective results indicated high expectations about participants' future.

Attitude to pregnancy was divided into categories: positive (45.5%), negative (30%), neutral (24.5%). No relation to other characteristics studied was observed, possibly due to a small sample. Identified categories can help in determining woman's pregnancy attitude in order to adjust behavior to more health-saving.

Pilot study results can be useful for preventive programs design and further implementation.

## Genome-wide DNA methylation analysis of the human postmortem nucleus accumbens identifies differential methylation in AUD individuals.

Rita Cervera-Juanes, PhD.

Genetics Division, Neuroscience Division. Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Oregon, 97006, USA.

Chronic alcohol use has been linked to alterations in synaptic plasticity thought to contribute to alcohol dependence, tolerance, craving and withdrawal. Clarifying the genes and regulatory mechanisms underlying such neuroadaptations is critical to fully understand and treat alcohol abuse. Here, we explore the methylome of the postmortem human nucleus accumbens (NAcc) from controls and AUD subjects. To identify differential methylation signals we conducted genome-wide DM (GWDM) analysis using the Agilent SureSelect Human MethylSeq kit on NAcc tissue from age-matched pairs of AUD and control subjects (32 males) obtained from the New South Wales Brain Tissue Resource Center (NSWBTRC). We obtained ~108 million raw reads per library that were aligned to the GRCh38/hg38 assembly of the human genome using Bismark. After quality control evaluation, we obtained ~2.5 million CpG sites per sample that were used for downstream analyses. Differential methylation was analyzed using the generalized linear modeling approach implemented in RnBeads including covariates of interest (i.e. batch sequencing effect, age, smoking status, etc.) and Comb-p analysis was used to identify differentially methylated regions (DMRs) between controls and AUD subjects. We identified a total of 914 DMRs (26% hypomethylated and 74% hypermethylated), with CpGs ranging in differential methylation from ~5-43%. As we observed in our prior nonhuman primate NAcc GWDM analysis, the majority of DMRs mapped intragenic locations ( $p_{(\text{hypergeometric})} < 1.02\text{e-}288$ ), mostly to intronic regions, while only 5% of the DMRs mapped to promoters. In terms of proximity to CpG islands, most of the DMRs overlapped with CpG islands (44%;  $p_{(\text{hypergeometric})} = 7.10\text{e-}1152$ ), followed by open sea (30%;  $p_{(\text{hypergeometric})} = 7.40\text{e-}502$ ) and CpG island shores (18%;  $p_{(\text{hypergeometric})} = 2.10\text{e-}122$ ). Cell-type enrichment analysis of the 707 DMRs mapping to genes or their promoters showed that chronic alcohol consumption significantly (hypergeometric test) affects the methylome of genes specific of astrocytes (Astroc,  $n=56$ ;  $p=8.9\text{e-}10$ ), endothelial cells (Endot,  $n=61$ ;  $p=4.7\text{e-}12$ ), microglia (Microg,  $n=54$ ;  $p=6.3\text{e-}92$ ), oligodendrocytes (Oligod,  $n=57$ ;  $p=3.2\text{e-}10$ ) and neurons (Neur,  $n=66$ ;  $p=1.6\text{e-}14$ ). Using Ingenuity pathway analysis, the following pathways were enriched: axonal guidance signaling (19 genes,  $p= 5.5\text{e-}3$ ), Wnt/B-catenin signaling (19 genes,  $p=1.4\text{e-}6$ ), synaptic long term depression (18 genes,  $p=1.9\text{e-}5$ ), CREB signaling in neurons (18 genes,  $p=1.5\text{e-}4$ ), gap junction signaling (17 genes,  $p=2.6\text{e-}4$ ), corticotropin releasing hormone signaling (13 genes,  $p=4.2\text{e-}4$ ), dopamine cAMP signaling (14 genes,  $p=4.6\text{e-}4$ ), opioid signaling pathway (18 genes,  $p=6.6\text{e-}4$ ) and synaptogenesis signaling pathway (19 genes,  $p=5.5\text{e-}3$ ). Some of these genes have been previously associated with alcohol abuse (i.e. *AGAP1*, *SEMA5A*), which reinforces the role of these genes in modulating alcohol abuse as well as the potential of our approach to not only identify these genes but also provide important details on the underlying epigenetic mechanisms potentially regulating their activity in the context of alcohol abuse. Other genes identified in this study have not yet been linked to addiction but their function is highly relevant in modulating alcohol-associated neuronal adaptations (i.e. *TCF7L2* involved in Wnt signaling). Altogether, our data suggests that a history of alcohol abuse is associated with differential DNA methylation in affecting synaptic plasticity mechanisms, similarly to what we observed in NHPs with long-term consumption of heavy doses of alcohol. This study not only provides genes, but equally important, it provides epigenetic information on how these genes may be regulated by alcohol, and how they could be targeted to revert such effect from a therapeutic perspective.

## **H<sub>2</sub>O<sub>2</sub>, a major reactive oxygen species of alcohol metabolism induces autophagy without involving mTOR**

Chen, Cheng<sup>1</sup>; Peccerella, Teresa<sup>1</sup>; Mueller, Sebastian<sup>1</sup> and Rausch, Vanessa <sup>1</sup>

<sup>1</sup> Center for Alcohol Research and Salem Medical Center, University of Heidelberg, Heidelberg, Germany

**Background and Aims:** Alcohol-mediated reactive oxygen species (ROS) formation in the liver, mainly H<sub>2</sub>O<sub>2</sub>, contributes to disease progression and eventually hepatocellular carcinoma development in patients with ALD. Enhancement or activation of autophagy, with the suppression of mTOR signaling, is likely to play an important role in early stages of the alcoholic liver disease (ALD). However, with the progression of the disease, the expression of mTOR increases dramatically leading to the suppression of autophagy. It is also known, that H<sub>2</sub>O<sub>2</sub> is involved in the regulation of autophagy in both acute and chronic ALD models, however, the exact underlying molecular mechanisms are still unclear. Therefore, we investigated in vitro and in vivo by using alcohol mouse model alterations in mTOR signaling as well as downstream effects induced by H<sub>2</sub>O<sub>2</sub> and low oxygen tension.

**Methods:** Huh7 hepatoma cells and VL-17A cells (stably transfected with CYP2E1 and ADH) were cultured with the GOX/CAT system, which allows an independent control of hydrogen peroxide as well as oxygen levels, in combination with different doses of ethanol. LC3B, p62, mTOR and autophagy related proteins (e.g. AMPK, AKT, STAT3) were analyzed by western blot. Same analyses were performed in liver tissues of C57BL/6 mice treated with acute (alcohol binge) and chronic ethanol (20% Ethanol in the drinking water) for 4 weeks (n=4).

**Results:** H<sub>2</sub>O<sub>2</sub> significantly increased LC3B activation and this effect could be efficiently blocked by N-acetyl cysteine (NAC), which is a ROS scavenger. Interestingly, even though the LC3B activation was increased by H<sub>2</sub>O<sub>2</sub>, the m-TOR expression was not suppressed as normally expected. Co-treatment of hepatoma cells with H<sub>2</sub>O<sub>2</sub> and the mTOR inhibitor Rapamycin led to an increased autophagic flux as compared to single H<sub>2</sub>O<sub>2</sub> and Rapamycin treatment. The in vivo experiments showed a combined activation of LC3B and suppressed p62 and AKT levels as well as enhanced p-AMPK expression in the livers of the acute alcohol group. In contrast, mice exposed to chronic alcohol showed blocked autophagic flux with dramatically increased LC3-I and p62 levels.

**Conclusion:** Our findings underscore an important role of H<sub>2</sub>O<sub>2</sub> in regulating autophagy during acute and chronic alcohol ALD exposure. Further studies will be needed to address the differences between acute and chronic alcohol-mediated effects as well as to identify H<sub>2</sub>O<sub>2</sub>-induced signaling pathways that regulate autophagy.

***Prevalence and demographic characteristics of alcohol use disorder in Chinese Shandong provincial adult residents: a cross-section epidemiologic survey***

*Xu Chen<sup>1,2</sup>, Ruzhan Wang<sup>2</sup>, Yuchen Zhang<sup>1</sup>, Jingxuan Zhang<sup>2</sup>*

**Author Affiliation:** 1 Binzhou Medical University, 2 Shandong Mental Health Center

***Abstract***

**Background & objectives:** This survey aimed to investigate the prevalence and demographic characteristics of alcohol use disorder (AUD) in Chinese Shandong provincial adult residents.

**Methods:** Multistage stratified random sampling was used to identify 34 urban communities and 62 rural administrative villages as the sampling sites in Shandong province, with the 300 sample size of each site. The trained psychiatric nurses completed the primary screening with General Health Questionnaire (GHQ), and the trained psychiatrists examined the risk individuals with a Chinese version of the Structured Clinical Interview for Diagnostic and Statistical Manual IV axis I disorders.

**Results:** There were 27917 enrolled, and 27489 completed in this survey. Adjusted for gender, age and other demographic items, the one month prevalence of AUD was 5.27% (95%CI 5.01-5.54), and at the head of mental diseases' prevalence, with the significant difference on gender ( $Z=45.29$ ,  $p=0.00$ ), but without the difference on residential place ( $Z=1.46$ ,  $p=0.14$ ).

**Conclusions:** The AUD prevalence in Shandong was high and should be highlighted as a public health problem.

**Key words:** Alcohol use disorder, Epidemiology, Shandong Province

# **Effect of trauma and family alcohol using situations in childhood on the occurrence of alcohol dependence in Chinese male patients**

Xu Chen<sup>1,2</sup>, Jiapei Yang<sup>1</sup>

*Author Affiliation:* 1 Binzhou Medical University, 2 Shandong Mental Health Center

## ***Abstract***

**Background & objective:** It was proved by some studies that childhood traumas have strongly effect on the arousing of alcohol dependence in males, but the role of family alcohol using situations is unclear. This cross-section was aimed to analyze the effects of trauma and family alcohol using situations in childhood on the occurrence of alcohol dependence. **Methods:** In this study, the questionnaires and the formulated structure interview were used. 120 patients with alcohol dependence and 103 healthy volunteers were assessed by Childhood Trauma Questionnaire (CTQ), self-made alcoholic using questionnaire for parents and the formulated interview. **Results:** The scores of CTQ, the fathers' frequency of drinking in the patients and inducing to drink in childhood were significantly higher than those of volunteers; and the rate of parents' opposed attitudes to drinking in patients were lower than that of the volunteers'. It was showed in the multiple-factors analysis that the relative risk factors of alcohol dependence were Parents' drinking frequency, No-opposed attitude to drink, Being induced to drink and Lower-level education. **Conclusions:** Compared to childhood trauma, parental alcohol using is the more important role in the formation of alcohol dependence.

**Key words:** Alcohol dependence, trauma, family, environment, risk factors

# **Cross-modal processing of emotions in Severe Alcohol Use Disorders: impaired discrimination of anger and fear under dynamic audiovisual conditions**

Coralie Creupelandt<sup>1</sup>, Fabien D'Hondt<sup>2,3,4</sup>, Federica Falargiarda<sup>1</sup>, Olivier Collignon<sup>1,5</sup>, and Pierre Maurage<sup>1\*</sup>

1 Institute of research in Psychology (IPSY) & Institute of Neuroscience (IoNS) - UCLouvain, Louvain-la-Neuve, Belgium

2 Univ. Lille, CNRS, UMR 9193 - SCALab - Sciences Cognitives et Sciences Affectives, F-59000 Lille, France

3 CHU Lille, Clinique de Psychiatrie, CURE, F-59000 Lille, France

4 Centre National de Ressources et de Résilience (CN2R), F-59000 Lille, France

5 Centre for Mind/Brain Studies, University of Trento, Trento, Italy

Severe alcohol use disorders (SAUD) are associated with a large variety of affective disturbances, among which a well-established decoding deficit for facial and vocal emotional expressions. This deficit has recently been found to be increased in cross-modal settings, namely when inputs from different sensory modalities have to be combined. Compared to unimodal emotional stimuli, cross-modal ones allow for faster and more accurate emotional predictions, and therefore constitute critical cues for social interactions. However, so far, studies exploring emotional cross-modal processing in SAUD relied on static faces, associated with voices from a different individual, largely hampering ecological validity. Besides, in real life conditions, emotions are often not fully expressed, so that we have to make guesses based on incomplete information. Accordingly, the aim of this study was to assess cross-modal emotional processing using a new ecological paradigm with dynamic audiovisual stimuli, manipulating the amount of emotional information available to the individual. Thirty individuals with SAUD and 30 matched healthy controls performed an emotional discrimination task requiring to identify emotions (anger, disgust, fear, happiness, sadness) expressed in short movies containing visual, auditory or auditory-visual information of various durations. The shortest excerpts revealed the very early emotional sketch (i.e., initial facial movements and prosody) while the longest ones depicted a more complete emotion. Sensitivity analyses ( $d'$ ) showed that discrimination levels varied across sensory modalities and emotions, and increased with stimuli duration in both groups. Individuals with SAUD's performances improved from unimodal to cross-modal conditions, but their discrimination for cross-modal stimuli was impaired for anger and fear. This deficit was not influenced by the amount of information displayed, suggesting that it persists even when more emotional information is available. Results are discussed in light of the predictive mechanisms underlying emotion recognition, and converge with earlier findings to ascribe a specific role for anger and fear in SAUD.

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\* Correspondence should be addressed to: Pierre Maurage (pierre.maurage@uclouvain.be)

## **A NEW ANIMAL MODEL OF PAIN-INDUCED ALCOHOL RELAPSE: INVOLVEMENT OF MU AND KAPPA OPIOID RECEPTORS IN THE MESOCORTICOLIMBIC SYSTEM**

Javier Cuitavi<sup>1</sup>, Jesús David Lorente<sup>1</sup>, Sandra Fernández-Rodríguez<sup>1</sup>, Yolanda Campos-Jurado<sup>1</sup>, Raquel Montón-Molina<sup>1</sup>, José Luís González-Romero<sup>1</sup> and Lucía Hipólito<sup>1</sup>

1. Department of Pharmacy and Pharmaceutical Technology and Parasitology, University of Valencia, Avda. Vicent Andrés Estellés s/n. 46100 Burjassot, Spain

**Key words:** Alcohol, Inflammatory pain, Relapse, Mu Opioid Receptor and Kappa Opioid Receptor

Several clinical studies have uncovered that pain may lead to alcohol relapse in patients with a previous history of alcohol use disorder. Unfortunately, we are still lacking a valid animal model to investigate the underlying neurochemical basis of this effect. According to that an alcohol intermittent administration animal model in combination with an inflammatory pain rat model has been created. Our model showed that all male rats increased the alcohol consumption after reintroduction, however in the case of female rats only the ones with inflammatory pain increased their intake over its baseline. That may represent that females have an increased vulnerability to relapse in the presence of pain. Besides we measured Mu and Kappa opioid receptors levels (MORs and KORs) in Ventral Tegmental Area (VTA), Prefrontal Cortex (PFC), Amygdala and Nucleus Accumbens (NAc) in abstinence and alcohol relapse phase. No differences were found in males. Nevertheless, we observed in females with pain a higher expression of KORs and MORs in NAc during both abstinence and the reintroduction periods. MORs expression was decreased in VTA and PFC in females during the abstinence. This effect on MORs expression was more pronounced in the presence of pain. Very interestingly our studies also revealed that these changes in VTA and PFC observed during abstinence are reverted after alcohol relapse, contributing to the understanding of the mechanisms involved in pain induced alcohol relapse-like behaviour in female rats.

### **Acknowledgements**

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**Title:** Adipokines (adipocytokines), selected clinical and nutritional variables in the patients with alcohol dependence

Damian Czarnecki, Marcin Ziółkowski\*, Ewa Żekanowska, Barbara Góralczyk, Jacek Budzyński  
Zofia Rosińska

**Introduction:** The hospitalized patients with alcohol dependence frequently have disturbances of nutritional status. The problems with the nutrition may be connected with changes of secretion of adipokines and the clinical status associated with alcoholism.

**Aim:** The purpose of the study was to assess the adipokines and clinical variables and anthropometric variables in the patients with alcohol dependence.

**Patients and methods:** The study was conducted among 59 men hospitalized in the unit of short-term therapy for addiction. In every subjects were assessed the clinical, anthropometric variables and the endogenous adipokines. The laboratory tests was performed using the ELISA.

**Results:** It was shown that the leptin concentration was lower in the patients who started treatment in hospital (with declared monthly abstinence) than in the patients after at least four weeks of hospitalization. In the patients at the beginning of hospitalization concentration of the apelin was lower than in the control group. The higher concentration of leptin was correlated with the higher BMI ( $r=0.460$ ) and the higher %FM ( $r=0.464$ ). The lower concentration of visfatin was correlated with the experiences of alcohol craving ( $r=-0.282$ ). The lower level of %FM, BMI, MAC and WHR was correlated with higher frequencies of alcohol craving.

**Conclusion:** The concentration of adipokines (leptin, apelin) are changing during hospitalization of patients with alcohol dependence. The adipokines (visfatin, leptin), selected clinical and nutritional variables are correlated with each other. It seems that the evaluate of factors such as adipokines, clinical and nutritional variables may be predictors of recovery of patient with addiction.

# **INVOLVEMENT OF NEUROINFLAMMATION IN THE EFFECTS OF TWO ETHANOL BINGE EPISODES DURING ADOLESCENCE ON CA1 HIPPOCAMPAL SYNAPTIC PLASTICITY**

Deschamps C., Vilpoux C., Naassila M., Pierrefiche O.

Binge drinking is characterised by an ethanol (EtOH) consumption in a short period of time leading to intoxication, drunkenness, blackouts or even coma. The binge drinking behaviour is commonly seen in adolescents and can be associated with memory impairment but the neurobiological mechanisms underlying EtOH-induced memory impairment remain unclear. However, memory impairment induced by intermittent EtOH exposure in young adult rats has been related to neuroinflammation in the hippocampus (*Vetreno et al., 2015*). We previously reported in hippocampus slices of male adolescent rats that 48h after Two Ethanol Binge Episodes (TEBE, EtOH, 3g/kg, i.p., 9h apart), long-term depression (LTD) of synaptic transmission - the cellular basis of learning and memory - was abolished and associated with memory impairments. Here we tested the effects of the anti-inflammatory agent minocycline (45mg/kg, i.p.), administered alone or 30 min before each EtOH exposure on LTD disruption in hippocampus slices from male adolescent rats, and we performed immunolabelling for TLR4 after EtOH. We found that minocycline alone had no effect on LTD while pretreatment completely prevented LTD abolition 48h after two binge episodes. In parallel, TLR4 expression was increased at that time point after EtOH. Our study demonstrated that synaptic plasticity impairment induced by two EtOH binge episodes during adolescence involves neuroinflammatory mechanisms.

## **The FASD Resource Center in Reunion Island : Back to 3 years of activity.**

**Bérénice Doray** 1,2,3, Barbara Delmotte 1, Karine Josse 1, Stéphanie Sotaca 1, Thierry Bafinal 1, Stéphanie Robin 4, Justine Lanneaux 4, Augustin Rousselle 4, Marilyn Tallot 4, Marie-Line Jacquemont 4, Alizé Payet 4, Lucie Rebourg 4, Sonia Henkous 4, Nathalie Penard 4, Marine Gayet 4, Agnès Cudenet 4, Michel Spodenkiewicz 4,5,6

1 – Centre Ressources ETCAF Fondation Père Favron – CHU de La Réunion – France

2 – CIC 1410 - CHU de La Réunion – France

3 - Centre de Référence Anomalies du Développement et Syndromes Malformatifs Sud-Ouest Occitanie Réunion – Site Constitutif de La Réunion – France

4 - Centre Diagnostic ETCAF – CHU La Réunion – France

5 – Pôle de Santé Mentale, CHU Réunion, Saint-Pierre, La Réunion, France

6 – CEPOI EA 7388, UFR Santé, Université de la Réunion, Saint-Pierre, La Réunion, France

Concerning 1 per 100 births, Fetal Alcohol Spectrum Disorders (FASDs) constitute a major but preventable cause of neurocognitive disorders and social maladjustment. Nevertheless, screening, diagnosis and management of patients and families remain difficult, due to a lack of knowledge of this condition by the various professionals concerned but also difficulties of access to care of these patients.

With the highest rate of FASDs in France (Public Health France, 2018), Reunion Island was selected in 2015 as a pilot region for prevention, screening, diagnosis and care for FASDs (Interministerial Mission against Drugs and Addictive behavior MILDECA 2013-2017 plan). The FASD Resource of Reunion Island constitutes the central link of this experimental Action Plan against FASD. It is funded by the Regional Health Agency of Indian Ocean (ARS-OI) and MILDECA and managed by the medicosocial Foundation “Père Favron” in partnership with the University Hospital. Its missions are multiple : 1 - to identify and put together the different actors of health, medico-social and social sectors, but also of education and Justice, to coordinate the formation of the different professionals and the information of the general public , 2 – to facilitate the diagnosis and care of the families in synergy with regional health networks about perinatality and addiction and the new FAS diagnosis center at the University Hospital, 3 - and finally to promote research with the creation of a cohort of patients.

After 3 years of activity and the training of more than 4000 students and 2000 professionals, the setting up of questionnaires and standards for professional use, a synergy between the actors of health, medico-social, social of the National Education and Justice has been created, allowing the identification of families and, in connection with the Center Diagnosis FASD, the diagnosis of about 150 patients. This cohort is a unique source in France of malformative, neuro-cognitive-behavioral and socio-demographic data. It is based on a biological collection in order to propose an integrative approach of the neurobiological mechanisms as genetics (presence of genomic variations in 13% of the patients) and epigenetics (search for a specific methylation profile for early screening).

Our region has developed a device that responds point by point to the recommendations of the new National Action Plan against Addictions MILDECA 2018-2022. It could be a model for setting up other centers, both in France and in other overseas regions.

## SUPPRESSION of ETHANOL INDUCED NEUROINFLAMMATION BY PPAR- $\gamma$ AGONISTS IN AN ANIMAL MODEL OF FASD

P.D. Drew, J.W. Johnson, T. Rafferty, C.J.M. Kane

Maternal alcohol consumption can lead to developmental maladies associated with Fetal Alcohol Spectrum Disorders (FASD). FASD is a leading cause of mental retardation and is associated with substantial lifetime disabilities. Unfortunately, there is no effective pharmaceutical treatment. Thus, the need for new therapeutic strategies to mitigate the consequences of FASD is of great importance. Using our third trimester-equivalent mouse model of FASD in which mice are treated with 4 g/kg ethanol per day via intra-esophageal gavage on postnatal days 4-9, we showed that ethanol produces prevalent neuronal and glial cell loss in the developing brain. Further, surviving microglia undergo a morphological change to an activated pro-inflammatory phenotype. This is accompanied by an increase in expression of cytokines and chemokines associated with neurodegeneration, neuroinflammation, and neuropathology. We further demonstrated that the FDA-approved PPAR- $\gamma$  agonist pioglitazone can attenuate ethanol-induced cellular toxicity, microglial morphological change, and expression of inflammatory molecules. This suggests that PPAR- $\gamma$  agonists may hold therapeutic potential for those affected by FASD. We also evaluated the effect of the PPAR- $\gamma$  agonist docosahexaenoic acid (DHA) on ethanol-induced neuroinflammation. DHA is an  $\omega$ -3 fatty acid that possesses anti-inflammatory activity, and is abundant in the brain. It is available in dietary sources, fish oil, and commercial baby formula. We treated postnatal mice with DHA 1-2 hours prior to ethanol treatment. Brain tissue was harvested on postnatal day 10 and gene expression was quantified. DHA suppressed ethanol induced expression of pro-inflammatory molecules including the cytokines IL-1 $\beta$  and TNF- $\alpha$  in the brain. Thus, PPAR- $\gamma$  agonists including DHA and pioglitazone are identifying new mechanisms of alcohol-induced brain pathogenesis. Further study is needed to evaluate their safe and effective use for treatment of the neuropathological consequences of FASD. (Supported by NIH AA026665, AA024695, AA027111)

## **Alcohol-related abnormalities in the early postnatal period can be corrected by multitarget low-affinity agonist of sigma-1, MT1 and MT3 receptors**

DURNEV Andrei

Federal State Budgetary Institution "Research Zakusov Institute of Pharmacology"

Alcohol intake leads to negative reproductive outcomes in men and complications in pregnancy followed by behavioral and physiological alteration in offspring. Previous studies showed the ability of fabomotizole (Afobazole®), a low-affinity agonist of sigma-1, MT1 and MT3 receptors, developed for treatment generalized anxiety disorder, to prevent ethanol-induced DNA damage in embryonic cells and fetal abnormalities. The aim of the work was to evaluate possibility of pharmacological treatment of ethanol-induced early postnatal disorders in outbred rat offspring from chronic ethanol-exposed male rats (CEE) or prenatal ethanol-exposed female rats (PEE).

In CEE model male rats had 10% v/v ethanol as the only source of liquid for 24 weeks. Sperm morphology was examined in stained slides (100×magnification, 200 sperms/rat). In PEE model dams had ethanol (4.3 g/kg/ day, 40% v/v, orally) from 10th to 19th day of pregnancy and were pretreated with fabomotizole (1-10 mg/kg, orally, daily) 15 min prior to ethanol. Newborns from rats after CEE and PEE were evaluated for unconditional reflexes formation ("turning on the plane" and "avoiding the edge" tests) and muscle tone ("horizontal rope" test) on 5th day of life.

CEE model resulted in significant increase of sperm abnormalities, however no changes in sensory-motor reflexes and muscle strength in offspring were revealed. In PEE newborns the main indices of reflexes and muscle tone were reduced by 1,5-2 times. Fabomotizole at anxiolytic doses prevented alcohol-induced neurodevelopmental damage.

Thus, early postnatal abnormalities in rats exposed to ethanol in utero can be corrected by fabomotizole perhaps due to cytoprotective, neuroprotective and antioxidative properties.

L.Fabry, A. Rogiers, C. Hanak

“Alcohol and you?” A two day assessment program with a multidisciplinary team

Introduction :

The prevalence of alcohol use disorders in Europe is 7,5% according to WHO data in 2010. Based on the health survey of L. Gisle and collaborators in 2013, the incidence of alcohol abuse in Belgium is 10%. In another Belgian survey in 2013, it is mentioned that 90% of alcohol abusers do not get specialized help. However, it is known that early intervention and ease of access to specialized care gives a better prognosis for people with alcohol dependence issues. This is also the clinical observation shared by our team, the alcohol rehabilitation center in CHU Brugmann (Unit 72). Hospitalized patients for withdrawal frequently arrive with few therapeutic levers. For example, their family is often exhausted by their long course of alcohol dependence. Patient resources are diminished in financial, social, professional and cognitive terms. And the negative impact of alcohol abuse on neurocognitive functions does not facilitate the patients ability to combat addiction.

Description of our program :

We have created a two-day program whose goal is to inform participants and assess both their physical and psychological health (in relation to alcohol use ). Our program is open to anyone concerned about his drinking behavior. Our primary objectives are to inform persons who are ambivalent about their alcohol consumption and to refer them if necessary to the appropriate services that could provide them with adapted assistance. Our aim is also preventing the development or aggravation of dependence and to favor intervention as early as possible with an accessible and attractive program.

Our program consists of an evaluation of alcohol consumption via a psycho-medical assessment, providing clear information, while being attentive to the persons. We want to provide participants with a complete assessment of their consumption. To do this, our method of communication and animation are based on Motivational Interviewing. We emphasize the importance of follow-up afterwards, our program has the objective to motivate and to incentivize the setup of a personalized care or prevention project.

We hope to be able to answer any questions clearly and to allow participants to discuss and think about their alcohol use in complete confidentiality and without any judgment.

# The role of Alcohol on Iron metabolism and Erythropoiesis in Acute and Chronic Alcohol Mouse model

Faraz Faiza<sup>1</sup>; Peccerella, Teresa<sup>1</sup>; Mueller, Sebastian<sup>1</sup>, Scheller-Wendorff, Marina<sup>2</sup> and Rausch, Vanessa<sup>1</sup>

<sup>1</sup>Center for Alcohol Research and Salem Medical Center, University of Heidelberg, Heidelberg, Germany

<sup>2</sup>Department of Medicine V, Hematology, Oncology and Rheumatology, University Hospital Heidelberg, INF 410, 69120 Heidelberg, Germany

**Background and Aims:** Chronic alcohol consumption leads to multiple illnesses as well as to deleterious effects on hematopoiesis. However, little is known about alcohol effect on erythropoiesis. Our aim was to investigate the relationship between alcohol consumption, iron overload and erythropoiesis.

**Methods:** The effect of ethanol ingestion on erythropoiesis was determined in male C57BL/6 wild-type mice (8 weeks old) treated with 2 gavages of alcohol 31.5 v/v (acute group), 20% alcohol in drinking water for 4 weeks (chronic group) and control group was given normal drinking water followed by 2 gavages of maltodextrin (45% w/v). At the end animals were sacrificed and peripheral blood, spleen, kidney, liver, and bone marrow were collected. Erythroid differentiation and erythroid maturation was analyzed by flow cytometry. Hpcidin, SMAD6, SMAD7, and HO-1 mRNA levels from liver and spleen were assessed by qRT-PCR and STAT3 and ferroportin by western blot. Paraffin embedded sections were also histologically analyzed.

**Results:** We observed reduced numbers of RBCs along with reduced cellularity in bone marrow, splenomegaly and increased liver weight in both short and long term alcohol mouse models. Number of megakaryocyte-erythroid progenitors (MEPs) was drastically reduced in acute group suggesting impaired early stages of erythropoiesis. However, in chronic ethanol exposure a high number of proerythroblast (Ter119<sup>neg</sup> CD71<sup>high</sup>) and low number of late erythroblasts (Ter119<sup>high</sup> CD71<sup>med</sup>) was detected. Acute as well as chronic alcohol exposure led to significant hepcidin suppression accompanied by suppression of SMAD6 and 7 mRNA and an induction in HO-1 levels suggesting heme degradation. Ballooned hepatocytes and a large number of erythrocytes were observed in liver during histological analysis.

**Conclusion and Outlook:** Hematopoietic tissues displayed a dramatic increase in early erythroblast numbers, but these fail to differentiate. This was accompanied by disturbances in systemic iron homeostasis mediated by hepcidin suppression in the liver.

# **Alcohol use disorder and comorbid depression: a randomised controlled trial investigating the effectiveness of supportive text messages in aiding recovery**

Conor K Farren, H O Reilly, A Hagerty, A Farrell, D MacLoughlin

*Department of Psychiatry, Trinity College Dublin, St Patrick's University Hospital, Dublin 8, Ireland*

**Aim:** The aim of this randomised controlled trial was to examine the impact of daily supportive text messages over a six-month treatment period on mood and alcohol consumption in individuals with a dual diagnosis of alcohol use disorder (AUD) and depression following completion of an inpatient treatment programme.

**Method:** 95 adult participants with AUD and comorbid depression were recruited. The intervention group (n=47) received twice-daily supportive text messages over 6-months while control participants (n=48) had treatment as usual for a 6-month period, with an added 6-month post-treatment follow-up for both groups. Drinking history in the previous 90 days as well as symptoms of depression, anxiety and stress were measured at baseline, 3- and 6-month treatment points and 6-month post treatment follow up.

**Results:** Depression scores ( $p = 0.02$ ) and perceived stress scores ( $p < .01$ ) were significantly reduced at 3-month treatment point in the intervention group relative to control participants with small to medium effect. The intervention group also showed a significantly greater reduction in units per drinking day from baseline to 6-month treatment point compared to the control group with a medium effect size ( $p = 0.03$ ). There were no differences in drinking or mood measures at 6-month post treatment follow-up.

**Conclusions:** Supportive text messages provide an early initial benefit in decreasing symptoms of depression and stress, with a further positive impact on alcohol consumption following a longer treatment period. Benefits did not persist six months after the intervention ended.

## **HISTORICAL KEYPOINTS IN THE CONCEPT, DEFINITION and PATHOGENESIS OF ALCOHOLIC CARDIOMYOPATHY.**

Fernández-Solà J<sup>1</sup> , Guitart-Mampel M<sup>2</sup>, Ferrer-Curriu G<sup>3</sup>, Tobias-Baraja E<sup>2</sup>, Garrabou-Tornos G<sup>2</sup>, Planavila A<sup>3</sup>.

- 1.- Alcohol Unit Hospital Clínic. IDIBAPS. University of Barcelona.
- 2.- Mucle & Mitochondria Research Group. CELLEX-IDIBAPS. University of Barcelona.
- 3.- Department of Biochemistry & Molecular Biology. University of Barcelona.

**BACKGROUND:** Alcoholic Cardiomyopathy (ACM) is at present a well defined clinical and pathological entity. However, over the years many previous doubts have emerged concerning to its existence, definition and physiopathological concept.

- AIM:** 1) To review historical key points in the establishment of concept and definition of ACM.  
2) On this basis, to perform a future overview projection on how to prevent future ACM in chronic consumers

**METHODS:** We analyze 12 different critical historic points on the scientific knowledge on ACM

- 1.- Hippocrates' recognition.
- 2.- First clinical modern descriptions.
- 3.-Ethanol itself or ethanol contaminants cause ACM?
- 4.-The nutritional hypothesis.
- 5.- Ethanol or acetaldehyde?
- 6.- The dose-dependent relationship between alcohol and heart function.
- 7.- ACM in women. The same as men?
- 8.- ACM and control drinking
- 9.- The multisite pathogenic hypothesis.
10. Heart Remodeling in ACM.
- 11- The heart's secretor role: alcohol and cardiomyokines.
- 12.- How to prevent future ACM in chronic consumers?

**CONCLUSIONS:** After the Hippocrates' definition of alcoholic cardiomyopathy, its modern clinical recognition delayed more than one millennium. Their pathogenic bases just have emerged 100 years ago and are still on construction. Possible effective pathogenic intervention are just planned for nearly future.

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# **N-acetylcysteine alters GLT-1 and $\Delta$ Fos B expression in the dorsolateral striatum of long-term ethanol-experienced rats during the abstinence period**

Fernández-Rodríguez S, Espósito C, Granero L, Polache A, Cano-Cebrián MJ, Zornoza T

Department of Pharmacy and Pharmaceutical Technology and Parasitology  
University of Valencia

Alcohol Use Disorder (AUD) is a chronic and recidivant neurobehavioural disorder which supposes a serious health as well as economic problem worldwide. Nowadays, relapse prevention is considered the main target for therapies against drug addiction, but after decades of research, few drugs have been marketed for this purpose. In the last decades, deregulation of glutamate homeostasis has been postulated as one of the critical points in cue-induced relapse. In previous research, our laboratory evidenced that N-acetylcysteine (NAC), a safe and well-tolerated marketed drug, is able to block the Alcohol Deprivation Effect (ADE) in long-term ethanol-experienced rats, but the mechanism underlying its anti-relapse efficacy is complex and still remains unclear. We hypothesized that the anti-relapse effect displayed by NAC could be related to a normalization of glutamatergic adaptations triggered by continuous ethanol experience. In the present research, we explored the expression of glutamate type 1 transporter (GLT-1) and  $\Delta$ Fos B (a transcription factor that is related to the mechanisms by which addictive drugs produce stable changes in the brain) in the dorsolateral striatum, a region implicated in the addiction process through the control of habit formation. During the fourth abstinence period, 30 male Wistar rats were subcutaneously administered 0, 60 or 100 mg/kg NAC once daily during 14 days. Animals were sacrificed before ethanol reintroduction and Western Blot analysis was performed. The obtained results may suggest a plausible mechanism for NAC previously demonstrated anti-relapse efficacy.

# HCC alcohol exposure inhibits the suppressor of tumor SLAMF3

Fouquet G., Marié C., Papillon C-A, Nguyen-Khac E., Naassila M., Bouhlal H., Marcq I.

**Background and Aims:** HepatoCellular Carcinoma (HCC) is one of the most frequent cancer worldwide and the fourth one in cancer-related deaths. One of the main important etiology of HCC is chronic alcohol consumption. A study from Costentin *et al.* described a decrease of global survival in alcoholic HCC patients compared to other etiologies. Interestingly, we identified in our laboratory, a receptor, SLAMF3 at the surface of hepatocytes. The expression of SLAMF3, a member of Signalling Lymphocytic Activation Molecules family, is lost in cancerous hepatocytes compared to healthy cells. SLAMF3 overexpression in HCC cells induces tumors regression in a xenograft model, which was explained in part, by the decrease of MAPK pathway activity. Furthermore, alcohol consumption is known to induce the MAPK pathway activation. In this context, we investigated the effects of alcohol exposure on the tumor suppressor effect of SLAMF3 and signalling pathways implicated in these mechanisms.

**Method:** HCC cell lines were exposed to increased concentrations of alcohol (0 to 160mM). Effect of alcohol on SLAMF3 expression was analyzed by flow cytometry. SLAMF3 expression was also studied by RTqPCR in HCC patients from different etiologies.

**Results:** We showed that alcohol exposure decreased SLAMF3 expression. Simultaneously, we observed a significant decrease of SLAMF3 expression by RTqPCR in alcoholic patients compared to patients with other etiologies.

**Conclusion:** We revealed the involvement of alcohol in the loss of expression of the tumor suppressor SLAMF3 in HCC. This observation might explain the aggressiveness of alcoholic HCC compared to others etiologies.

# Perceptions of alcohol use during pregnancy in France, Spain and Portugal - a cross-cultural qualitative study

1. Renata Franco, 2. Belén Charro & 3. María Raul Xavier

1. Department of Psychology, Institut Catholique de Toulouse, 31 rue de la Fonderie - B.P. 7012 | 31068 Toulouse Cedex 7, France. Mail: [fran\\_re@yahoo.com.br](mailto:fran_re@yahoo.com.br)
2. Department of Psychology, Universidad Pontificia Comillas (Madrid), Rua C. Alberto Aguilera 23, 28015, Madrid Mail: [bcharro@comillas.edu](mailto:bcharro@comillas.edu)
3. Universidade Católica Portuguesa, CEDH -Centre for Studies in Human Development, Faculdade de Educação e Psicologia, Rua Diogo Botelho, 1327, 4169-005 Porto, Portugal. Mail: [mxavier@porto.ucp.pt](mailto:mxavier@porto.ucp.pt)

**Background and aims:** Considering children prenatally exposed to alcohol present substantial challenge to parents, schools, and societies and considering minimum safe dose of alcohol during pregnancy is unknown, WHO, EU and different countries suggest zero consumption. Despite, research shows that there is a substantial number of women who continue to drink.

Taking into consideration that information is needed to make an informed decision about alcohol use during pregnancy, understanding the accessibility and quality of information available to pregnant women is an issue for research. This work presents a qualitative study exploring attitudes of Portuguese, Spain and French pregnant women regarding alcohol use during pregnancy, knowledge about the impact of alcohol use during pregnancy, accessibility and quality of information available.

**Methods:** Semi-structured interviews were conducted with 20 French (Toulouse), 19 Portuguese (Porto) 30 Spanish (Madrid) pregnant women. Interviews were audio recorded and transcribed verbatim. Data were qualitative analyzed using a semi-inductive approach. Theoretical saturation was achieved in both groups.

**Results:** six of the twenty French, six of the nineteen Portuguese and nine Spanish pregnant women reveals to drink at some point during pregnancy: in both countries during festive events. Pregnant women (French, Portuguese and Spanish) described mixed messages and confusions about consequences of alcohol consumption during pregnancy. In Portugal, participants reported several limitations concerning accessibility and quality of information available for pregnant women and social pressure to drink in festive occasions. French and Spanish participants argued that it is easy to find information related to alcohol and pregnancy.

# A MULTI-LEVEL APPROACH FOR PREVENTION OF UNDERAGE DRINKING ON CALIFORNIA INDIAN RESERVATIONS: EFFICACY AND MECHANISM OF BEHAVIOR CHANGE IN ADULTS AND ADOLESCENTS.

D. Gilder, J. Geisler, J. Luna, D. Calac, J. Lee, R. Moore, C. Ehlers

The Scripps Research Institute, Molecular and Cellular Neuroscience Department, 10550 North Torrey Pines Road, TPC-32, La Jolla, CA 92037 USA

**Purpose:** The individual and community level interventions of a successful multi-level prevention of underage drinking program were assessed for efficacy and mechanism of behavior change in a community sample of reservation dwelling Southern California American Indian adults and adolescents.

**Methods:** The four interventions were: (1) youth participatory community mobilization to develop a billboard message, (2) convenience store checks to discourage alcohol sales to minors, (3) Motivational Interviewing (MI) vs. Psycho-education (PE) in 112 youth, and (4) 298 community education and outreach events to adults and youth aimed at preventing underage drinking. One hundred and twenty adults and 100 youth were surveyed. Frequency analysis, Wilcoxon signed ranks tests, and logistic regression were used to analyze survey results.

**Data and Results:** Adults (awareness of the intervention, took action to reduce teen drinking as a result of that awareness): Billboard (49%, 75%), Store Checks (77%, 75%), MI vs. PE (63%, 80%), Outreach (65%, 76%). Relative strengths of interventions (z score, p-value): Outreach > Billboard (2.52, 0.012), Outreach > MI vs. PE (2.70, 0.007), no other differences. Mechanism of behavior change associated with the overall program (OR, p-value): movement from Precontemplation to Contemplation with awareness of problem (6.04, 0.001), from Precontemplation to Contemplation with considering change (1.94, NS), from Contemplation to Preparation (10.80, 0.001). Teens (awareness of the intervention, reduced drinking as a result of that awareness, doesn't drink): Billboard (31%, 30%, 61%), Store Checks (68%, 32%, 52%), MI vs. PE (61%, 32%, 52%), Outreach (74%, 29%, 58%). Relative strengths of interventions (z score, p-value): Outreach > Billboard (2.11, 0.035), no other differences. Mechanism of behavior change associated with the overall program (OR, p-value): movement from Precontemplation to Contemplation with awareness of problem (18.1, 0.006), from Precontemplation to Contemplation with considering change (9.5, 0.027), and from Contemplation to Preparation (9.5, 0.027).

**Conclusions:** All four interventions were associated with high levels of awareness and intervention to reduce teen drinking (adults) and reducing drinking (teens). With some exceptions, perceived strengths of interventions were similar. For both adults and teens, interventions were associated with movement along the transtheoretical model of stages of behavior change.

Support: National Institute on Alcohol Abuse and Alcoholism (NIAAA) grant R01 AA023755.

## INCIDENT NEOPLASIA AMONG HEAVY ALCOHOLICS: RELATIONSHIP WITH BODY COMPOSITION

González-Navarrete Lourdes, Ribot-Hernández Iván, Vera-Delgado Víctor, Martín-González Candelaria; Sánchez-Pérez M<sup>a</sup> José, Alvisa-Negrín Julio, Martínez-Riera Antonio; González-Reimers Emilio.

Servicio de Medicina Interna. Hospital Universitario de Canarias. Tenerife, Canary Islands (Spain)

In a series of 408 alcoholic patients consecutively admitted to the hospitalization unit of the Internal Medicine Service and followed up for 12 years we observed that the proportion neoplasia among cirrhotic and non-cirrhotic was similar (21.20 % among cirrhotic and 21.43% among non-cirrhotic ( $\chi^2=0$ ). Most cancer affected the oropharyngeal area (28.57%), colon (20.24 %) and prostate (13.10 %). Fourteen patients developed a multiple neoplasia. Of the 87 patients with cancer, 14 were already diagnosed with a neoplasm when they entered the study, whereas 73 developed an incidental neoplasia along the study period. The incidence of neoplasia was not related to ethanol consumption, the presence of liver cirrhosis, the consumption of tobacco or liver function impairment, but with age over the median (LR=5.94; p=0.015). In addition, altered body composition (assessed in 313 patients by total body densitometry) was related with the time at which neoplasia developed, so that patients with trunk fat below the median (LR=3.59; p=0.058; B=4.06; p=0.044), total BMD over the median (LR=3.32, p=0.066; Breslow=4.22; p=0.04), or lumbar t-score over the median (LR=6.31; p=0.012; Breslow=6.93; p=0.008) developed cancer earlier, especially among the 149 cirrhotic (LR=7.16; Breslow=9.71; p<0,001) . These results are similar to those observed among women with breast cancer, but to our knowledge, they have not been reported among alcoholics.

## Neural responses to multisensory alcohol cues in heavy-drinking smokers

**Authors:** Kimberly Goodyear, Ph.D., Robert M. Swift, M.D., Ph.D., Lorenzo Leggio, M.D., Ph.D., and Carolina L. Haass-Koffler, Pharm.D.

**Corresponding Author:** Kimberly Goodyear, 121 South Main Street, Providence, RI, 02903, 401-863-6626, kimberly\_goodyear@brown.edu

**Background:** Approximately 80% of individuals with an alcohol use disorder (AUD) are also cigarette smokers, and despite previous research on functional magnetic resonance imaging (fMRI) cue-reactivity, the behavioral and neural responses to alcohol cues in heavy-drinking smokers have not been investigated.

**Study Objectives:** The goal of this pilot study was to examine the effects of visual and olfactory alcohol cues on blood-oxygen-level-dependent (BOLD) activity in heavy-drinkers during fMRI scan.

**Methods:** Heavy-drinking smokers ( $n = 10$ ) participated in the alcohol fMRI cue-reactivity task. We implemented an alcohol cue-reactivity task, where participants, after being exposed to alcohol and neutral cues (visual and olfactory), rated their craving for alcohol and cigarettes with visual analog scales.

Independent samples  $t$ -tests were implemented to compare alcohol and cigarette craving during alcohol and neutral cues. Further, whole-brain and region of interest (ROI) analyses were done to compare BOLD responses to alcohol and neutral cues. Lastly, correlation analysis was done on activation in ROIs and baseline craving and drinking and smoking behaviors.

**Results:** Our behavioral results showed that participants had higher alcohol craving during alcohol cues compared to neutral cues ( $p < .05$ ). Further, our whole-brain analysis revealed significant activation in the right lingual gyrus ( $p < .005$ ). The ROI analysis showed significant activation in the right orbitofrontal cortex (OFC) ( $p < .05$ ) when comparing alcohol to neutral cues. Correlation analysis indicated that there was a positive associations with baseline alcohol craving and activation in the right ventral striatum (VS) ( $p < .05$ ) and the left anterior cingulate cortex (ACC) ( $p < .05$ ). There were also positive associations with total alcohol drink in the ninety days prior to the experiment and activation in the right VS ( $p < 0.0001$ ), left VS ( $p < .01$ ) and left ACC ( $p < .0001$ ).

**Conclusions:** We have provided preliminary evidence that there are distinct behavioral and neural patterns in response to alcohol cues in heavy-drinking smokers.

## **A Polymer-curcumin conjugate ameliorates the neuroinflammation associated with chronic alcohol treatment in mice**

Cuesta CM<sup>1</sup>, Ibañez F<sup>1</sup>, Lopez-Hidalgo R<sup>1</sup>, Urena J<sup>1</sup>, Duro-Castaño A<sup>1</sup>, Armiñán<sup>1</sup> A., Vicent MJ<sup>1</sup>, Pascual M<sup>1,2</sup> and Guerri C<sup>1</sup>, <sup>1</sup>Príncipe Felipe Research Center, Valencia (Spain) and <sup>2</sup>Physiology Department, University of Valencia, Valencia (Spain).

Several evidences demonstrated that alcohol, by activating the brain immune receptors TLRs and NLRs, can induce inflammatory mediators and cytokines/chemokines triggering neuroinflammation and brain damage. Therefore, it is important to develop effective therapies to reduce or ameliorate the neuroimmune system activation. Considering that curcumin has important anti-inflammatory and antioxidant properties, but low bioavailability, we have used a polymer-curcumin conjugate (PCC) derivatised to be able to cross the blood-brain barrier through the LRP-1 receptor in order to block neuroinflammation. The conjugation of curcumin to a biodegradable polymeric carrier enhances curcumin efficiency and controls drug release by the presence of bioresponsive polymer-drug linkers (pH-labile esters). We used glial cells in culture incubated with and without ethanol and in the presence or absence of PCC. For the *in vivo* experiments, mice treated with or without ethanol during two months were administered PCC intravenously, two times/week. *In vitro* results experiments demonstrated that PCC is not toxic for glial cells and protects against ethanol-induced cell toxicity. Our *in vivo* result shown that PCC administration protects ethanol-induced the up-regulation of inflammatory mediators (TLR4, iNOS, COX-2, IL-1 $\beta$ , fractalkine), in prefrontal cortex and in medial cortex of chronic ethanol mice. We further observed that chronic ethanol-treatment significantly up-regulated some miRNAs (miRs 146a-5p and let-7b-5p) that modulate neuroinflammation in the medial cortex. PCC administration supresses' ethanol-induced changes in these miRNAs. In summary, our results support the beneficial effects of PCC administration by attenuating the neuroinflammation associated with chronic alcohol abuse. (Supported by SAF2015-69187R)

***Ocimum sanctum* suppresses alcohol abstinence-induced depression-like behavior through regulation of biochemical and *GRIN2A*, *GRIN2B* gene expression of NMDA receptor signaling in rats**

Gupta Girdhari Lal<sup>1</sup> and Sharma Anamika<sup>2</sup>

<sup>1</sup>Department of Pharmacology, Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, Deemed to be University, Vile Parle (W), Mumbai-400 056, Maharashtra, India.

<sup>2</sup>Department of Pharmacy, Jaypee University of Information Technology, Wagnaghat-173 234, Solan, Himachal Pradesh, India.

India's Queen of herbs Tulsi (*Ocimum sanctum* Linn., family Labiatae) have huge medicinal uses and traditionally being used for the treatment of alcohol disorders. However, its underlying mechanism(s) of action have not been adequately addressed. Therefore, we evaluated the effect of *Ocimum sanctum* in alcohol abstinence-induced depression, developed following long-term voluntary alcohol intake in rats. The hydro-alcoholic extract of *Ocimum sanctum* leaves (EOS) was first characterised for the presence of oleanolic acid (0.54% w/w), eugenol (0.39% w/w) and caryophyllene (0.02% w/w) and subsequently acute, sub-acute toxicity studies were also performed. For evaluation of the effects of EOS in ethanol abstinence syndrome, healthy Wistar rats were enabled to voluntary drinking of 4.5%, 7.5% and 9% v/v alcohol for fifteen days. The behavior studies were conducted employing tail suspension test and forced swim test on day 16<sup>th</sup>, 17<sup>th</sup> & 18<sup>th</sup> and peak ethanol withdrawal syndrome was determined. EOS (100, 300, and 500 mg/kg) and standard drug fluoxetine were administered orally during withdrawal symptoms. Alcohol biomarkers like ALT, AST, ALP, GGT, and MCV were estimated by using commercially available kits. Serotonin concentrations were measured in the plasma, hippocampus and prefrontal cortex using the rat ELISA kit. The gene expression analysis of *GRIN1*, *GRIN2A*, and *GRIN2B* of N-methyl-D-aspartate receptors (NMDAR) subunits in the hippocampus and the prefrontal cortex were also examined by RTqPCR. The results displayed that no observed adverse effect level (NOAEL) for EOS was higher than 2000 mg/kg, orally. The deregulated levels of alcohol markers and serotonin following ethanol abstinence in the plasma, hippocampus, and prefrontal cortex were also reversed by EOS at doses 300 and 500 mg/kg. EOS exerted a significant protective effect at doses 300 and 500 mg/kg, but 100 mg/kg showed insignificant protection against alcohol abstinence-induced depression like behavior in both FST and TST. The increased expression levels of *GRIN2A* and *GRIN2B* following ethanol abstinence were also reversed with a higher dose of EOS (500 mg/kg) treatment. Thus, the results of the study reveal that EOS has a remarkable protective role in the ethanol abstinence-induced depression by modulating alcohol markers, serotonin levels, and expression of *GRIN2A*, *GRIN2B* gene of NMDAR signaling in rats.

**Title:**

Nucleus reuniens of ventral midline thalamus is highly susceptible to permanent neuron loss in the rat model of binge drinking during third trimester.

**Authors:**

Z.H. Gursky, A.Y. Klintsova

*Department of Psychological & Brain Sciences, University of Delaware, Newark, DE 19716, USA*

**Abstract:**

Individuals with fetal alcohol spectrum disorders often have difficulty performing high-demand cognitive tasks (i.e., have impaired “executive function”). Executive function is supported by communication between 2 parts of the brain: hippocampus and prefrontal cortex. We hypothesized that midline thalamic nucleus reuniens (responsible for coordinating activity between prefrontal cortex and hippocampus) could be affected by developmental alcohol exposure (AE). We use 3 AE paradigms in Long Evans rat during a period comparable to human third trimester to demonstrate the mechanism by which AE results in short- and long-term neuroanatomical damage within nucleus reuniens, and what cell types are most vulnerable.

The first paradigm, high-dose (5.25 g/kg/day) AE on single postnatal day (PD) 7 demonstrated that alcohol-induced cell loss in reuniens in adulthood is caused by alcohol-induced cell death in males and females. Additionally, AE reduced reuniens volume. The second paradigm, high-dose AE on PD4-9 caused a selective loss of neurons, but not non-neurons, reducing neuron-glia ratio, as well as a reduction in volume of reuniens. No alterations were observed in the neighboring rhomboid nucleus. The third paradigm examined the impact of high-dose (5.25 g/kg/day) and moderate-dose (3.00 g/kg/day) AE on reuniens. We observed significant neuron loss in both sexes at both doses, but volume was only reduced following high-dose AE (replicating and extending findings from both prior paradigms). Neither dose of alcohol altered the number of microglia in reuniens. Taken together, these experiments indicate that reuniens is highly susceptible to damage, over various levels of drinking, even following brief exposure.

# Chemogenetic Manipulation of Nucleus Accumbens and Insula Activity Modulates Alcohol Consumption in Rats

Haaranen, Mia (1); Schäfer, Annika (1); Häkli, Sara (2); Hyytiä, Petri (1)

(1) University of Helsinki, Faculty of Medicine, Department of Pharmacology

(2) University of Eastern Finland

Functional brain imaging in humans and rodents has implicated two brain regions in the development and maintenance of alcohol use disorders (AUDs), the nucleus accumbens (Acb) and the anterior insula (Ai). Both structures are part of the mesocorticolimbic reward system: The Acb is located in the striatum and has been associated with mediating emotionally rewarding sensations elicited by natural rewards as well as drugs of abuse. The Ai is a cortical structure implicated in the generation of interoceptive cues and decision making during goal-directed actions.

To characterize the functional role of Acb and Ai in the regulation of voluntary alcohol consumption, we used chemogenetic manipulation of neuronal activity through designer receptors exclusively activated by designer drugs (DREADDs). The viral construct carrying either an excitatory (G(q)-) or inhibitory (G(i)-) DREADD was injected bilaterally into the Acb or Ai of alcohol-preferring AA (Alko, Alcohol) rats trained to voluntarily self-administer alcohol (intermittent drinking paradigm, 2-bottle choice test, 2h access to 10% EtOH qad). After a four week expression time, DREADDs were activated by clozapine-N-oxide application (CNO, 10 mg/kg, ip). Neuronal Acb activation resulted in an increase in alcohol consumption while neuronal silencing lead to a decrease of alcohol intake. Neuronal Ai stimulation produced a decrease in alcohol drinking. Neuronal silencing did not show a change in drinking habits. Water consumption was not affected in either of the groups.

As the Ai has direct afferent connections to the Acb, we decided to additionally examine the effect of pathway specific manipulation targeting the connections originating from the Ai and terminating in the Acb. Here, Cre-dependent DREADDs were injected into the Ai while the corresponding Cre-factor was applied to the Acb. The results showed a statistically significant increase in alcohol consumption after CNO administration in the excitatory G(q) group while drinking in the G(i) group remained unaltered.

The results presented here show that both Acb and Ai contribute to voluntary alcohol consumption. However, while activation of Acb or the Ai->Acb projection increased alcohol intake, the Ai activation decreased it. This suggest that Ai may partly provide the Acb with the excitatory input enhancing alcohol drinking, whereas the effects of Ai stimulation are mediated by other, still unknown circuits.

## **(±)-baclofen in alcohol use disorder: identification of responders and of the role of dopamine release in the nucleus accumbens in the efficacy of the different enantiomers**

J. Jeanblanc<sup>1</sup>, V. Echeverry-Alzate<sup>1</sup>, P. Sauton<sup>1</sup>, V. Jeanblanc<sup>2</sup>, M. Naassila<sup>1</sup>.

<sup>1</sup>GRAP – UMR INSERM 1247, Université de Picardie Jules Verne, Amiens, France.

<sup>2</sup>Plateforme animalerie PlatAnN, Université de Picardie Jules Verne, Amiens France.

Studies to evaluate the efficacy of (±)-baclofen (the racemic R(+) and S(-) form) in the treatment of alcohol dependence have yielded mixed results and lively debate about the benefit/risk ratio at the international level. Recent studies have suggested that different enantiomers may help to explain, at least in part, the contrasting results and the great variability in treatment response. We investigated the effectiveness of each of the enantiomers on self-administration of alcohol in either alcohol-dependent rats or binge drinker rats. We have shown that the R(+) form is more effective in reducing alcohol consumption, craving and relapse than the racemic form and at a lower dose (1.5 mg / kg vs. 2 mg / kg). Almost 30% of rats significantly increased their alcohol consumption (+ 50%) after the administration of either the racemic or the S(-) forms of baclofen. R(+)-baclofen only leads to a sharp decrease in alcohol consumption in both rat populations. We also found that the racemic and the R(+)-baclofen are both more efficient in males than in females rats. Finally, using the fast cyclic voltammetry technic on nucleus accumbens containing brain slices, we found that the racemic and the R(+)-baclofen reduced the DA release whereas the S(-)-baclofen increases the release of dopamine. Therefore, the S(-)-baclofen seems to induce opposite effects both on the behavior and the dopaminergic release than the R(+)-baclofen and thus, the R(+)-baclofen should be a better medication in order to treat AUD than the currently prescribed racemic form.

# Correlation between self-reported alcohol-intake (AUDIT-C) and PEth-concentrations in somatic patients admitted to hospitals in Oslo and Moscow

**JOERGENRUD Benedicte**

*Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway*

**Background:** AUDIT-C has traditionally been one of the most commonly used screening tools for identification of harmful alcohol use. In recent times, the use of the biomarker phosphatidylethanol (PEth) (16:1/18:0) has been applied in several clinical settings for detecting harmful alcohol use, as it corresponds directly with alcohol consumption. However, there have been few studies that investigate the relationship between AUDIT-C and PEth-concentrations. In our study we wanted to see the correlation between PEth concentrations and self-reported alcohol-consumption during the last 12 months.

**Methods:** AUDIT-C data and PEth concentrations was collected from 1897 Norwegian and Russian somatic patients during a period from 11/2016 to 12/2017. The AUDIT-C data was converted to weekly grams of alcohol consumption by multiplying drinking events (AUDIT item 1) with the average number of alcoholic units consumed in a normal drinking event (AUDIT item 2), and adding the alcoholic units from binge drinking (AUDIT item 3). We subsequently correlated the weekly consumed alcohol with PEth concentrations.

**Results:** When dividing the patient-population by country and gender we found that most patients drink from 12.8-99.9 grams of alcohol per week. We also found that men drink proportionally more alcohol compared to women on a weekly basis. Mean and interquartile range of PEth increased with higher self-reported alcohol consumption during the last 12 months, and a medium correlation effect size was found.

**Discussion:** Converting AUDIT-C scores into alcohol consumption in grams per week makes for a more practical and feasible approach in correlating with PEth concentrations. Our study showed that self-reported grams of alcohol consumed each week correlated well with PEth concentrations within two different hospital populations. However, a major limitation is that the patients self-reported alcohol consumption referred to the last 12 months, while PEth concentrations reflects alcohol use only during the last 4 weeks.

## Effect of Chronic Stress on Alcohol Consumption is Mediated by Genetics in BXD Recombinant Inbred Mice

Byron C. Jones<sup>1</sup>, Megan K. Mulligan<sup>1</sup>, Lu Lu<sup>1</sup>, Wenyuan Zhao<sup>1</sup>, Robert W. Williams<sup>1</sup>, Sonia A. Cavigelli<sup>2</sup>, Elena Terenina<sup>3</sup>, Pierre Mormède<sup>3</sup>

<sup>1</sup> The University of Tennessee Health Science Center, Memphis, Tennessee, USA

<sup>2</sup>The Pennsylvania State University, University Park, Pennsylvania, USA

<sup>3</sup>GenPhySE, Université de Toulouse, INRA, ENVT, Castanet-Tolosan, FRANCE

**Background.** The effect of stress on alcohol consumption in humans is highly variable and underlying processes are not yet understood. Attempts to model an association between stress and altered ethanol consumption in animals have not been successful. Our hypothesis is that individual differences in stress effects on ethanol consumption are mediated by genetics.

**Methods.** We measured alcohol consumption, using drinking-in-the-dark (DID) in females from two inbred mouse strains, C57BL/6J (B6) and DBA/2J (D2) and 35 of their inbred progenies (the BXD family). A control group was maintained under normal housing and a stress group was exposed to chronic mild stress (CMS), consisting of unpredictable stressors over seven weeks. Alcohol intake was measured over sixteen weeks in both groups during Baseline (preceding 5-week period), CMS (intervening 7-week period), and post-stress (final 4-week period).

**Results.** There was a strong effect of CMS on alcohol intake. A few strains demonstrated CMS-related increased alcohol consumption; however, most showed decreased intake. We identified one suggestive quantitative trait locus on chromosome 5 that contains the neuronal nitric oxide synthase gene (*Nos1*). The expression of *Nos1* is frequently changed following alcohol exposure and variants in this gene segregating among the BXD population may modulate alcohol intake in response to stress.

**Conclusions.** These results are the first to show a genetic basis for individual differences in the effects of chronic stress on alcohol consumption and nominated a likely candidate gene. Future work will involve validating *Nos1* and discovering other genes underlying stress-related alcohol consumption in humans.

# Changes in the metabolome of human post-mortem brain samples associated with excessive alcohol use

Olli Kärkkäinen<sup>1</sup>, Kati Hanhineva<sup>1</sup>, Pekka J. Karhunen<sup>2,3</sup>, Jari Tiihonen<sup>4,5</sup>, and Eloise Kok<sup>2,3</sup>

<sup>1</sup> University of Eastern Finland, Finland; <sup>2</sup> Tampere University, Finland; <sup>3</sup> Fimlab Laboratories Ltd, Finland, <sup>4</sup> Niuvanniemi hospital, Finland; <sup>5</sup> Karolinska Institutet, Sweden

**Aims:** Alcohol exposure has been shown to alter metabolite levels in the brain in rodents. Here our aim was to investigate if the brain metabolome of humans is altered in association with excessive alcohol use.

**Methods:** We analyzed frozen human post-mortem frontal cortex samples from persons with history of excessive alcohol use (n = 97) and controls (n = 107). We used non-targeted liquid chromatography mass spectrometry method for the metabolomics analyses.

**Results:** We observed differences between the study groups in the metabolite levels in the post-mortem frontal cortex samples. For example, we observed decreased levels of acetylcholine ( $p < 0.001$ ) and GABA ( $p < 0.001$ ) in the alcohol group when compared to the controls, indicating alterations in the neurotransmitter metabolism. Moreover, we observed increased levels of S-adenosyl-L-methionine (SAM,  $p < 0.001$ ) in the alcohol group when compared to the controls, indicating alterations in the methylation processes since SAM is an important cofactor in the methyl group transfer reactions.

**Conclusions:** Overall these results show that the metabolome of human post-mortem frontal cortex samples is altered in persons with history of excessive alcohol use when compared to controls.

## Effects of a Mindfulness Based Relapse Prevention Intervention on Alcohol Consumption and Gut Microbial Diversity: Preliminary Findings

Hollis C. Karoly, Sarah L. Hagerty, Raeghan L. Mueller, Angela D. Bryan & Kent E. Hutchison

Emerging research suggests that mindfulness-based interventions (MBIs) influence the inhibitory control network, which is critical to the etiology and maintenance of alcohol use disorders (AUDs). Mindfulness is associated with alterations in the microbiota-gut-brain-axis (MGBA) across numerous patient populations<sup>1-3</sup>, and gut and immune alterations especially impact brain regions involved in executive function and inhibition<sup>4,5</sup>. We compared an 8-week Mindfulness Based Relapse Prevention program to a Relapse Prevention (RP) intervention on alcohol consumption and gut microbial diversity in an AUD sample. 36 participants have completed treatment so far. From baseline to post-treatment, participants reduced monthly binge drinking ( $M=7.0$  to  $3.0$ ;  $t(60)=2.25$ ,  $p=.03$ ), total drinks ( $M=112.2$  to  $74.7$ ;  $t(62)=2.41$ ,  $p=.02$ ) and alcohol-related problems using the Alcohol Use Disorder Identification Test (AUDIT), ( $M=19.3$  to  $13.2$ ;  $t(69)=3.51$ ,  $p<.001$ ). No significant group differences were observed. Although sample sizes at 3-month follow-up are small, preliminary analyses show greater reductions for MBRP participants for total drinks ( $d=0.56$ ) and AUDIT ( $d=0.30$ ). Post-treatment, gut microbial diversity was negatively associated with AUDIT ( $r=-.288$ ,  $p=.021$ ). Change in microbial diversity pre to post treatment was negatively associated with alcohol consumption on the Alcohol Dependence Scale ( $r=-.246$ ,  $p=.050$ ), indicating that as consumption decreased, microbial diversity increased. While these preliminary analyses are encouraging, the full sample is needed to determine whether MBRP is superior to RP and whether effects are mediated in part by alterations within the MGBA. Future work characterizing the microbiota at each level of the phylogenetic tree will allow for characterization of species that are differentially altered by alcohol use and MBIs.

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# Depression mediating the effect of social support and Alcoholics Anonymous on alcohol use disorder recovery in Korea: A 2-year longitudinal study

## Author

Seon Wan Ki, MD. PhD, Department of Psychiatry, School of Medicine, Catholic Kwandong University International Saint Mary's Hospital, Korea

Il Ho Park, MD. PhD, Department of Psychiatry, School of Medicine, Catholic Kwandong University International Saint Mary's Hospital, Korea

Won Mi Jung, Department of Social Welfare, Ewha Womans University, Seoul, Korea

Jeong Seok, Seo MD., PhD, Department of Psychiatry, School of Medicine, Konkuk University, Chung-ju, Korea

Soo Bi Lee, Department of Social Welfare, Chung-Ang University, Seoul, Korea

## Purpose

In order to understand the factors contributing to the course of alcohol use disorder in South Korea, we conducted a nation-wide longitudinal follow-up study of alcohol use disorder in South Korea. The mediating effect of depression on factors influencing the recovery of alcohol use disorder was examined in this study.

## Methods

Biannual survey and clinical follow-up were conducted in patients with alcohol use disorder from the hospitals/clinics and community mental health centers representing 6 districts in South Korea between 2016 and 2017. Data of 120 individuals who complete all four surveys were analyzed. Path analysis was conducted with duration of AA participation and extent of social support system from the 1st survey as predictor variables, depression score from the Patient Health Questionnaire (PHQ-9) as the mediating variable, and Alcohol Use Disorders Identification Test (AUDIT-C) score as the dependent variable.

## Results

The degree of social support system establishment from the 1st survey negatively correlated with the depression severity in the 3rd survey. Moreover, the duration in AA from the 1st survey and the degree of depression from the 3rd survey correlated with the severity of alcohol problem from the 4th survey. The model's goodness of fit ( $\chi^2=12.927$ ,  $df=10$ ,  $P=0.228$ ,  $IFI=0.926$ ,  $CFI=0.898$ ,  $RMSEA=0.050$  (90% CI: [0.0000-0.117])) satisfied the acceptance criteria proposed by Hu & Bentler (1999). The regression coefficient from this model show that the degree of depression from the 3rd survey is decreased as the degree of social support system establishment from the 1st survey increases ( $\beta=-3.186$ ,  $P<0.01$ ). Increased severity of depression, resulting from weak social support system, increased the severity of alcohol problem ( $\beta=0.152$ ,  $P<0.01$ ). Increases in the duration in AA decreased severity of alcohol problem without the mediation of depression ( $\beta=-0.039$ ,  $P<0.05$ ). Among the control variables, the alcohol problem severity from the 1st survey showed positive auto-regression effect ( $\beta=0.311$ ,  $P<0.01$ ). When the auto-regression effect by alcohol problem was controlled, the degree of social support system establishment from the 1st

survey affected later alcohol problem through the mediation of depression.

### **Conclusion**

Adequate social support system relieves depression and improvement in depression helps the recovery process of patients with alcohol use disorder. Longer participation in AA can have a persisting effect on alleviating alcohol problem. Therefore, combining support for establishing sufficient social support system and psychosocial interventions, such as AA, is important for the recovery of alcohol use disorder. Particularly, screening and providing treatment for patients who are at high risk for depression are needed in order to achieve a successful recovery.

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# Does a glucagon-like peptide 1 (GLP-1) receptor agonist reduce alcohol intake in patients with alcohol dependence?

## Author(s):

Mette K. Klausen<sup>1</sup>(Presenter), Mathias E. Jensen<sup>1</sup>, Marco Møller<sup>1</sup>, Anne-Marie Østergaard Grindsted Jensen<sup>1</sup>, Nina le Dous<sup>1</sup>, Kamilla W. Miskowiak<sup>2</sup>, Patrick M. Fisher<sup>3</sup>, Gerda K. Thomsen<sup>3</sup>, Sabine Vollstädt-Klein<sup>4</sup>, Ulrik Becker<sup>5</sup>, Claus Ekstrøm<sup>6</sup>, Gitte M. Knudsen<sup>3</sup>, Tina Vilsbøll<sup>7</sup>, Anders Fink-Jensen<sup>1</sup>

1. Psychiatric Centre Copenhagen, Rigshospitalet, University Hospital of Copenhagen, Denmark
2. Department of Psychology, University of Copenhagen, Copenhagen, Denmark
3. Neurobiology Research Unit, Copenhagen University Hospital, Denmark
4. Department of Addictive Behaviour and Addiction Medicine, Mannheim/Heidelberg, Germany
5. National Institute of Public Health, University of Southern Denmark and Gastro unit, Medical Division, Copenhagen University Hospital Hvidovre, Denmark
6. Section of Biostatistics, University of Copenhagen, Copenhagen, Denmark
7. Steno Diabetes Centre Copenhagen, University of Copenhagen, Gentofte, Denmark

**Aims:** Glucagon-like peptide-1 (GLP-1) receptor stimulation has proven to reduce alcohol consumption in preclinical experiments with rodents and non-human primates. However, the effect of GLP-1 receptor agonists on alcohol reduction in humans with alcohol dependence has to our knowledge, not yet been investigated.

**Methods** The effect of the once-weekly GLP-1-receptor-agonist, exenatide will be investigated in a double-blinded, placebo-controlled, randomized clinical trial. 114 outpatients, age 18-70 years will be randomized to either placebo or exenatide once-weekly for 26 weeks as a supplement to cognitive behavioural therapy. *The primary endpoint* is reduction in number of 'heavy drinking days', measured by the Time Line Follow Back (TLFB) method. Secondary endpoints include changes in total alcohol consumption, days without consumption, changes in brain activity and function, smoking status, cognition, measures of quality of life and changes in phosphatidylethanol (PEth) as a biomarker of alcohol consumption from baseline to follow-up at week 26.

In addition to these clinical outcome parameters, we will explore the possible neurobiological underpinnings by use of functional Magnetic Resonance Imaging (fMRI) and the possible neuromolecular changes in striatal dopamine transporter (DAT) availability by use of the Single photon emission computed tomography (SPECT).

**Results:** 103/114 patients are recruited.

**Conclusions:** The potential as a new treatment is being tested. If successful, this could be a new revolutionary treatment for alcohol dependence.

**Financial Support:** The study is financed by *Region Hovedstadens Forskningsfond*, *Region Hovedstadens Psykiatri* and *Fonden Novavi*. The manufacturer of Bydureon<sup>®</sup>, AstraZeneca A/S, has no financial interest or involvement in this project.

## **Why me? One midline thalamic nucleus is critically important for hippocampo-prefrontal cortex communication and vulnerable to alcohol exposure during third trimester equivalent**

KLINTSOVA Anna  
(University of Delaware)

Children exposed to alcohol *in utero* display physical and behavioral irregularities, including impairment in “executive function” (EF) behaviors that require coordination between prefrontal cortex (PFC) and hippocampus (HPC). Non-human primate and rodent studies have demonstrated that the midline thalamic nucleus reuniens (Re) is essential in coordinating PFC-HPC activity, as selective Re inactivation impairs PFC-HPC synchrony and behavioral performance. To unveil the structural deficits in HPC-Re-mPFC circuitry, one needs to consider that Re is a critical intermediary with reciprocal connections to mPFC and HPC, while mPFC connects with HPC via Re.

Rodent model of binge drinking during third trimester was used in our studies: rat pups were intubated with moderate or high doses of alcohol (AE) or sham-intubated (SI) on PD4-9. Unbiased stereology was used to estimate cell death after AE and cell/neuronal loss in midline thalamus in adulthood, after the rats underwent comprehensive behavioral testing.

Our data indicate that AE during third trimester equivalent leads to 30-fold increase in cell death and cell loss ( $\approx 30\%$ ) in Re but not in neighboring thalamic nuclei (mediodorsal and rhomboid nuclei). This cell loss is driven by loss of neurons that persists into adulthood. AE rats display alterations in object-in-place memory and impairments in rule switching in a plus maze-based operant conditioning task in adulthood.

These data suggest that Re is specifically targeted by postnatal AE and that this damage persists into adulthood. The integrity of RE may be a structural indicator of impaired executive functioning observed in some manifestations of FASD.

## Tuftsins peptide analog prevents ethanol-induced cognitive impairment in aged alcohol-withdrawn rats through modulation BDNF signaling pathway

Kolik L.G., Nadorova A.V., Antipova T.A., Kruglov S.V., Kudrin V.S., Durnev A.D.  
*Federal State Budgetary Institution "Research Zakusov Institute of Pharmacology",  
Moscow, Russia*

**Background.** Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), as tuftsins analogue, is safe anxiolytic with cognitive enhancing properties. However, there is no data about its use in patients with comorbid alcohol use disorders, so, work aimed to study selank effects in early alcohol withdrawal.

**Methods.** Male albino rats were administered 10% (v/v) ethanol (EtOH) as the only source of drinking water within 30 weeks (n=20). Then EtOH-withdrawn rats were treated saline («EtOH») and selank 0.3 mg/kg, i.p. («EtOH+selank») for 7 days. EtOH-naïve age-matched rats (n=20) were treated by saline («Control») and selank («Selank»). Learning capacities were measured in novel object recognition task 24 hours after selank treatment, then rats were sacrificed. Selected brain neurochemicals were measured by HPLC, expression of BDNF protein in particular brain structures was analyzed using Western blot.

**Results.** Selank prevented decrease of discrimination index of novel object ( $p<0.05$ ) in aged EtOH-naïve and EtOH-withdrawn rats indicating its positive impact on cognitive performance. Selank restored increased 5-HT and reduced turnover 5-HIAA/5-HT in *frontal cortex*, prevented alcohol-induced increased aspartic acid, glycine and taurine levels in *hypothalamus*, GABA in *n.Acc.* and aspartic acid and glycine in *striatum*. Forced alcohol intake with subsequent withdrawal led to significant increase in BDNF level in *hippocampus* («Control»  $2.0\pm 0.2$  R.D.U., «EtOH»  $3.4\pm 0.6$  R.D.U.) and *frontal cortex* («Control»  $1.1\pm 0.2$  R.D.U., «EtOH»  $1.8\pm 0.3$  R.D.U.). Selank prevented BDNF increase in *hippocampus* and *frontal cortex*.

**Conclusions.** The obtained results indicate pronounced effect of tuftsins analog on age-related changes associated with memory impairment, accompanied by chronic alcohol intoxication possibly through modulation BDNF signaling pathway.

## **Alcohol-related mortality in the WHO European region: sex-specific trends and predictions**

Daniel König<sup>1</sup>, Nathalie Pruckner<sup>1</sup>, Andrea Gmeiner<sup>1</sup>, Barbara Hinterbuchinger<sup>1</sup>, Matthäus Fellinger<sup>1</sup>, Thomas Waldhör<sup>2</sup>, Otto M. Lesch<sup>1</sup>, Stephan Listabarth<sup>1</sup>, Andreas Wippel<sup>1</sup>, Sandra Vyssoki<sup>3</sup>, Benjamin Vyssoki<sup>1</sup>

1 Clinical Division of Social Psychiatry, Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria

2 Center for Public Health, Department of Epidemiology, Medical University of Vienna, Austria

3 Department of Health Sciences, St. Pölten University of Applied Sciences

### Introduction

According to data from the WHO Health For All Database, alcohol-related deaths have significantly decreased within the European region between 1979 and 2015. Yet, there are still pronounced differences between regions and burden of alcohol consumption and dependence remains high.

### Aims

Alcohol is an important risk factor for morbidity and mortality, especially within the European region. Differences in per capita consumption and drinking patterns are possible reasons for regional differences and diverging trends in alcohol-related health outcomes.

### Methods

For 29 countries within the WHO European region the last four decades were evaluated for trends and predictions in alcohol-related deaths using data available from the WHO Health For All Database.

### Results

Between 1979 and 2015, age-standardised death rates for both sexes due to selected alcohol-related causes decreased significantly in all included countries of the WHO European region, but regional differences were still pronounced. Assuming a similar trend in the future, the model predicted a further decrease until the year 2030.

### Conclusions

Even though alcohol-related mortality might have decreased within the last decades, the detrimental effects of alcohol consumption and alcohol dependence remain a considerable burden of disease within Europe.

This study provides information on possible reasons why some countries show greater and others show lower decreases on alcohol-related mortality. To put light on these various –and especially- influenceable factors, further research is recommended. Findings on this area, such as certain legal regulations or adequate interventions to reach potentially alcohol burdened people earlier, are from utmost importance to establish essential preventive strategies.

# **BRIEF INTERVENTION AIMED AT FETAL ALCOHOL SYNDROME PREVENTION: EFFICACY STUDY**

**Ekaterina A. Burina, Almara K. Kulieva**

**Saint Petersburg State University  
Saint Petersburg, Russia**

This study focuses on the psychological effects of brief interventions aimed at preventing Fetal Alcohol Syndrome (FAS) and Fetal Alcohol Spectrum Disorders (FASD).

The sample of the study consisted of 280 women of childbearing age: 140 women entered the experimental group and 140 – the control group. All participants were screened; a basic interview and three follow-up interviews at 3, 6 and 12 months were conducted. All women received information materials (a brochure) about the alcohol effects on the fetus and fetal alcohol syndrome. With women of the experimental group, after a baseline interview, twice in the period from 2 weeks to one and a half months, specially trained OBGYN physicians carried out a dual-focused brief intervention.

The dynamics of the actual alcohol consumption by women of childbearing age under the influence of dual-focused brief intervention and passive informing indicates a significant decrease in the frequency of alcohol consumption. At 3 months follow-up, significant differences were found between the experimental and control groups: 47% of the women in the experimental sample and 62% in the control group were at risk. After 6 months, the differences are found at the level of the statistical tendency (45% and 55%, respectively), and after 12 months no significant differences were revealed (46% and 49%, respectively), which indicates a faster effect achieved with the brief intervention method.

Thus, the results of the study indicate the effectiveness of the brief intervention designed to prevent FAS and FASD.

# What does impact health related quality of life in alcohol use disorder: cognitive deficits, anxiety or depression?

Najlaa LAHBARI\*<sup>1</sup>, Alice LANIEPCE<sup>1</sup>, Nicolas CABE<sup>1,2</sup>, François VABRET<sup>1,2</sup>, Céline BOUDEHENT<sup>1,2</sup>, Géraldine RAUCHS<sup>1a</sup>, Anne-Lise PITEL<sup>1a</sup>

(1) Normandie Univ, UNICAEN, PSL Université, EPHE, INSERM, U1077, GIP Cyceron, NIMH, Caen, France

(2) Service d'Addictologie, Centre Hospitalier Universitaire de Caen, Caen, France

\*Mail: [najlaa.lahbairi@unicaen.fr](mailto:najlaa.lahbairi@unicaen.fr)

<sup>a</sup>: Both authors contributed equally to the work.

Alcohol Use Disorder (AUD) results in multiple social and cognitive problems with a poor health related quality of life (HRQoL). The association between HRQoL and cognition is well-known in various diseases (stroke, dementia...). While HRQoL is crucial to maintain abstinence, it remains little studied in AUD. Depression and anxiety also affect HRQoL and cognition, potentially exacerbating the risk of relapse. The objective of this study was to investigate the relationships between HRQoL, cognition and mood in AUD. Thirty-three recently detoxified AUD inpatients and 28 healthy control (HC) subjects were included. An extensive neuropsychological assessment was conducted and the intensity of depressive and anxiety symptoms was measured using the Beck Depression Inventory (BDI) and the Spielberg State-Trait Anxiety Inventory (STAI). In AUD patients, HRQoL was evaluated using the Alcohol Quality of Life Scale (AQoLS), which focuses on 7 different domains: activities, relationships, living conditions, negative emotions, looking after self, control and sleep. Compared to controls, AUD patients showed higher levels of depression, anxiety and more severe cognitive impairments. All patients complained on at least 6 of the 7 domains of HRQoL. Contrary to our expectations, HRQoL did not relate to cognition, but to depression ( $r=0.53$ ,  $p=0.02$ ) and anxiety ( $r=0.42$ ,  $p=0.01$ ). Our results confirm previous findings suggesting altered HRQoL, impaired cognitive abilities and altered mood in AUD patients early in abstinence. The pattern of a relationships between HRQoL, cognition and mood suggests that improving HRQoL in AUD patients may require prioritizing the treatment of anxiety and depression.

## **Withdrawal history is associated with sleep and cognitive alterations in recently detoxified alcohol use disorder patients.**

Alice Laniepce<sup>1</sup>, Nicolas Cabé<sup>1,2</sup>, Françoise Bertran<sup>1,3</sup>, Claire André<sup>1</sup>, Céline Boudehent<sup>1,2</sup>, François Vabret<sup>1,2</sup>, Francis Eustache<sup>1</sup>, Géraldine Rauchs<sup>1\*</sup>, Anne-Lise Pitel<sup>1\*</sup>

- (1) Normandie Univ, UNICAEN, PSL Research University, EPHE, INSERM, U1077, CHU de Caen, Cyceron, Neuropsychologie et Imagerie de la Mémoire Humaine, 14000 Caen, France
- (2) Service d'Addictologie, Centre Hospitalier Universitaire de Caen, 14000 Caen, France.
- (3) Unité d'exploration et de traitement des troubles du sommeil, Centre Hospitalier Universitaire de Caen, 14000 Caen, France

\*: equally contributed to this work.

**Background:** Early in abstinence, patients with Alcohol Use Disorder (AUD) frequently present brain alterations, cognitive deficits and sleep disturbances<sup>1,2</sup>. Considering the crucial role of sleep in cognitive functioning, we aimed at investigating whether objective sleep disturbances contribute to cognitive deficits in AUD patients recently detoxified. Given the short delay since drinking cessation, relationships with withdrawal history were also explored.

**Methods:** 18 AUD patients underwent a neuropsychological battery and sleep examinations (including a 1-week continuous actigraphy recording and one night of polysomnography). Withdrawal history was also documented. While patients were early in abstinence, none of them presented physical symptoms of alcohol withdrawal<sup>3</sup> nor were under medication by benzodiazepines. Regressions analyses were performed between cognition, sleep, and withdrawal history.

**Results:** Longer sleep duration was associated with a lower amount of slow-wave sleep and executive deficits in recently detoxified AUD patients. Alcohol withdrawal history, especially the duration of benzodiazepines prescription and the total amount of benzodiazepines prescribed, was related to sleep abnormalities and executive deficits.

**Discussion:** Our results suggest that alcohol withdrawal and associated benzodiazepines prescription result in poor restorative sleep and executive deficits in recently detoxified AUD patients. The duration and severity of alcohol withdrawal should be taken into account when a neuropsychological assessment is conducted early in abstinence. Further studies are required to disentangle the neurotoxic effect of withdrawal *per se* from the consequences of the benzodiazepines treatment.

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# **What can we learn from epidemiology in alcohol research? Recent findings from large population-based cohorts**

LEMOGNE Cédric

AP-HP, Hôpitaux Universitaires Paris Ouest, Department of Psychiatry and Addictology.  
Inserm, U894, Centre Psychiatrie et Neurosciences, Paris, France

(Missing abstract)

# Baclofen Modulates Psychophysiological Responses to Appetitive Cues in Treatment-seeking Alcohol Use Disorder Individuals

## Authors:

WARREN LOGGE<sup>1,2</sup>, ANDREW BAILLIE<sup>3</sup>, PAUL HABER<sup>2,4</sup>, KIRSTEN MORLEY<sup>2</sup>.

<sup>1</sup>*Sydney Local Health District, Sydney, Australia*, <sup>2</sup>*Discipline of Addiction Medicine, University of Sydney, Sydney, Australia*, <sup>3</sup>*Faculty of Health Sciences, University of Sydney, Sydney, Australia*, <sup>4</sup>*Drug Health Services, Sydney Local Health District, Sydney, Australia*,

[warren.logge@sydney.edu.au](mailto:warren.logge@sydney.edu.au)

## Introduction

Baclofen is an emerging potential pharmacotherapy for alcohol use disorder. Little research has investigated how baclofen affects psychophysiological responses to alcohol cues, and subsequent effects upon drinking behaviours. We assessed whether baclofen-treated alcohol dependent participants show different subjective and psychophysiological responses to appetitive cues during an alcohol cue reactivity task compared to placebo participants, and whether these responses are associated with prospective drinking outcomes.

## Method:

Forty-two alcohol dependent participants (placebo: n = 12, low-dose baclofen [30 mg/day] n = 18, high-dose baclofen [75 mg/day]: n = 12) completed an alcohol cue reactivity task, whereby water and alcohol beverage cues were presented, with subsequent respective recovery periods. Subjective alcohol craving and psychophysiological indices (skin conductance; cardiovascular measures: heart rate, high-frequency heart rate variability) were recorded across the task.

## Results:

High-dose baclofen-treated participants showed both overall cue reactivity to both water and alcohol cues and greater recovery effects during recovery periods, revealed by high-frequency heart rate variability levels, when compared to low-dose- and placebo-treated participants. There were no medication effects on subjective alcohol craving. In high-dose baclofen participants only, there was a predictive effect of lower baseline resting heart rate variability and fewer post-test percentage of heavy drinking days.

## Discussions and Conclusions:

There was a dose-specific rescuing effect of high-dose baclofen on the dynamic modulation of reactivity and regulation of responses to eliciting cues. Using psychophysiological techniques to detect treatment responses may elucidate baclofen's mechanisms of action, and potentially identify alcohol use disorder subgroups that may best benefit from this pharmacotherapy.

## Disclosure of Interest Statement:

The Australasian Professional Society for Alcohol and other Drugs (APSAD) recognises the considerable contribution that industry partners make to professional and research activities. We also recognise the need for transparency of disclosure of potential conflicts of interest by acknowledging these relationships in all written publications.

*There are no competing interests related to this study.*

## **Affect preceding drinking sessions predicts increased alcohol consumption in University students: an experience sampling approach.**

### **Authors:**

**WARREN LOGGE**<sup>1,2</sup>, BENJAMIN RIORDAN<sup>2</sup>, KIRSTEN MORLEY<sup>2</sup>, ANDREW BAILLIE<sup>3</sup>, PAUL HABER<sup>2,4</sup>, TAMLIN CONNER<sup>5</sup>.

<sup>1</sup>*Sydney Local Health District, Sydney, Australia*, <sup>2</sup>*Discipline of Addiction Medicine, University of Sydney, Sydney, Australia*, <sup>3</sup>*Faculty of Health Sciences, University of Sydney, Sydney, Australia*, <sup>4</sup>*Drug Health Services, Sydney Local Health District, Sydney, Australia*, <sup>5</sup>*Department of Psychology, University of Otago, Dunedin, New Zealand*

[warren.logge@sydney.edu.au](mailto:warren.logge@sydney.edu.au)

### **Introduction**

University students are a high-risk group for developing alcohol problems. Positive and/or negative affect is associated with increased consumption, but there are mixed results. Impulsivity, which is a key risk factor for initiation of and excessive alcohol use, may explain the link between affect and drinking. This study used experience sampling to assess whether reported affect prior to drinking was associated with increased consumption, and whether impulsivity moderated this association.

### **Method:**

We recruited 694 University students (18-25 years) for a micro-longitudinal daily diary study, with impulsivity (BIS/BAS) measured at baseline. Students reported affect (positive, negative) via text message four times per day for 13 days, and daily alcohol use.

### **Results:**

Linear mixed models found a three-way interaction between positive affect, number of drinking days, and the BIS/BAS Drive subscale score. For participants who drank less frequently, those with higher Drive scores reported a higher number of drinks per session with increasing positive affect, while those lower Drive scores showed less pronounced increase. For participants who drank more frequently, those with higher Drive scores showed little change regardless of positive affect, whereas those with lower Drive scores showed a marked increase in drinks per session according to positive affect. There were no effects found related to negative affect.

### **Discussions and Conclusions:**

Positive affect, but not negative affect, has a key role in consumption levels according to drinking session frequency and level of goal-directed motivation in university students. This association is complex and dependent on drive and established patterns of drinking.

### **Disclosure of Interest Statement:**

*There are no competing interests related to this study.*

# Brain metabolites and hypothalamic-pituitary-adrenocortical activity during baclofen treatment in alcohol dependent patients: modulation by the GABA<sub>B</sub> receptor polymorphism rs29220

## Authors:

KIRSTEN C. MORLEY<sup>1</sup>, NATASHA LUQUIN<sup>2</sup>, JIM LAGOPOULOS<sup>3</sup>, WARREN LOGGE<sup>1</sup>, ANDREW BAILLIE<sup>4</sup>, RONALD J. TRENT<sup>2</sup>, PAUL S. HABER<sup>1,5</sup>.

<sup>1</sup> *NHMRC Centre of Research Excellence in Mental Health and Substance Use, Central Clinical School, Sydney Medical School, University of Sydney, NSW, Australia.*

<sup>2</sup> *Department of Medical Genomics, Royal Prince Alfred Hospital, NSW, Australia*

<sup>3</sup> *Mind and Neuroscience, University of Sunshine Coast, NSW, Australia.*

<sup>4</sup> *NHMRC Centre of Research Excellence in Mental Health and Substance Use, Central Clinical School, Sydney Medical School, University of Sydney, NSW, Australia.*

<sup>5</sup> *Drug Health Services, Royal Prince Alfred Hospital, NSW, Australia.*

[warren.logge@sydney.edu.au](mailto:warren.logge@sydney.edu.au)

## Introduction

We have previously shown that the *GABBR1* rs29220 polymorphism is associated with response to baclofen, a GABA<sub>B</sub> agonist, in the treatment of alcohol dependence. In the current study, we aimed to further examine the role of the *GABBR1* rs29220 polymorphism on hypothalamic-pituitary-adrenocortical activity and neurometabolites following administration of baclofen (BAC) or placebo (PL) in alcohol dependent individuals.

## Method:

Parietal GABA, Glutamate, Glutathione and N-Acetyl Aspartate levels were measured in N = 25 alcohol dependent patients using *in vivo* proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) 120 minutes following administration of PL or BAC. Blood samples were obtained for analysis of the single nucleotide polymorphism (rs29220) in the GABA<sub>B</sub> receptor subunit 1 gene (*GABBR1*) (CC = 15, G- = 10). Plasma cortisol levels were also measured at two time points including pre and post scan.

## Results:

There was a significant effect of medication (BAC vs PL) on cortisol levels ( $F = 10.18$ ,  $p = 0.007$ ) but there were no significant main effects of genotype (G- x CC) or medication (BAC vs PL) x genotype (G- x CC) interaction effect. There was a significant medication (BAC vs PL) x genotype (G- x CC) interaction effect for parietal concentrations of glutamate ( $F = 4.87$ ,  $p = 0.04$ ) but not for the other metabolites.

## Discussions and Conclusions:

Our data demonstrate that the *GABBR1* rs29220 polymorphism does not moderate baclofen induced changes in HPA axis activity. The *GABBR1* rs29220 polymorphism did moderate cortical concentrations of glutamate following baclofen treatment in alcohol dependent individuals.

## Disclosure of Interest Statement:

*There are no competing interests related to this study.*

## The active ingredients of *Bupleurum falcatum*, saikosaponins A and D, but not C, reduce alcohol self-administration in rats

Paola Maccioni<sup>1</sup>, Federica Fara<sup>1</sup>, Irene Lorrain<sup>1</sup>, Young-Won Chin<sup>2</sup>, Jung Hwan Lee<sup>3</sup>, Hak Cheol Kwon<sup>3</sup>, Giancarlo Colombo<sup>1</sup>

<sup>1</sup>Neuroscience Institute, National Research Council of Italy, Section of Cagliari, Monserrato, Italy; <sup>2</sup>College of Pharmacy, Dongguk University-Seoul, Goyang, Gyeonggi-do, Republic of Korea; <sup>3</sup>Korea Institute of Science and Technology, Gangneung Institute of Natural Products, Gangneung-si, Gangwon-do, Republic of Korea

Treatment with saikosaponin A (SSA) – an active ingredient of the medicinal herb, *Bupleurum falcatum* – has been reported to suppress i.v. self-administration of morphine and cocaine and oral self-administration of alcohol in rats. It has been demonstrated that these *anti*-addictive properties of SSA occur, at least in part, *via* a GABA<sub>B</sub> receptor-mediated mechanism. This lab has recently started a research program aimed at investigating whether ingredients of *Bupleurum falcatum* other than SSA affect alcohol self-administration in rats. Accordingly, the present study investigated whether the *anti*-alcohol properties of SSA extend to saikosaponin C (SSC) and saikosaponin D (SSD; an epimer of SSA). To this end, adult female Sardinian alcohol-preferring (sP) rats were trained to lever-respond for alcohol (15%, v/v) on a fixed ratio 5 (FR5) schedule of reinforcement. Once responding had stabilized, rats were tested under the same schedule after treatment with saikosaponins. Treatment with SSA (0.25-1 mg/kg, i.p.) and SSD (0.25-1 mg/kg, i.p.) resulted in highly similar, marked reductions (50-60% at the highest dose tested) in lever-responding for alcohol and amount of self-administered alcohol. Conversely, treatment with SSC (0.25-1 mg/kg, i.p.) failed to alter lever-responding for alcohol and amount of self-administered alcohol. Future experiments will investigate the effect of other saikosaponins on alcohol self-administration in sP rats, with the intent of establishing a possible structure-activity relationship. These results confirm that *Bupleurum falcatum* is a valuable source of compounds with *anti*-alcohol potential.

Is it relevant to postpone psychosocial treatment of alcohol dependence by one month to favor early neuropsychological recovery?

Angéline MAILLARD<sup>2</sup>, Hélène POUSSIER<sup>1</sup>, Céline BOUDEHENT<sup>2,3</sup>, Coralie LANNUZEL<sup>2</sup>, Angel VICENTE<sup>1</sup>,  
François VABRET<sup>2,3</sup>, Nicolas CABE<sup>2,3</sup>, Anne-Lise PITEL<sup>2</sup>

- (1) Clinique de Soins de Suite et de Réadaptation Korian Côte Normande, 14123 Ifs
- (2) Normandie Univ, UNICAEN, PSL Research University, EPHE, INSERM, U1077, CHU de Caen, Neuropsychologie et Imagerie de la Mémoire Humaine, 14000 Caen, France
- (3) Service d'Addictologie, Centre Hospitalier Universitaire de Caen, 14000 Caen, France

Many recently detoxified Alcohol Use Disorder (AUD) patients early in abstinence exhibit neuropsychological impairments which limit the benefit of treatment and increase the risk of relapse. While psychosocial alcohol treatment may not be clinically relevant in AUD patients with impaired neuropsychological abilities, it is now clear that these neuropsychological deficits can be partially or totally reversible with drinking cessation. The main purpose of this retrospective clinical study was to investigate whether a three-week stay as inpatients in a convalescent home enables neuropsychological deficits observed in recently detoxified AUD patients to recover and even to return to normal.

Neuropsychological data were collected in 84 AUD patients. Five neuropsychological components were assessed before and after a three-week multidisciplinary treatment in convalescent home. Baseline and follow-up performance was compared using Wilcoxon's and Chi-square tests.

The comparisons between baseline and follow-up performance revealed a significant improvement for the five cognitive components. The ratio of patients with preserved or impaired performance was significantly different between the baseline and follow-up sessions for three components, indicating that there were fewer patients with impaired performance at follow-up than at baseline.

In recently detoxified AUD patients, impaired cognitive functions recover with a three-week stay in a convalescent home ensuring sobriety and healthy nutrition. Such therapy seems favoring cognitive recovery and even performance to return to normal. It is thus crucial to postpone alcohol treatment for AUD patients with neuropsychological impairments in order to make them cognitively able to benefit from it.

## **Development of a peripherally restricted CB1 receptor antagonist for alcohol induced liver disease**

George Amato, Amruta Manke, Robert Wiethe, Vineetha Vasukuttan, Rodney Snyder, Yun Lan Yueh, Ann Decker, Scott Runyon, Nayaab Khan and Rangan Maitra

RTI International, 3040 East Cornwallis Road, Research Triangle Park, NC 27709

Antagonists of peripheral type 1 cannabinoid receptors (CB1) can treat various diseases including alcoholic liver disease (ALD). Unfortunately, inhibition of human CB1 (hCB1) receptors in the central nervous system (CNS) produces adverse effects including depression, anxiety and suicidal ideation. Therefore, efforts are underway to develop peripherally restricted antagonists of hCB1. Recent crystal structures of hCB1 and docking studies with the purine otenabant, a centrally acting CB1 inverse agonist developed by Pfizer that was clinically tested but abandoned due to concerns related to adverse effects, indicated that the piperidine group of this compound could be functionalized at the 4-position to access a binding pocket that might accommodate both polar and nonpolar groups. Therefore, we proceeded to examine the piperidine as a linker, which was functionalized with alkyl, heteroalkyl, aryl and heteroaryl groups using a urea connector. These studies resulted in orally bioavailable and peripherally selective compounds that were potent inverse agonists of hCB1 with exceptional selectivity for hCB1 over hCB2. The lead compound from this series presented good ADME properties, clean selectivity profile against >40 high-risk receptor targets (SafetyScreen, Eurofins), and was advanced into in vivo efficacy studies in the Lieber DeCarli model of alcohol-induced steatosis. Once a day oral dosing with this lead compound blocked alcohol-induced liver steatosis in mice and reduced expression of several molecular biomarkers associated with hepatic inflammation and metabolism. In conclusion, a promising peripherally selective CB1 receptor antagonist has been identified that is suitable for further clinical development for ALD and other disorders.

# TELOMERE LENGTH AND POLYMORPHISMS IN TELOMERASE GENES AMONG PATIENTS WITH ALCOHOL USE DISORDERS

*C. Carbonell<sup>1,2</sup>, H. Llorente<sup>1</sup>, E. Bueno<sup>2,3</sup>, J. A. Pérez Rivera<sup>4</sup>, M. A. Pérez<sup>2</sup>, C. Cieza<sup>1</sup>, J. Fernández Mateos<sup>1</sup>, R. González-Sarmiento<sup>2,3</sup>, F. Laso<sup>1,2,3</sup>, M. Marcos<sup>1,2,3</sup>*

<sup>1</sup>University Hospital of Salamanca, Spain. <sup>2</sup>Institute of Biomedical Research of Salamanca (IBSAL), Spain. <sup>3</sup>University of Salamanca, Spain. <sup>4</sup>University Hospital of Burgos, Spain.

**Introduction:** Telomeres are repetitive DNA sequences located at the ends of chromosomes, protecting cells from loss of genomic material during replication. Telomere length (TL) variation is associated with many inflammatory diseases. The relationship between alcohol consumption and TL has been previously studied, but, to date, no clear relationship is established regarding alcohol use disorders (AUDs) and TL. Polymorphisms in telomerase genes have also been associated with susceptibility to AUDs. Here, we have analysed TL and the polymorphisms TERC rs2293607, rs12696304, and rs16847897, TERT rs2735940, rs2736100, and rs2736098 in patients with AUDs.

**Patients and methods:** 99 men with AUDs (according to DSM IV criteria) and 99 healthy age and sex-matched controls were included. DNA was extracted from peripheral blood leukocytes using phenol/chloroform procedure and genotyped using TaqMan 5'-exonuclease allelic discrimination assays (Applied Biosystems). Relative mean TL was measured from DNA by a qPCR assay. Statistical analysis was performed using GenEx v6 software and SPSS v25.

**Results:** Mean telomere length (T/S) was  $4.38 \pm 3.18$  in alcoholic patients and  $6.28 \pm 1.94$  in controls ( $p < 0.001$ ). Alcoholic patients with alcoholic liver cirrhosis or without liver disease had also shorter TL than their respective controls. Area under ROC curve to study correlation between telomere length and alcoholism was 0.70 ( $p < 0.001$ ) and the best cut-off-point of telomere length (T/S) was 5.91 (sensitivity 70% and specificity 64%). The presence of the G allele of allelic variant TERC rs2293607 polymorphism was associated with increased risk of AUDs and shorter TL.

**Conclusion:** Our study supports the correlation between TL attrition and chronic and excessive ethanol intake, which has not been previously analyzed in this population. In addition, the possession of the G allele of TERC rs2293607 allelic variant is associated with both shorter TL and increased risk of AUDs and therefore may be associated with genetic susceptibility to develop alcoholism through modulating TL.

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# **Impact of Chronic Alcohol Exposure and Withdrawal on Hepatocellular Carcinoma Aggressiveness**

C. Marié, C-A. Papillon, G. Fouquet, H. Bouhlal, E. Nguyen-Khac, M.Naassila, I.Marcq

Groupe de Recherche sur l'Alcool et les Pharmacodépendances (GRAP), INSERM UMR 1247, Université Picardie Jules Verne (UPJV), Amiens, France

In France, 9.7% of the population drinks alcohol every. Furthermore, 50% of liver disease mortality is due to alcohol. Indeed, chronic alcohol exposure can lead to HepatoCellular Carcinoma (HCC) development. HCC is the 6th cancer in incidence but the 2nd cancer in mortality. HCC patients have a 5-year survival lower than 10 percent.

The study of C-E Costentin (et al, 2018, Cancer) showed a decrease of survival in patients with alcoholic HCC. These observations determined that alcoholic HCC are more aggressive than other etiologies of HCC. This study also demonstrates the importance of withdrawal in patient survival. Our project aims to determine the physiopathological mechanisms explaining these clinical observations.

We developed a protocol of Chronic Alcohol Exposure (CAE). For this, HCC cells were exposed to alcohol during several months at different doses. We also devised a withdrawal method simultaneously. We investigated the impact of alcohol on cellular viability, mortality and proliferation. Furthermore, we studied cancer stem cell markers in cells exposed to CAE and after withdrawal, and finally their effects on migratory and invasive cell potentials.

Our results show that CAE decreases cellular viability and promotes aggressiveness by an increase of metastatic potential and cancer stem cell markers. Interestingly, withdrawal partially reverses these modifications due to CAE.

Our results obtained in HCC cellular model exposed to CAE allow a better understanding of the mechanisms underlying decreased survival of patients with alcoholic HCC. They also demonstrate the importance of looking for alcohol abstinence in patients.

# The Effects of Environmental Enrichment on Intermittent 20% Ethanol Intake in Long-Evans Rats

Shehata, L.<sup>1</sup>, Jeanblanc, J.<sup>2</sup>, Naassila, M.<sup>2</sup>, & Martinetti, M. P.<sup>1</sup>

<sup>1</sup>*The College of New Jersey, Ewing, NJ, 08618, USA*

<sup>2</sup>*INSERM U1247, GRAP, CURS, Univ. Picardie Jules Verne, Amiens, FRANCE*

Environmental enrichment (EE), such as toys or novel objects, is recommended practice for laboratory animal research; however, in animal models of alcohol consumption, EE has produced varying effects on ethanol (EtOH) intake. The current study examined the effects of EE on EtOH intake in male Long-Evans rats using an intermittent two-bottle choice (2BC) paradigm. Animals were randomly assigned to either an EE group ( $n = 12$ ), with a packet of crinkle paper in the home cage, or non-enriched control ( $n = 12$ ). A 20% EtOH solution was provided concurrently with water in the home cage for 24-hr periods on Mon, Wed, and Fri each week for 13 weeks. Overall, EE rats consumed less absolute EtOH (g/kg/24 hrs) compared with CTRL rats. This effect was specific to EtOH, as water consumption on the days between the two-bottle choice periods did not differ, nor did the groups differ with respect to body weight. These findings suggest that EE reduces 20% EtOH consumption in an intermittent 2BC paradigm, and these results underscore the experimental implications of environmental enrichment in animal models of alcohol use disorder.

# **Effect of Behavioral “Super-Intervention” in Adolescence on Cortically-Projecting Cholinergic Neurons in a Rodent Model of Fetal Alcohol Spectrum Disorders (FASD)**

K.A. Milbocker; Z.H. Gursky, MS; A.Y. Klintsova, Ph.D.

*University of Delaware, Dept. of Psychological and Brain Sciences, Newark, DE, 19716, USA*

One in twenty infants in the United States are affected by prenatal alcohol exposure (PAE) resulting in a range of disorders categorized as Fetal Alcohol Spectrum Disorders (FASD). PAE during late-stage pregnancy can produce lasting deficits of cortical-dependent behaviors in affected individuals (executive function, i.e. decision-making and inhibitory control). Evidence from human cases and animal models of FASD suggest that cognitive impairments are mitigated by non-invasive intervention such as choline supplementation (Fuglestad et al., 2013; Idrus et al., 2017).

Our study examines the effects of a behavioral “super-intervention” on the integrity of cortically-projecting cholinergic neurons from the nucleus basalis of Meynert (NBM) in a rat model of FASD. Male Long-Evans rats were exposed to binge-like alcohol (5.25 g/kg/day) on postnatal days (PD) 4-9. Control groups included sham-intubated (SI, no liquid) and suckle-control (SC) rats. Rats were weaned on PD23. On PD30, behavioral “super-intervention” began, consisting of twelve days of voluntary running in a wheel (WR) followed by four weeks of housing in a complex environment (EC). Immunocytochemical visualization of ChAT+ neurons was performed on brain tissue collected at PD72. Preliminary analyses of unbiased stereological estimates show a significant increase in the number of cholinergic neurons following adolescent intervention ( $F(1, 27) = 6.63, p = 0.02$ ; interaction with treatment  $p = 0.08$ ) and volume of NBM ( $F(1, 27) = 4.65, p = 0.04$ ). However, it appears that the intervention affects postnatal treatment groups differently (SC:  $t(8) = 2.86, p = 0.02$ ), indicating decreased neuroplasticity in AE rats long-term.

# **Violations of a healthy behavior of pregnant women: pilot study**

**Valentina A. Moshkivskaya, Ekaterina A. Burina**

**Saint Petersburg State University**

**Saint Petersburg, Russia**

Most women know that proper nutrition, avoiding the use of various psychoactive substances, moderate exercise, prolonged sleep during pregnancy is a necessary condition for the normal development of the fetus, the course of pregnancy and the health of mother and child. However, a number of studies reveal a violation of the principles of healthy behavior. In this regard, it was decided to conduct a study that allows investigating some of the habits of a violation of the healthy lifestyle of pregnant women.

Materials and research methods. 25 pregnant women took part in the study: 20 – had a physiological pregnancy, 5 – got pregnant with the help of reproductive methods. The sample was enrolled on the base of a maternity hospital. Social interview, analysis of medical notes and Questionnaire of Disorders of Healthy Behavior (Lutsenko, Gabelkova) was used in the study due to research aim. The questionnaire includes 9 scales: smoking, eating disorders, neglecting safety, alcohol use, chasing a trendy image, low self-control, emotional incompetence, self-destructive behavior and general performance.

Study results indicated low and average level of violation in general. Detailed analysis showed a tendency of women with first pregnancy to have normal eating and drinking habits in comparison to women who already have one or two children ( $W=113.5$ ,  $p\text{-value}=0.0549$ ). Participants with higher level of emotional incompetence tend to have more eating problems or disorders ( $r(25)=0.63$ ,  $p=0.002$ ). Current study should be continued to gain more crucial information that can be used for preventive measures.

## VALIDITY OF THE SELFPERCEPTION OF ALCOHOL CONSUMPTION AMONG FRESHMEN

MOURE-RODRIGUEZ Lucia

*Complexo Hospitalario Universitario de Ourense, Spain*

**Aim:** Our aim is to assess the importance of the perception of university students about their own alcohol consumption at 18 years of age on risky alcohol consumption (RAC) and binge drinking (BD) at that same age and during the following 10 years.

**Methods:** a cohort study among university students (Compostela Cohort 2005, Spain), was carried out between November 2005 and February 2015. Participants were selected by cluster sampling, going to at least one 1<sup>st</sup> year class of every faculty of the Universidade de Santiago de Compostela. The Alcohol Use of Disorder Identification Test was used for measuring both RAC, -total score  $\geq 5$  for women and  $\geq 6$  for men-, and BD -drinking  $\geq 6$  alcoholic beverages in one single occasion at least monthly-. Students answered questions related to their expectations about alcohol (Low/High) and their perceptions of their own alcohol consumption (nothing/little/fair amount/large amount). A multilevel logistic regression was performed and predictive values were calculated with SPSSv20 statistical software.

**Results:** 99% of students in class the day of the survey participated (n=1,382). As subject's perception of their own alcohol consumption at 18 years old increases, so does the proportion of them with positive expectations regarding alcohol (55.2% vs 14.3%) and the proportion of them who practice RCA (100% vs 14% among females and 100% vs 37.7% for men) and BD (84.4% vs 10% among females and 85.7% vs 12.3% on men) at this same age. This tendency is maintained in relation to their RCA and BD during 20, 22, 24 and 28 years old among both genders. Taking in to account subjects who perceive their consumption was nothing or little at 18 years old compared to those who perceive that they consume a fair amount or large amount of alcohol, this last group maintains much higher prevalence of RCA and BD all through the study period. Understanding one's alcohol consumption perception as a diagnostic tool, they were observed high negative and positive predictive values for RAC and BD at 18, 20, 22, 24 and 28 years old for both genders.

**Conclusions:** The perception of a freshmen college student about the amount of alcohol they consume, is an important variable, easy and quick to assess and that can offer more information than we expected to. At this study we do not only confirm that the amount of alcohol consumption that a student who practices alcohol assesses of him/herself highly corresponds with his/her real consumption, but we observed how this self-perception of alcohol use at 18 years old is an important predictor of the RAC and BD at 20, 22, 24 and 28 years old.

## Relationship between liver fibrosis and liver iron in patients with alcoholic liver disease

Mueller J., Raisi H., O. Elshaarawy, Rausch V., Silva, I., Seitz H.K., Mueller S.

Dept. of Internal Medicine, Salem Medical Center and Center for Alcohol Research, University of Heidelberg, Germany

**BACKGROUND & AIMS:** Alcoholic liver disease (ALD) is one of the most common liver diseases and can cause hepatic iron overload.

**AIM:** To investigate the relationship between fibrosis stage and liver iron in ALD patients.

**METHODS:** 358 Patients were prospectively recruited between 2007 and 2018 at the department of Internal Medicine at Salem Medical Center, Heidelberg. In 224 patients with ALD, non-invasive liver stiffness (LS) (FibroScan, Echosense, Paris) and iron determination (room temperature susceptometry, RTS) was performed. 134 patients with liver biopsy had histological assessment of liver fibrosis (Kleiner) and iron (Prussian Blue stain). Additionally, all patients had routine laboratory tests and abdominal ultrasound.

**RESULTS:** Mean age was 52.4 years and mean alcohol consumption was 176 g/day. Biopsied patients showed more fibrosis than non-invasively characterized patients (fibrosis stage 1.7 to 2.6,  $P < 0.001$ ). No significant correlation between fibrosis/LS and histological iron or RTS was observed, while ferritin was moderately associated with LS ( $r = 0.45$ ,  $P < 0.01$ ). Serum transferrin decreased continuously with LS/fibrosis ( $r = -0.51$ ,  $P < 0.001$ ). The relationship between fibrosis stage and liver iron was non-trivial in both cohorts. Liver iron (histology and RTS) was elevated in both cohorts in fibrosis stages up to stage 3 while it was significantly decreased in fibrosis stage 4 in comparison to stage 3 ( $P < 0.05$ ). This decrease was also observed in ferritin values, however, non-significantly. Accordingly, RTS was significantly associated with LS in patients with stiffness less than 12 kPa ( $r = 0.22$ ,  $P < 0.05$ ), while no significant correlation was observed in patients with high LS  $> 20$  kPa.

**CONCLUSIONS:** In contrast to common perception, ALD patients with liver cirrhosis showed significantly lower liver iron in histology and RTS. The highest iron concentrations were found in intermediate fibrosis stages F1 to F3. Elevated ferritin or lower transferrin in liver cirrhosis patients is more likely due to a release of iron from cell death instead of increased body iron stores.

# **Identification of novel epigenetic biomarkers of alcohol dependence in brain and blood**

NIERATSCHKER Vanessa

Department of Psychiatry and Psychotherapy, University of Tuebingen

## Background:

Alcohol dependence (AD) is a severe disorder accompanied by mental and physical health problems. The complex pathogenesis includes environmental and genetic factors. Evidence is emerging that epigenetic mechanisms might contribute to gene environment interactions which seem to play a major role in the manifestation of addiction. In previous studies, we already identified several genes as being differentially methylated in patients compared to healthy controls. Interestingly, our top hits are both involved in the cellular stress response and in neurodevelopment.

One of the biggest challenges in psychiatric epigenetics is the inaccessibility of living brain tissue. Therefore, in this study we tried to replicate our previous hits, which had been identified in human blood, in human post mortem brain samples as well as in blood and brain samples derived from a rat model for AD.

## Methods:

We investigated post mortem human brain samples originating from Brodman area 9 either from AD patients (n=13) or healthy controls (n=10). In addition, brain and blood samples from a rat model of AD were investigated. To induce AD, rats had been exposed to daily intermittent cycles of alcohol vapor intoxication and withdrawal. Rats were weight-matched and assigned to two groups which were either exposed to ethanol vapor (n=16) or normal air (n=16). Samples from blood and from different brain regions of the same animal were obtained one day after alcohol abstinence. DNA methylation levels in our top hits were assessed by pyrosequencing.

### Results:

Interestingly, for the human brain samples both genes showed differential DNA methylation when comparing patients with healthy controls. However, for both genes the changes in methylation were in opposite direction to our previous results comparing human whole blood samples. In the rat model, we found differential DNA methylation levels for both genes in the PrLC.

### Discussion:

Our results further facilitate the potential role of the two investigated candidate genes in AD. Interestingly, both genes play a role in the cellular stress response as well as in neurodevelopment. However, more research is needed to examine on a cellular level how the regulation of these two genes might be involved in AD. Furthermore, in the rat brain we found differential methylation patterns in the PrLC, a brain region thought to be involved in drug-seeking behavior. Our results further emphasize the tissue and cell type specificity of epigenetic changes. Although we can sometimes assume a correlation of DNA methylation changes between different cell types, further studies are needed to identify which genes do correlate between certain tissue and cell types and which seem to be regulated independently.

## Représentations socioculturelles et stigmatisation liée au Trouble de l'Usage d'alcool : le point de vue de patients, de leurs proches, et de professionnels d'un Pôle universitaire d'Addictologie à Limoges (France)

Thibaut Dumontheil <sup>1,2</sup>, Jean-Jacques Yonga <sup>1,2</sup>, Murielle Girard <sup>1,2,3</sup>, Philippe Nubukpo <sup>1,2,3</sup>

1- Unité de recherche et de Neurostimulation, Centre Hospitalier Esquirol, Limoges, France

2- Pôle Universitaire d'Addictologie en Limousin, Centre Hospitalier Esquirol, Limoges, France

3- INSERM 1094 NET, Faculté de Médecine, Limoges, France

En France, l'usage d'alcool est très fréquent, inscrit dans une habitude culturelle. Cette substance psychoactive est traditionnellement associée à la notion de plaisir et de convivialité dans tous les milieux.

Cependant, les représentations sociales portant sur les « alcooliques » ont toujours été paradoxales et ambivalentes. L'une des stigmatisations la plus « populaire » aujourd'hui est l'idée générale que les personnes souffrant d'alcoolisme ne sont pas malades, mais sont responsables de leur sort. Relativement aux personnes présentant d'autres maladies mentales, celles dépendantes à l'alcool sont fortement rejetées et souffrent de stéréotypes négatifs.

Une source importante de stigmatisation et de discrimination est trouvée par certains, dans le personnel de santé lui-même (incluant les infirmier(e)s), considéré comme en étant le premier contributeur. Pour mieux comprendre et identifier les déterminants de la stigmatisation dans le Trouble de l'Usage de l'Alcool (TUA), nous avons effectué deux études entre novembre 2018 et Juin 2019 afin de décrire les représentations socio-culturelles et le stigma associés au TUA chez 24 patients accueillis au Centre Hospitalier Esquirol, et leurs proches, et chez 594 professionnels travaillant dans le même hôpital. Nous avons utilisé le questionnaire « Explanatory Model Interview catalogue » (EMIC) qui est un outil d'entretien semi-structuré permettant une évaluation quantitative et qualitative des représentations de la maladie, des connaissances, du stigma associé et de la recherche d'aide.

Les résultats montrent que 36% des patients et 43% des proches ne connaissent pas la maladie, et les différents types d'aide disponibles ne sont pas connus pour plus de la moitié. L'analyse du discours des malades et des proches montre la présence d'une stigmatisation importante envers les personnes souffrant de TUA. Par ailleurs, l'enquête chez les professionnels hospitaliers montre une expression plus faible du stigma chez les personnes travaillant au sein du Pôle Universitaire d'Addictologie (PUAL). Ceci met en avant l'importance de la formation sur la maladie, ou du moins d'un contact plus important avec les malades pour la diminution de la stigmatisation de ces derniers.

Les actions de prévention visant à mieux identifier les sources d'aide aux patients et familles doivent être entreprises. La formation addictologique apparaît indispensable auprès des professionnels pour mieux combattre la stigmatisation.

Mots clés : alcool, trouble de l'usage, représentations sociales, stigmatisation, psychiatrie

## Clinical and biological monitoring of subjects with Alcohol Use Disorder after alcohol withdrawal treatment

Murielle Girard<sup>1,4</sup>, Marine Pareaud<sup>2</sup>, Paul Carrier<sup>3</sup>, Philippe Nubukpo<sup>1,2,4</sup>.

1. Unité de recherche et de Neurostimulation, Centre Hospitalier Esquirol, Limoges, France
2. Pôle Universitaire d'Addictologie, Centre Hospitalier Esquirol, Limoges, France
3. Service d'Hépto- Gastroentérologie, CHU, Limoges, France
4. INSERM 1094 NET, Faculté de Médecine, Limoges, France

The diagnosis and follow-up of the subjects with alcohol use disorder (AUD) are based on clinical examination and biological measure of alcohol use toxicity indicators. The rigorous examination of the symptomatology corresponds to a moment of the expression of the disorder, and does not make it possible to predict the success of the treatment, or to make an evolutionary prognosis, or characterize dependence. Biological indicators could help to objectify them. We summarize here the contributions of different studies that we carried out in a 6-month follow-up of a cohort of subjects with AUD who came for alcohol withdrawal at the psychiatric hospital. Follow-ups at 1, 2, 4 and 6 months after alcohol withdrawal focused on clinical indicators (relapses, comorbidities, depression, anxiety, craving), and specific biological indicators : liver stiffness, and serum levels of molecules of interest in the pathophysiology and expression of AUD: neuronal plasticity indicator (Brain Derived Neurotrophic Factor), pro-inflammatory cytokines, and neuronal damage markers (S100 beta and Neuron Specific Enolase). The variations of these indicators indicate a long-term improvement independent of alcohol consumption status (relapse, abstinence). The results allow an overview of some dysfunctions at specific times of the disorder, and reflect the characteristics of the problematic alcohol use and not those from the addictive component of AUD.

Source of funding: Inter-regional Clinical Research Hospital Program 2011 - Esquirol Hospital Center

Key words: alcohol withdrawal – BDNF – liver stiffness

## Sociocultural Representations and Stigmatization Related to Alcohol Use Disorder : The Perspective of Patients, their Relatives, and Professionals at a University Addiction Center in Limoges (France)

Thibaut Dumontheil <sup>1,2</sup>, Jean-Jacques Yonga <sup>1,2</sup>, Murielle Girard <sup>1,2,3</sup>, Philippe Nubukpo <sup>1,2,3</sup>

1- Unité de recherche et de Neurostimulation, Centre Hospitalier Esquirol, Limoges, France

2- Pôle Universitaire d'Addictologie en Limousin, Centre Hospitalier Esquirol, Limoges, France

3- INSERM 1094 NET, Faculté de Médecine, Limoges, France

In France, the use of alcohol is very frequent, and is placed in a context of a cultural habit. This psychoactive substance is traditionally associated with the notion of pleasure and conviviality in all environments.

However, social representations of "alcoholics" have always been paradoxical and ambivalent. One of the most "popular" stigmas today is the general idea that people with alcoholism are not sick, but are responsible for their trouble. Relative to people with other mental disorders, those dependent on alcohol are strongly rejected and suffer from negative stereotypes.

An important source of stigma and discrimination is found in the care teams themselves (including nurses), which are considered to be the first contributors. To better understand and identify the determinants of Alcohol Use Disorder (AUD), we conducted two studies between November 2018 and June 2019 to describe the socio-cultural representations and stigma associated with AUD at the Esquirol Hospital Center in Limoges (France) with 24 patients and their relatives, and 594 professionals working in the same hospital. We used the "Explanatory Model Interview Catalog" (EMIC) questionnaire which is a semi-structured interview tool allowing quantitative and qualitative evaluation of the representations of illness, the knowledges, the associated stigma and the help seeking.

The results show that 36% of patients and 43% of relatives do not know the disease. The analysis of the speech of patients and relatives shows the presence of a significant stigma towards people suffering from AUD. In addition, the survey of hospital professionals shows a weaker expression of stigma among people working in the University Service of Addictology in comparison with the other health care workers. This highlights the importance of formation on the disease for care workers, or at least greater contact with patients to reduce the stigma of the latter.

Prevention actions to better identify sources of support for patients and families should be undertaken. The training on AUD for the professionals appears essential to better fight the stigmatization.

Key words: alcohol use disorder, social representations, stigma, psychiatry

# IMPACT OF ADDICTOLOGY EXPERT PATIENTS ON MEDICAL STUDENTS: A QUALITATIVE STUDY

C.OBADIA<sup>1</sup>, A.NGUYEN VAN NHIEU<sup>2</sup>, D. MOISAN<sup>3</sup>, M.CLAUDON<sup>4</sup>, M.LEJOYEUX<sup>4</sup>, A. REYRE<sup>5</sup>

[chanaelle.obadia@aphp.fr](mailto:chanaelle.obadia@aphp.fr)

<sup>1</sup> UFR médecine Paris Diderot, UMR-S-1123 ECEVE, 75010, France

<sup>2</sup> UFR médecine Paris Diderot, France

<sup>3</sup> Unité de Traitement Ambulatoire des Maladies Addictives, Hôpital Beaujon, Clichy, France

<sup>4</sup> Psychiatrie-addictologie, Hôpital Bichat-Claude Bernard, Paris, France.

<sup>5</sup> APHP-Avicenne University Hospital, Department of Psychiatry and Addictology, Paris 13 SPC University, 125 rue de Stalingrad, 93000 Bobigny, France

## Abstract

**Background:** The expert patient (EP) participation in medical education is growing in France. They are involved in the topic of addictions, but the literature is poor on this subject. However, excessive alcohol consumption is the second leading cause of preventable death in France and screening for alcohol use disorders is one of the least common. This study evaluates the impact of EPs' intervention on medical students.

**Design:** It's a qualitative study. Semi-directed interviews were conducted with 11 students, 4 weeks and 4 months after an addiction training involving 4, conducted in October 2017. The analysis of transcribed data follows the thematic method.

**Findings:** The study identifies 3 dimensions along which students express themselves in different postures: the student appreciates the innovative, enriching and playful nature of this training, through the philanthropic contribution that seems to be missing in the usual curriculum. They understand and retain better. They project themselves more into their clinical practice. They also adopt a citizen's posture by seeing their representations of dependent individuals upset, which leads to a questioning of their relationship with others. These reflections lead the physician to question his own way of practicing medicine and to improve his clinical practice.

**Conclusions:** This study reveals the interest of a collective reflection about the contribution of EPs during medical education, and then about the pedagogical models of the medical studies. Prospectively, this work makes it possible to consider the integration of EPs into medical education concerning taboo subjects or even more globally in medical education

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## **Alcohol-related Brain Injury in Alcohol Use Disorder Patients Attending a UK Secondary Care Hospital**

Lynn Owens<sup>1,2,3</sup>, Andrew Thompson<sup>1,3</sup>, Munir Pirmohamed<sup>1,3</sup>, Paul Richardson<sup>2,3</sup>

<sup>1</sup> Wolfson Centre for Personalised Medicine, University of Liverpool, United Kingdom

<sup>2</sup> Hepatology, Royal Liverpool University Hospital Trust, United Kingdom

<sup>3</sup> Liverpool Centre for Alcohol Research University of Liverpool, United Kingdom

### **Introduction:**

Alcohol Related Brain Injury (ARBI) is an umbrella term for a number of neuropsychiatric conditions caused by headrinking. This condition is under-recognised and under-treated, often resulting in readmissions leading to increased physiological and psychological harm for patients and relatives.

### **Methods:**

A 12-month cohort study on the implementation of the Montreal Cognitive Assessment (MoCA) tool for detection of ARBI in heavy drinkers. Primary measure of interest was MoCA  $\leq 23$ . **Participants** were all in-patients aged  $\geq 18$  years who were reviewed by the Alcohol Care Team's Specialist Nurses (n=1276), and need for MoCA screening was based on pre-determined criteria.

### **Results:**

In 12 months, 205 patients were screened, 38% were initiated due to concerns about cognition raised by relatives. Of those screened, the directly standardised period prevalence rate for MoCA  $\leq 23$  was 36.1% (n=74). Drinking measures were not predictive of either; the need for screening or the presence of ARBI. The most common (27%) co-morbidity was Alcohol Related Liver Disease (ARLD). In patients with a MoCA  $\leq 23$  at 12-month follow-up, 17.5% had died of which 61.5% had no evidence of ARLD. Mean hospital attendances were significantly reduced from 8 to 5.6 (95%CI: 1.1 to 6.4; P=0.08) as were admissions from 3.2 to 2.4 (CI 95%: 0 to 1; P=0.03)

**Conclusions:** Screening for ARBI in acute hospital settings is an important first step in improving identification and management of this patient group with complex medical histories. Further work is required to optimise screening processes and to develop appropriate treatment pathways.

# Assessing the genetic aspects of subjective level of response to alcohol in an American Indian population through whole genome association and pleiotropic analyses

Q. Peng<sup>1</sup>, K.C. Wilhelmsen<sup>2</sup>, C.L. Ehlers<sup>1</sup>

<sup>1</sup>The Scripps Research Institute, Department of Neuroscience, La Jolla, CA 92037 USA;

<sup>2</sup>University of North Carolina, Chapel Hill, NC 27599 USA

## Abstract

Subjective level of response (LR) to alcohol is considered a risk factor for alcohol use disorders (AUD). LR varies by ethnicity. Previous studies showed that American Indians (AI) were less sensitive to the alcohol effects, which could partially contribute to their elevated AUD rates. In this study, we assessed how LR related to AI ancestry and AUD severity, conducted GWAS and pleiotropic study of LR measures in 684 admixed AI participants. Three LR traits from Subjective High Assessment Scale Expectancy for alcohol (SHAS-E) were evaluated: SHAS-E-total, -great, -terrible. AUD severity was derived from SSAGA interview.

SHAS-E-total was anti-correlated with AI ancestry ( $r=-0.10$ ,  $p$ -value= $9.5E-3$ ) and AUD severity ( $r=-0.16$ ,  $p=3.3E-5$ ). GWAS identified several revealing suggestive findings: Rs547109 on *GCLC* was negatively associated with SHAS-E-total ( $p=3.6E-7$ ). Rs547109 has MAF 26% in general population, but 41% in AI. *GCLC* belongs to glutathione pathway crucial to ethanol detoxification. *CACNA1B* variant was associated with SHAS-E-great ( $p=1.2E-7$ ). Variants on *SCML4*, *PLD5*, *GADL1*, *PCDH7* were associated with SHAS-E-terrible ( $p=6.3E-8$  -  $4.3E-7$ ). Top pathways included adherens-junction for SHAS-E-total, toll-like-receptor and neurotrophin signalings for SHAS-E-terrible, immune-response-signaling for SHAS-E-great. 217-232 pleiotropic SNPs were selected from GWAS Catalog. Among these, inflammatory-measurement was the most enriched for SHAS-E-total, immune-system-disorder and neurological-disorder for SHAS-E-great, and neurological-disorder for SHAS-E-terrible. Enriched networks in pleiotropic variants included fatty-acid-metabolic-process for SHAS-E-total, neurotrophin-signaling-pathway for SHAS-E-great and -terrible.

Our study identified a glutathione metabolic gene to be suggestively associated with SHAS-E-total in AI, and suggests that the genetic substrates underlying LR might relate to cell adhesion, neurotrophin, and inflammatory responses.

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## Comparing non-treatment-seeking participants in alcohol medication research studies across demographic and clinical variables by geographic location

Roberta Perciballi, MD, Bianca Persaud, Victoria Long, BA, Daria Piacentino, MD, PhD, MSc,  
Xiaobai Li, PhD,  
Lorenzo Leggio, MD, PhD, MSc, and Carolina Haass-Koffler, PharmD

Center for Alcohol and Addiction Studies, Department of Psychiatry and Human Behavior and Department of Behavioral and Social Sciences, Brown University, Providence, RI, USA; Sapienza, University of Rome, Italy

**Background:** Pharmacotherapy development for Alcohol Use Disorder (AUD) treatment is a long and challenging process. One of the challenges in early stage clinical research for AUD medication is that typically the enrolled participants are heavy drinkers, but they are not seeking treatment for AUD. This may impact the translational effort to move medications from clinical research to a clinical setting. One study found various significant differences between non-treatment-seeking participants with AUD from human laboratory studies conducted at the University of California, Los Angeles (UCLA) when compared to treatment-seeking participants with AUD from a national, multi-site clinical trial, the COMBINE study, which evaluated naltrexone and acamprosate with or without a combined behavioral intervention (CBI). The UCLA studies were confined to one homogenous geographic location, which showed significantly less variability between participants compared to the COMBINE study, which was conducted at 11 sites across the country.

**Objectives:** This present analysis aims to compare non-treatment-seeking individuals who participated in human laboratory studies for medication development at Brown University to non-treatment-seeking individuals that participated in similar studies at UCLA across demographic and clinical variables.

**Methods:** Participants from the Brown University group ( $n=240$ ) were compared to participants from the UCLA ( $n=213$ ) group. All participants from all studies were non-treatment-seeking individuals who met DSM-IV criteria for AUD and were enrolled in a medication development trial. The Brown studies were compared across multiple demographic, clinical, and alcohol-related variables to the UCLA studies. All studies at both sites gave participants same baseline questionnaires and assessments that were used for comparison in this analysis.

**Results:** Analysis comparing non-treatment-seeking participants from the Brown studies to the participants from the UCLA studies revealed significant differences between the populations across demographic and alcohol-related variables. Participants in the Brown studies were older ( $p<0.0001$ ), had fewer years of education ( $p=0.005$ ), and were more likely to be in a committed relationship or previously married ( $p<0.0001$ ) when compared to the UCLA population. Participants in the Brown studies also had less alcohol dependence ( $p=0.01$ ) and consumed fewer drinks 30 days prior to their baseline session ( $p<0.05$ ) than the participants in the UCLA studies.

**Conclusions:** Significant differences between the non-treatment-seekers within different geographic locations raises further questions and the need for more research to further define AUD populations. Future directions will compare non-treatment-seekers in AUD medication studies at Brown University to the treatment-seekers in the COMBINE study.

# Effects of smoking on endogenous hormones in individuals with alcohol use disorder

Bianca Persaud, Roberta Perciballi, MD,  
Lorenzo Leggio, MD, PhD, MSc, and Carolina Haass-Koffler, PharmD

*Center for Alcohol and Addiction Studies, Department of Psychiatry and Human Behavior and  
Department of Behavioral and Social Sciences, Brown University, 121 South Main St. G-S121- 5,  
Providence, RI, USA*

**INTRO:** Among individuals with alcohol use disorder (AUD), smoking is prevalent. Smoking has multiple effects on hormone secretion, some of which are associated with important clinical implications. Cotinine represents the most reliable biomarker of smoking behavior. Some significant correlations exist between this biomarker and endogenous hormones. Beta-endorphins, is an endogenous opioid neuropeptide known to be involved in stress responses and maintain homeostasis, released in the peripheral circulation are affected by nicotine stimulation; nicotine can affect beta-endorphins concentration resulting in changes in pain threshold and immune response. Melatonin, a pineal hormone, exerts potential effects on smoking induced oxidative stress. Plus, melatonin can help to counteract the acute effects of smoking cessation on mood. Alpha-Melanocyte-stimulating hormone (alpha-MSH) an endogenous peptide hormone and neuropeptide of the melanocortin family, production is stimulated by nicotine. Substance P neuropeptide, represents a key responder to preserve biological integrity, likely resulting in stress responses. Oxytocin is a hypothalamic peptide hormone and a neuropeptide. It is involved in empathy, sexual reproduction and childbirth. Smoking indirectly modulates oxytocin neuronal activity determining changes in environmental and stress responses. Also, nicotine can affect orexin activity, increasing appetite. Orexin regulates the release of noradrenaline, one of the neurotransmitters involved in stress. Orexin also is implicated in the induction of behavioral response to stressors.

**METHOD:** We performed a pilot study with 18 patients with alcohol use disorder to investigate the relationship between cotinine and endogenous hormones.

**RESULTS:** We found strong positive correlations between cotinine and the following hormones: Beta-endorphin ( $r_{16} = .604$ ;  $p = .008$ ), melatonin ( $r_{16} = .509$   $p = .031$ ), alpha-MSH ( $r_{13} = .645$   $p = .009$ ), Substance P ( $r_{16} = .470$   $p = .049$ ), oxytocin ( $r_{16} = .667$   $p = .002$ ), orexin ( $r_{14} = .742$   $p = .001$ ).

**CONCLUSION:** We found that most of these hormones, correlated with cotinine, are involved in stress systems. Thus, smoking may affect stress responses resulting in a possible impairment of homeostasis and dysregulation of the central feedback response.

## Relationships between hepatic function, cognitive status and cerebral macrostructure in Alcohol Use Disorder patients (AUD)

A-L .Pitel<sup>1</sup>, A. Lanquetin<sup>2</sup>, S. Segobin<sup>1</sup>, S. Wilson<sup>1</sup>, A. Laniepce<sup>1</sup>, F. Vabret<sup>3</sup>, N. Cabé<sup>1</sup>, L. Coulbault<sup>4</sup>, F. Eustache<sup>1</sup>, D. Vivien<sup>2,5</sup>, M. Rubio<sup>2</sup>

1. Normandy Univ, UNICAEN, PSL Université, EPHE, INSERM, U1077, CHU de Caen, GIP Cyceron, Neuropsychologie et Imagerie de la Mémoire Humaine, Caen, France.

2. Normandie Univ, UNICAEN, INSERM, UMR-S U1237, Physiopathology and Imaging of Neurological Disorders, 14000 Caen, France

3. Addiction department, Centre Hospitalier Universitaire de Caen, France.

4. Caen University Hospital, Biochemistry department, Normandie University, UNICAEN, Caen, France.

5. Department of Clinical Research, CHU Côte de Nacre, Caen, France.

**Introduction:** Alcohol Use Disorder (AUD) has been associated with cognitive impairments, brain macrostructure alterations and liver diseases but their relationship remains unclear. Moreover, their inherent pathophysiological mechanisms are still unknown. Some studies found no relationship between biological markers of liver function and structural brain abnormalities while others reported correlations between increased GGT levels and both gray and white matter atrophy. GGT level is not a specific measure of alcoholic liver disease, unlike liver fibrosis (estimated by Fibrometre®) which has, however, never been compared to structural brain alterations or cognitive impairment. Our aim was to explore the relationships between liver function and brain structure and cognitive function in AUD patients recently after detoxification.

**Mat & met:** Thirty-two recently detoxified AUD patients and 20 healthy controls (HC) underwent a neuropsychological evaluation, a volumetric T1-weighted MRI examination and blood sample tests. First, between-group comparisons were conducted between AUD and HC on the neuropsychological scores, measures of GGT levels, fibrosis scale and gray matter volumes using voxel-based morphometry (SPM12). Second, to establish the relationship between these measures, correlations were carried out between hepatic enzyme levels and liver fibrosis scales, gray matter volumes and cognitive performance.

**Results:** The neuropsychological evaluation showed lower results in AUD patients than in HC in executive functions, balance and working memory. AUD patients presented decreased gray matter volume in the frontal cortex, cerebellum, cingulate and limbic structures including the thalamus and the hippocampus (FWE,  $p < 0.05$ ,  $k = 100$ ). Compared to HC, AUD patients presented GGT levels and mild liver fibrosis. Liver fibrosis negatively correlated with motor abilities of the upper limb and the putamen volume. GGT levels negatively correlated with gray matter volumes in the left middle frontal gyrus and the right temporal gyrus, as well as with cerebellar ataxia.

**Conclusion:** First, our results confirm previous findings regarding GGT levels, liver fibrosis, gray matter abnormalities and neuropsychological deficits in AUD patients. To our knowledge, this is the first study describing the profile of gray matter and neuropsychological alterations in AUD patients with mild liver fibrosis. We find a correlation between liver alteration (GGT levels or fibrosis) and global motor impairment without associated neuropsychological alterations. Our data also reveal a link between the level of GGT in serum and alteration of some brain regions presenting with a particular sensitivity. Taken together, the two different profiles of gray matter alterations observed between GGT levels and hepatic fibrosis may reflect two different pathophysiological mechanisms. While hepatic fibrosis might be caused by oxidative stress in the liver, increased GGT levels might reflect oxidative stress not only in the liver, but also in the brain as a result of chronic alcohol consumption. Neuropsychological and neuroimaging correlations with liver impairment both support the brain-liver axis role in AUD.

**Key words:** Alcohol use disorders, liver fibrosis, neuroimaging, neuropsychology.

# **Acute IP administration of N-acetylcysteine prevents the activation of the mesocorticolimbic system triggered by intra-VTA ethanol administration in rats**

Fernández-Rodríguez S, Esposito C, Granero L, Polache A, Zornoza T, Cano-Cebrián MJ  
Department of Pharmacy and Pharmaceutical Technology and Parasitology  
University of Valencia

Several studies have explored the potential efficacy of prolonged N-acetylcysteine (NAC) treatments, to regulate/modify ethanol-related behaviours. Its effects has been attributed, at least in part, to the ability of prolonged NAC treatment to reverse ethanol induced plasticity. In this sense, one of the most widely accepted hypotheses assumes that chronic NAC treatment is able to restore the expression of glial transporters, such as xCT and GLT-1, contributing to the normalization of the glutamate function in the striatal system. However, on the other hand, recent research has also shown that an acute dose of NAC is enough to limit motivation, seeking behaviour and reacquisition after ethanol self-administration in rats. In order to give a plausible explanation to these latest results, in the present work, we have explored whether NAC is able to counteract the activation of mesocorticolimbic system triggered by acute intra-VTA ethanol administration. To this end, we combined local administration of 150 mM of ethanol in the posterior Ventral Tegmental Area (pVTA), with the systemic administration (IP) of NAC (60 or 120 mg/Kg) 30 min before. Afterwards, we measured the neuron cFOS immunoreactivity in the Nucleus Accumbens by immunohistochemical analysis. Our results show that 120 mg/kg of NAC, but not 60 mg/kg, prevented the increment in the cFOS immunoreactivity induced by ethanol. In this scenario, an alternative mechanism of action of NAC, apart from the above mentioned, cannot be ruled out. Therefore, further experiments are necessary to elucidate the mechanism of action of NAC.

## Real-time Drinking, Age of Drinking Onset, and Other Drug Use Predict Adverse Alcohol Use Consequences of Tertiary Students

Antoinette Poulton<sup>1</sup>, Adrienn Mata<sup>1</sup>, Jason Pan<sup>1</sup>, Loren Richard Bruns<sup>2</sup>, Richard O. Sinnott<sup>2</sup>, Robert Hester<sup>1</sup>

<sup>1</sup>Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Australia

<sup>2</sup>Computing and Information Systems, University of Melbourne, Parkville, Australia

**Background:** Compared to their non-university enrolled peers, tertiary students consume greater quantities of alcohol, are at increased risk of injury/harm, and have higher rates of alcohol use disorders. The Brief Young Adult Alcohol Consequences Questionnaire (BYAACQ) is often utilised to explore adverse alcohol-related outcomes among tertiary students. Alcohol consumption behaviour assessed via retrospective summary measures has been linked to BYAACQ score. It is unclear, however, how drinking assessed in real-time, in conjunction with variables such as age of drinking onset and other drug use might predict severity of adverse alcohol consequences as captured by the BYAACQ.

**Methods:** The psychometric properties of the BYAACQ were explored using a large Australian sample of tertiary students ( $N = 893$ ). A subsample ( $n = 504$ ) provided alcohol intake information in real-time (21 days) via a smartphone app (CNLab-A) plus age of drinking onset, drug use, and parental alcohol/drug use details.

**Results:** Average BYAACQ score for the full sample was 7.23 ( $SD = 5.47$ ). Classical and item response theory analyses revealed some inconsistencies related to progressive item severity and male/female differential item functioning. Current drinking – namely, frequency of intake and quantity per drinking occasion – plus age of drinking onset and other drug use accounted for 33.9% of the variance in BYAACQ score after controlling for age and depression symptomology.

**Conclusions:** Information related to current drinking, age of drinking onset, and drug use is useful for predicting severity of alcohol use consequences. These markers might enable tertiary institutions to target students for prevention/intervention programs.

# **DNA Methylation Analysis of the GABRA2 receptor subunit in alcohol dependence**

Ulrich W. Preuss<sup>1,2</sup>, Gabriele Koller<sup>3</sup>, Peter Zill<sup>3,4</sup>

<sup>1</sup> *Department of Psychiatry, Psychotherapy and Psychosomatics, Martin-Luther-University, Halle-Wittenberg, Julius-Kühn Str. 7, 06112 Halle/Saale;* <sup>2</sup> *Vitos Hospital Psychiatry and Psychotherapy, Austr. 40, 35745 Herborn;* <sup>3</sup> *Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University Munich, Nussbaumstrasse 7, 80336 Munich, Germany;* <sup>4</sup> *Division of Psychiatric Genetics and Neurochemistry, Ludwig-Maximilians-University Munich, Nussbaumstrasse 7, 80336 Munich, Germany;*

**Background:** Variants of GABRA2 have been repeatedly associated with alcohol dependence risk. However, no study investigated potential epigenetic changes in GABRA2 CpGs between alcohol-dependent (AD) subjects and controls and relationship to AD characteristics.

**Methods:** In the present study, blood samples for promoter-related GABRA2 CpG and genome wide global methylation were obtained from n = 57 AD subjects and 51 controls which were clinically assessed by structured interviews. Global 5-methylcytosin (5-mC) methylation was measured using ELISA kits for 5-mC. Assessment of random DNA methylation status was accomplished using the [3H] methyl group incorporation assay.

**Results:** While no significant difference in GM was detected across groups, after controlling for age and gender, measures of GABRA2 epigenetic changes yielded a significant hypomethylation of several CpG sites. Hypomethylation was related to recent alcohol intake, duration of AD and withdrawal severity.

**Summary:** The results indicate a significant epigenetic change in GABRA2 methylation which is also related to AD severity. Further studies are needed to determine the effects of epigenetic changes on GABRA2 expression and longitudinal changes of epigenetic regulation over time to clarify the pathophysiological relevance of the findings.

# **In search of perfection: defining new approaches to improve the EIBI-culture in general practice.**

PUSSIG Bram  
*KU Leuven*

## Background

Early Identification and Brief Interventions (EIBI) in primary care is a proven (cost-)effective method for reducing harmful drinking in society<sup>(1-5)</sup>. Unfortunately, integration in daily practice is low<sup>(6-8)</sup>. It is suggested that a more integrated EIBI-culture is necessary to fully utilise its effect<sup>(9-11)</sup> and that community involvement might provide a tool to stimulate that <sup>(10,12)</sup>. The still suggestiveness of this approach makes the stakeholders' point of view essential for defining community-oriented actions to stimulate the negotiability of alcohol use in primary care; creating a beneficial setting for EIBI delivery.

## Aim

The aim of this study is to define community-oriented strategies to stimulate the negotiability of alcohol use in general practice.

## Method

Stakeholders will be asked to define community-oriented strategies to normalise the negotiability of alcohol use in general practice. The nominal group technique will be applied, it allows generating ideas within a stakeholder population while creating consensus. Forty-eight stakeholders from the municipality of Leuven will be divided into four heterogeneous nominal group sessions. These sessions combine individual and group work that comprises generating ideas, sharing ideas, discussing ideas and voting on ideas; resulting in immediate action planning. All results will be merged into an overarching list, based on validated implementation frameworks. A member check session will be conducted to ensure correct interpretation of the results.

## Results

A prioritised list of scientific robust and stakeholder-inspired strategies, for stimulating the negotiability of alcohol use in general practice, is generated. Developed community-oriented actions will be presented at the conference.

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## Role of Estrogen in the gender effect of Binge-drinking on hippocampus LTD in young adult rats

K. Rabiant, J. Antol, M. Naassila and O. Pierrefiche

INSERM UMR 1247 Groupe de Recherche sur l'Alcool et les Pharmacodépendances,

UPJV, CHU, Amiens

Binge-drinking is responsible for memory impairments especially during adolescence. Recent studies highlighted a higher sensitivity of women brain regarding the effects of ethanol. However, the reason behind such difference remains unclear. Here, we tested the hypothesis that estrogen level (E2) play a role in the gender difference observed in the effects of ethanol on hippocampus plasticity, the cellular mechanisms implicated in learning and memory processes. Long-Term Depression (LTD) was recorded in hippocampus slices 24h after 2 binge-like ethanol exposure (3g/kg, i.p.) applied during the different phases of the oestrus cycle. We also used exogenous E2 (180µg/kg) with and without concomitant presence of ethanol during specific phases of the oestrus cycle in postpubertal rats and prepubertal female rats. We found that neither oestrus cycle nor ethanol binge alone had an effect on LTD magnitude in postpubertal female rats at 24h delay. However, LTD was abolished at 24h delay only when ethanol was injected during the endogenous peak of E2. Such abolition of LTD was also obtained when co-injection of E2 and ethanol was performed in either postpubertal female rats in low E2 phase or prepubertal rats. Finally, we measured a similar abolition of LTD in male rats at 24h delay when ethanol was injected with a higher dose of ethanol (3.75g/kg, i.p.). Our results showed that for the same dose of ethanol, LTD in female rats is more sensitive than in males, especially when ethanol was present at the same time that a high level of estrogen. These results suggests that E2 plays a role in the gender difference of ethanol effects in hippocampus plasticity.

Key words: Binge-Drinking, Estrogen, Gender difference, Plasticity, Hippocampus

# Alcohol consumption and gender gap in cardiovascular mortality in Europe

YURY Razvodovsky

Grodno State Medical University

**Background:** Cardiovascular disease (CVD) is the largest contributor to the morbidity and mortality in Europe. Mortality from cardiovascular disease remains substantially higher among men than among women. The level of alcohol-related problems differs substantially across Europe, with Eastern European countries experiencing higher burden of alcohol-attributable morbidity and mortality than Western European countries. **Objective:** This study aims to test the hypothesis that alcohol plays an important role in explaining the gender gap in CVD mortality in Eastern Europe. **Methods:** The male-to-female ratio of CVD mortality and the level of alcohol consumption per capita in Western (n 21) and Eastern European (n 24) countries were compared. The male-to-female ratio of CVD mortality (the five-year average from 2010 to 2014) was calculated. The comparison in the gender gap in CVD mortality was made between Western (n 21) and Eastern (n 24) European countries (t-test). **Results:** The results of the correlation analysis indicate statistically significant relationship between alcohol consumption per capita and gender gap in CVD mortality in Eastern Europe. The relationship between alcohol consumption and gender gap in CVD mortality in Western Europe is also positive, but statistically non-significant. **Conclusion:** Alcohol appears to play an important role in the gender gap in CVD mortality in the countries of Eastern Europe.

# Physical activity reverses the increase in ethanol oral self-administration induced by social defeat in male mice

Marina D Reguilón, Carmen Ferrer-Pérez, José Miñarro and Marta Rodríguez-Arias  
*Unit of Research on Psychobiology of Drug Dependence, Facultat de Psicologia,  
Universitat de València, Avda Blasco Ibáñez 21, 46010, Valencia, Spain.*

Preclinical and clinical studies have shown that exposure to stress increases drug-seeking and relapse into ethanol (EtOH) consumption by modifying the activity of brain areas involved in the rewarding effects of EtOH. In a previous study we demonstrated that exposure to repeated social defeat (RSD), a model of social stress, produced a long-term increase in the consumption of EtOH. The aim of the present work was to evaluate if exposure to physical activity can block the increase in EtOH consumption induced by RSD. Mice were exposed to 4 sessions of repeated social defeat in which they were confronted with an aggressive animal (resident), while the control groups were exposed to a similar situation without an aggressive opponent (exploration). During the whole procedure, half of the mice were exposed to controlled physical activity, being allowed 1h access to a low-profile running wheel three times a week. Three weeks after the last social defeat, animals began oral self-administration (SA) of ethanol (6% EtOH). Our results show that the socially defeated animals not exposed to physical activity consumed greater amounts of ethanol and showed greater motivation to obtain the substance than the non-stressed group. Defeated animals that performed physical activity behaved similarly to the non-stressed exploration group. Therefore, our results confirm that controlled physical activity can reverse the effects of social stress on EtOH consumption.

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## INCIDENTAL NEOPLASIA AND MORTALITY AMONG HEAVY ALCOHOLICS

Iván Ribot, Victor E. Vera Delgado, Lourdes Gonzalez Navarrete, Paula Reyes Suárez, Ana Godoy Reyes, Candelaria Martín González, Esther Martín Ponce, Emilio González Reimers.

Servicio de Medicina Interna. Hospital Universitario de Canarias. Tenerife, Canary Islands (Spain)

We included 255 alcoholic patients (aged  $59 \pm 11$ ; drinkers of  $200 \pm 150$  g/day during  $32 \pm 12$  years), consecutively admitted to the Internal Medicine Service of our hospital due to organic complications of alcoholism. who were followed up during a period of 5 years. During this time 103 died. 110 were cirrhotics; 61 of them died during this period, and mortality was clearly related to liver cirrhosis (Log rank (LR)=9.26;  $p=0.002$ ). Thirty nine patients developed neoplasia, 18 among cirrhotics and 21 among non cirrhotics ( $\chi^2=0.034$ ; NS). The development of neoplasia was not related to liver function (assessed by child-Pugh's score, LR=2.5; NS), but it was marginally related to the amount of ethanol ingested (LR=2.97;  $p=0.08$ ). Survival was significantly related with cancer (LR=5.46;  $p=0.019$ ), especially among non-cirrhotics (LR=13.026;  $p<0.0001$ ), but *not* among cirrhotics (LR=0.016; NS). The total amount of ethanol ingested was not related to mortality either in the whole group (LR=0.85; NS), non-cirrhotics (LR=0.51), or cirrhotics (LR=2.1), but mortality was associated with Child-Pugh's classification (LR=9.81;  $p=0.007$ ). By Cox regression model the variable cirrhosis entered in the first place in relation with survival, followed by the variable cancer. 150 patients underwent assessment of handgrip strength using a Collins dynamometer. Mortality was clearly related to handgrip strength (LR=8.1;  $p=0.004$ ). However, handgrip was displaced by liver function and cancer using Cox regression model. Therefore, we conclude that neoplasia is a common finding among heavy alcoholics, and is related to mortality both in the whole group and, especially, among non-cirrhotics. If cirrhosis has already developed, an incidental neoplasia has no effect on mortality. Although handgrip strength was associated with mortality in the univariate analysis, it was displaced by liver function and neoplasia in the Cox regression analysis.

## **EFFECTS OF ALCOHOL HANGOVER ON WORKING MEMORY PROCESS IN UNIVERSITY STUDENTS - AN ELECTROENCEPHALOGRAPHY STUDY**

Rui Rodrigues<sup>1</sup>, Natália Antunes<sup>1</sup>, Eduardo López-Caneda<sup>1</sup>, Adriana Sampaio<sup>1</sup>, Alberto Crego<sup>1</sup>

<sup>1</sup>Psychological Neuroscience Lab, Research Center in Psychology (CIPsi), School of Psychology, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal

Binge drinking (BD), characterized by an excessive intake of alcohol -5 or more drinks for males and 4 or more for females in two hours or less-, leading to elevated blood alcohol concentration (BAC), equal or higher than 0,08 g/dL over a brief period followed by abstinence, has acquired great attention due to its negative social and health consequences (car crashes, assaults, low academic performance, cardiovascular diseases), as well as its high prevalence among adolescents. These data, alarming by themselves, become even more worrying considering that adolescence is an especially vulnerable period to the neurotoxic effects of alcohol due to the structural and functional changes undergoing in the brain at this stage.

It is also known that alcohol intoxication has major detrimental effects on functioning the following day (e.g., lentification, difficulties in maintaining focus, poor decision making). Furthermore, after full metabolism of the alcohol consumed, symptoms like headache, diarrhea, tremulousness, fatigue or nausea may appear. The presence of two or more of these symptoms as the outcome of excessive alcohol intake is called hangover. Literature has reported that hangover can significantly decrease alertness and arousal performance which can be caused by a decrease in the sleep quality, affecting the ability to react quickly, and psychomotor ability in drive-tasks. However, despite the importance of the immediate consequences of BD to daily life, few are the studies that so far have evaluated the short-term effects of a BD session on neurocognitive functioning of the adolescent/young brain and, in our knowledge, no studies have used electrophysiological methods.

The aim of the present investigation was to assess the behavioral and electrophysiological consequences on the working memory processes in the real day after a BD session, during hangover state, in young people. For that purpose, we recorded EEG of 10 university students (6 females) with a BD consumption pattern while they execute a one-back task, in both a normal and a hangover day. The reaction times and percentages

of correct responses, as well as the latency and amplitude of P2, N2, P3 and Late Positive Component (LPC) were compared in both moments.

Despite having found no significant differences at the behavioural level, the hangover was associated with electrophysiological anomalies. The amplitude of ERP components analyzed was smaller in the hangover state. The lower amplitude of early ERP components as P2, N2 and P3 may indicate that during hangover subjects showed difficulties to cope as well with the attentional demands of the working memory task in regards of the constant updating of information; while smaller amplitude of LPC can suggest that subjects show latent deficits in the ability to recollect or maintain information during the hangover state. Further studies with a larger sample are needed to clarify the detected electrophysiological anomalies.

**Gut barrier disruption in alcohol-related liver disease is characterised by broad alterations of physiological transcriptional profiles and increased bacterial translocation**

Riva A<sup>1,2</sup>, Bajaj J<sup>3</sup>, Fagan A<sup>3</sup>, Williams R<sup>1,2</sup>, Chokshi S<sup>1,2</sup>

<sup>1</sup>Institute of Hepatology (Foundation for Liver Research), London, UK; <sup>2</sup>Faculty of Life Sciences and Medicine (King's College London), London, UK; <sup>3</sup>Department of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University and McGuire VAMC, Richmond, Virginia, USA

Liver cirrhosis induces profound immune suppression (cirrhosis-associated immune dysfunction, CAID) accounting for increased susceptibility to bacterial infection and mortality. We have previously shown the 'leaky' gut as pivotal in driving this phenomenon in Alcohol-related Liver Disease (ALD) (Riva et al, Gut 2018; Markwick et al, Gastroenterology 2015), with bacterial translocation due to increased gut permeability stimulating systemic/intrahepatic inflammation. However, the pathways that underlie gut hyper-permeability in ALD are not well understood.

As part of a "European Foundation for Alcohol Research" (ERAB)-funded study, we performed colon transcriptomics in 10 alcohol-cirrhotic patients (ARC) and 10 healthy controls (HC) (~20,000 genes, ArrayStar). We investigated differential gene expression and functional clustering by "Gene Ontology" (GO), "Kyoto Encyclopaedia of Genes and Genomes" (KEGG) pathways and "Gene Set Enrichment Analysis" (GSEA). We also measured surrogate markers of bacterial translocation (D-Lactate, Endotoxin) as indicative of gut barrier disruption.

~8,400 genes were differentially expressed in ARC vs HC colon. Most upregulated genes were functionally involved in transcriptional processes, ribosomal/chromatin structure, intercellular adhesion and energy metabolism. GSEA identified upregulated adhesion pathways in ARC vs HC, driven by tight junction genes including claudin 3, occludin and ZO-1. Conversely, downregulated genes did not cluster significantly. Quantification of plasma D-Lactate and endotoxin (Lps) indicated the presence of gut barrier disruption and bacterial translocation in ARC vs HC.

In conclusion, transcriptional profiling highlights major alterations in intestinal cell adhesion pathways in ALD patients. We are currently investigating these pathways in experimental models as new targets to restore barrier integrity in ALD.

**Don't stress the amygdala – the role of pro- and anti-stress amygdalar systems in alcohol use disorder (AUD)**

Marisa Roberto PhD

*The Scripps Research Institute, Department of Neuroscience*

(Missing abstract)

# Chronic alcohol exposure in alcohol-preferring (sP) rats provokes mild brain and liver inflammatory responses and a specific atrophy of the corpus callosum

M. Rubio<sup>1</sup>, A. Lanquetin<sup>1</sup>, M. Naveau<sup>2</sup>, P. Maccioni<sup>3</sup>, I. Lorrain<sup>3</sup>, G. Colombo<sup>3</sup>, D. Vivien<sup>1,4</sup>, A.L. Pitel<sup>5</sup>

1. Normandie Univ, UNICAEN, INSERM, UMR-S U1237, Physiopathology and Imaging of Neurological Disorders, 14000 Caen, France

2. Normandie Univ UNICAEN, CNRS, UMS 3408, GIP Cyceron, Caen, France

3. Neuroscience Institute, Section of Cagliari, National Research Council of Italy, 09042 Monserrato (CA), Italy

4. Department of Clinical Research, CHU Côte de Nacre, Caen, France

5. Normandy Univ, UNICAEN, PSL Université, EPHE, INSERM, U1077, Neuropsychologie et Imagerie de la Mémoire Humaine, CHU de Caen, GIP Cyceron, Caen, France

**Introduction:** Neuroimaging and neuropsychological studies reveal structural and functional brain alterations associated with chronic, excessive alcohol consumption. In 50 to 80% of Alcohol Use Disorder (AUD) patients, these brain alterations result in cognitive and/or motor impairments. In addition to this, 70 to 80% of AUD patients show hepatic damage that can vary from steatosis to cirrhosis. The relationship between hepatic damage and brain dysfunction in AUD is not well understood to date and has been mainly investigated within the context of acute hepatic encephalopathy. Here, we have studied macroscopic and microscopic brain and liver alterations induced by voluntarily consumed alcohol in selectively bred Sardinian alcohol-preferring (sP) rats, a validated model of excessive alcohol consumption.

**Mat & met:** *Post mortem* brain and liver studies were performed in sP rats exposed to the homecage, 2-bottle “alcohol (10% v/v) vs water” choice regimen with unlimited access for 12 months. Control rats were exposed to 2 water bottles. Liver inflammation was assessed by immunohistochemical analyses. Brain anatomical measurements were conducted by T2-weighted MRI scans. Regional brain astrogliosis and microgliosis were evaluated by immunohistochemical analysis.

**Results:** Weekly alcohol intake averaged 35-45 g/kg over the 12-month period.

Our results showed mild but significant inflammatory responses in the liver of alcohol-drinking sP rats, with (i) increased numbers of Kupffer cells (Iba1+); and (ii) overactivation of hepatic stellate cells (GFAP+).

Concerning the brain, we observed a generalized inflammatory response revealed by increased numbers of microglial cells and astrocytes in cortex, corpus callosum, and hippocampus of alcohol-drinking sP rats. These inflammatory responses were accompanied by a specific atrophy of the corpus callosum in alcohol-drinking sP rats, with no changes in hippocampus, cerebellum nor the total brain volume.

**Conclusion:** We describe here, for the first time, inflammatory responses to long-term alcohol drinking in liver and brain of alcohol-preferring sP rats. These inflammatory responses could be triggered by increased plasmatic LPS levels, which has been recently described in the same set of alcohol-drinking sP rats (Posteraro *et al.*, 2018). Interestingly, the specific atrophy of the corpus callosum suggests a vulnerability of this region to chronic alcohol exposure, and is in accordance to human studies (Oscar-Berman, 2003). The absence of anatomical changes in other regions of the brain could, on the contrary, suggest a specific resistance to alcohol damages. Behavioral studies are needed to determine the impact of these macro- and microscopic alcohol-induced alterations.

**Key words:** Alcohol use disorder, sP rats, liver, brain, MRI, inflammation

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# Nalmefene alleviates alcohol-induced neuroinflammation: a PET imaging study with [<sup>18</sup>F]DPA-714 in an adolescent rats

W SABA, G POTTIER, M GOISLARD, C LEROY, J NEGRONI, S DEMPHEL, F CAILLE, C COULON, E JAUMAIN, B JEGO, N TOURNIER

IMIV, CEA-SHFJ, 4, place du Général Leclerc 91400 Orsay, France

## Introduction

Alcohol exposure during adolescence induces important and long-lasting brain damages, which are assumed to involve neuroinflammatory processes. [<sup>18</sup>F]DPA-714 PET imaging targeting the glial biomarker TSPO (Translocator Protein 18kDa) was performed to i) non-invasively assess the neuroimmune component of alcohol-related neurotoxicity in a binge-like ethanol exposure in adolescent rats and ii) to evaluate the impact of nalmefene treatment on alcohol-induced neuroinflammation.

## Materials and Methods

Adolescent rats (n=6-10 animals /group)) received an i.p. injection of ethanol [3 g/kg in 25% (v/v)] or saline (control) in a validated intermittent administration pattern (two consecutive days at 48-h intervals over a 14-day period)<sup>(1)</sup>. In another group, nalmefene (0.4 mg/kg, s.c) was injected 1 hour prior to ethanol. MicroPET imaging with [<sup>18</sup>F]DPA-714 (~37 MBq, i.v.) was performed 24h after the last alcohol/saline injection. In each experiment, the brain distribution of [<sup>18</sup>F]DPA-714 was estimated in different brain areas using the Logan Plot analysis and the metabolite-corrected arterial input function. Blood alcohol levels obtained in the model were measured in an independent group using gas chromatography.

## Results

Ethanol administration to adolescent rats induced a blood alcohol concentration of  $2.40 \pm 0.5$  g/L at 5 min after injection. The regional  $V_{Ts}$  of [<sup>18</sup>F]DPA-714 in alcohol exposed animals ( $V_{T \text{ hippocampus}} = 21.1 \pm 2.7$  and  $V_{T \text{ accumbens}} = 25.3 \pm 6.1$ ) were significantly increased when compared to control animals ( $V_{T \text{ hippocampus}} = 5.9 \pm 0.8$  and  $V_{T \text{ accumbens}} = 7.07 \pm 1.3$ ). Nalmefene significantly alleviated the alcohol-induced increase in [<sup>18</sup>F]DPA-714 binding ( $V_{T \text{ hippocampus}} = 14.02 \pm 5.22$  and  $V_{T \text{ accumbens}} = 18.5 \pm 5.4$ ). The effects of alcohol and nalmefene were homogeneously observed in all brain areas.

## Discussion

These results support the neuroinflammatory hypothesis of alcohol-related brain toxicity and suggests that nalmefene may protect from this neuroinflammation. PET imaging using [<sup>18</sup>F]DPA-714 is a relevant technique to investigate the neuroinflammatory component of alcohol exposure in animal models and patients.

**Funding** : Fondation pour la recherche en alcoologie

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## Dimensionality and Scale Properties of DSM-5 Alcohol Use Disorder

Tulshi D Saha and Patricia Chou  
National Institute on Alcohol Abuse and Alcoholism  
National Institutes of Health  
6700 B Rockledge Drive, Bethesda, Maryland, USA  
Email: [sahatd@mail.nih.gov](mailto:sahatd@mail.nih.gov)

### Abstract

Changes to the DSM-5 criteria for alcohol use disorder (AUD) included eliminating the legal problems criterion and adding a new criterion, alcohol craving. DSM-IV abuse and dependence were based on discrete sets of 4 abuse and 7 dependence criteria, but all 11 criteria apply toward DSM-5 mild (2-3 criteria), moderate (4-5 criteria) and severe (6 or more criteria) AUD diagnoses.

Using Item Response Theory (IRT), the purpose of this study was to evaluate these cutoff points for DSM-5 AUD severity using US adult data from NESARC, IRT analyses provided test and information functions to examine the different level of AUD severity and evaluate the interpretation of these severity level.

There was no significant gain of information from DSM-5 criteria compared to DSM-IV criteria. The IRT information function curve showed that equal ranges in raw scores did not correspond to equal ranges in the latent AUD severity measure. For a cutoff score of 2+ (mild AUD), the latent severity level was between 1.2 and 1.6 (a range of 0.7 in logit units), for a cutoff score of 4+ (moderate AUD), the latent severity level was between 1.9 and 2.1 (a range of just 0.2 in logit units). That is, as latent severity increased, the contribution of each additional criterion diminished. For cutoff score of 6+ (severe AUD), the latent severity level was greater than 2.4 in logit units.

The interpretability and practicality of the DSM-5 AUD severity cut points can be enhanced through the evaluation of IRT test and information functions.

## Long lasting epigenetic marks of alcohol on circadian and stress regulatory genes.

Dipak K. Sarkar

Rutgers Endocrine Research Program, Department of Animal Sciences, Rutgers University, New Brunswick, NJ 08901, USA

Epigenetic modifications of a gene have been shown to play a role in maintaining a long-lasting change in gene expression. DNA methylation occurring at CpG dinucleotides is the most common epigenetic modification that constitutes an important regulatory element in human genome. Epigenetic alterations believed to occur early in disease state, thus providing the possibility of early diagnosis. As stress and circadian physiological systems governing many body functions are often dysregulated in alcohol dependent patients, we sought to test whether epigenetic changes of proopiomelanocortin (*Pomc*) and period 2 (*Per2*) genes, critical for stress and circadian regulation, is long-lasting and may serve as measures of behavioral motivation for alcohol. To test this, we first studied the dose response and time course effects of alcohol on *Per2* and *Pomc* gene methylation and gene expression in isolated mouse-derive POMC cells in cultures, and found binge-like ethanol concentrations increase DNA methylation while decrease mRNA expression of *Per2* and *Pomc* genes for several days beyond the day of ethanol exposures. In human, we found pregnant women who consumed moderate to high levels of alcohol and gave birth to prenatal alcohol exposed (PAE) children had higher DNA methylation of *POMC* and *PER2*. PAE children also had increased methylation of *POMC* and *PER2*. In adult humans, non-smoking moderate, non-binging compared to binge and heavy alcohol drinkers, we found increased methylation of the *PER2* and *POMC* DNA, reduced expression of these genes in the blood samples of the binge and heavy drinkers relative to the moderate, non-binge drinkers. Increased *PER2* and *POMC* DNA methylation was also significantly predictive of both increased levels of subjective alcohol craving immediately following imagery, and with presentation of the alcohol (2 beers) prior to the alcohol taste test, as well as with alcohol amount consumed during the alcohol taste test. These data establish significant association between binge or heavy levels of alcohol drinking and elevated levels of methylation and reduced levels of expression of *POMC* and *PER2* genes. Furthermore, elevated methylation of *POMC* and *PER2* genes is long-lasting and is associated with greater subjective and behavioral motivation for alcohol. (Supported by NIH/NIAAA grants U24 AA014811, AA08757, AA025359)

# Assessing decision-making impairments in a rodent model of Binge Drinking using relevant tools : The Rat Gambling Task and ex-vivo Fast-Scan Cyclic Voltammetry

P. SAUTON, E. NEGRIA, V. JEANBLANC, J. JEANBLANC and M. NAASSILA

Groupe de Recherche sur l'Alcool et les Pharmacodépendances INSERM UMR 1247, UPJV, CHU, Amiens

Decision-making (DM) is an essential cognitive process resulting in the most advantageous choice among several alternatives. DM has been shown to be altered in alcohol-dependent patients and the few reports in Binge Drinking (BD) subjects are contradictory. The aim of this study was to test whether the BD pattern of consumption is able to alter the DM performances as well and study the dopaminergic correlates within the nucleus accumbens.

In this study, we used a Rat Gambling Task (RGT) paradigm, which is based on the Iowa Gambling Task used in Humans, and two models of BD administration: the first one is an operant self-administration model of BD that we developed and the second is a commonly used intermittent forced administration of intoxicating doses of ethanol. Secondly, we used ex-vivo fast-scan cyclic voltammetry (FSCV) and dopamine D2 receptors (D2R) pharmacology in order to identify changes in the dopaminergic transmission in the NAc involved in these deficits. We show that BD rats made significantly less advantageous choices than control rats, and that the BD significantly increases the proportion of rats with a poor level of DM. Our FSCV results allowed us to identify a characteristic profile of bad DM and the involvement of D2R in the effect of alcohol on the dopaminergic transmission.

Being able to model the altered DM caused by alcohol and finding relevant therapeutic targets leads to important perspectives in order to study the neurobiological mechanisms involved in such a deleterious behavior that BD is.

Keywords : Binge Drinking, Rat Gambling Task, Dopamine, Nucleus Accumbens, Voltammetry

# **Measuring alcohol craving after virtual reality exposure: A method comparison in social drinkers**

Jessica Simon, Léonie Schroder & Etienne Quertemont  
*Psychology & Neuroscience of Cognition – PsyNCogn, ULiège, Belgium*

Craving contributes to the maintenance and relapse of alcohol dependence. Some methods, more or less explicit, are used to estimate this subjective state: on one hand, single items or validated questionnaires and on the other hand, ad-libitum taste test. In this second paradigm, participants are invited to taste and evaluate the organoleptic properties of several alcoholic beverages. The total amount of liquid drunk is an indirect measure of craving. The objective of the present study is to compare self-reported measures of craving with measurements from the ad-libitum tasting test. We hypothesize a relationship between the implicit and explicit measures of craving. In addition, we will attempt to determine the most valid measure of craving. To do this, we will determine which measure is most strongly correlated with the AUDIT score and the obsessive compulsive drinking scale score. 46 social drinkers will be recruited for this experiment and immersed in a virtual environment including alcohol-related cues, supposed to generate craving, before evaluating it with single items and ad-libitum taste test.

## **Impact of Chronic Alcohol Consumption and Withdrawal on Hippocampus or Striatum-dependent Learning and Related Synaptic Plasticity**

Léa Tochon<sup>1,2</sup>, Rose-Marie Vouimba<sup>1,2</sup>, Jean-Louis Guillou<sup>1,2</sup>, Daniel Béracochéa<sup>1,2</sup> and Vincent David<sup>1,2</sup>

<sup>1</sup> CNRS UMR 5287, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux, France

<sup>2</sup> Université de Bordeaux, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux, France

The hippocampus and striatum have dissociable roles in memory: while the former is necessary for spatial/declarative forms of learning, the latter underlies cued/procedural learning. An emerging hypothesis suggests that drug addiction could lead to a functional cognitive imbalance, which would maintain addictive behaviour and support the risk of relapse by promoting habit learning while concurrently disrupting spatial memory. We examined, in C57BL/6J male mice, whether chronic ethanol consumption (5 months) or withdrawal might modulate the use of spatial memory vs cued memory, and related hippocampal and striatal synaptic plasticity. Using a competition protocol in the Barnes maze assessing the respective use of hippocampus vs striatum-dependent learning strategies, we first show that alcohol withdrawal, and also to a lesser extent alcoholization, drastically increase the use of non-flexible, striatum-dependent cued strategies in a task solved with spatial memory in 95% of non-alcohol exposed mice. Concurrently, by performing *in vivo* electrophysiological studies in freely-moving mice to assess learning-induced synaptic plasticity in both the dorsal hippocampus (CA1) and dorsolateral striatum (DLS), we found that task-induced synaptic plasticity activity was reduced in the CA1 and increased in the DLS of withdrawn mice, and to a lesser extent, in alcohol mice as compared with controls. Furthermore, the capacity to induce LTP in the CA1 was impaired in both withdrawn and alcoholized mice. We conclude that early alcohol withdrawal and moderate chronic alcoholization, have disrupting effects on spatial memory processes and synaptic plasticity in the CA1, leading to the compensatory use of striatum-dependent learning strategies.

**Lack of alpha5 nicotinic receptors increases alcohol self-administration at high dose and reverses the pattern of alcohol-induced neuronal activity in VTA and IPN**

TOCHON Léa

*CNRS UMR 5287, Université de Bordeaux, Institut des Neurosciences Cognitives et Intégrative d'Aquitaine, Bordeaux, France*

(Missing abstract)

# Impact of alcohol exposure on the development and maturation of the cerebral cortex

Laura Van Hees, Sophie Laguesse, Laurent Nguyen

GIGA-Stem Cells, Interdisciplinary Cluster for Applied Genoproteomics (GIGA-R), University of Liège, C.H.U. Sart Tilman, Liège, Belgium.

Prenatal alcohol exposure (PAE) is known to damage the fetal brain and leads to life-long cognitive and behavioral dysfunctions. Fetal Alcohol Spectrum Disorders (FASD), which collectively describes the constellation of effects resulting from alcohol consumption during pregnancy, is a complex syndrome that affects up to 5% of children and is the leading cause of preventable intellectual disability. Despite prevention campaigns discouraging alcohol drinking during pregnancy, the number of children suffering from FASD has not decreased over the past years. The consequences of PAE have become a global public health problem and understanding the alcohol-related mechanisms is crucially needed to develop new pharmacological strategies and treatments. Studies have shown that alcohol interferes with the cerebral cortex development in a variety of ways, including defects in neurogenesis, impaired cell proliferation and cell migration, reduced survival and disrupted neurotransmission. However, the precise pathophysiological mechanisms underlying alcohol's actions on cortical development are yet poorly understood. In this study, we set up a mouse model of FASD, using an alcohol consumption paradigm in which mice voluntarily drink high amounts of alcohol throughout pregnancy. Importantly, this model avoids any bias resulting from maternal stress that could be introduced by stressful alcohol consumption procedures such as gavage or injection. We first showed that this model accurately reflects alcohol consumption in human, as mice reach blood alcohol concentration levels comparable to those reported in binge-drinking humans. In order to investigate alcohol-dependent corticogenesis defects, we are analyzing the number, proliferation and specification of glutamatergic projection neurons during embryonic development and at postnatal stages. By using in utero electroporation, we are investigating the migration pattern of projection neurons during neurogenesis. Our preliminary results reveal an abnormal accumulation of neurons in deep layers of the cortex of alcohol-exposed embryos, suggesting impaired neuronal migration or dysregulated layer specification. Analysis of radial migration at postnatal stage showed that projection neurons have finally reached the upper layer, similar to control. However, the morphology of neurons seems to be affected by prenatal alcohol exposure, especially at the level of apical dendrites. We thus plan to investigate more specifically the terminal differentiation and dendritogenesis of projection neurons of alcohol-exposed pups. We will also evaluate adult mice behavior and alcohol consumption in order to determine whether PAE has a long-term impact on adult behavior and drinking pattern.

# **Characterization of the behavioral sensitization and the conditioned response induced by long-term daily exposure to alcohol in DBA2/j and Swiss mice.**

VAN INGELGOM Théo

*Liege University*

Studies on the locomotor sensitization induced by repeated ethanol administrations in mice use typically experimental designs where duration of alcohol exposure is limited to a maximum of 21 days. Consequently, little or nothing is known about sensitization induced by a more prolonged ethanol exposure (exceeding 21 days). Therefore, the first aim of the present study is to characterize the behavioral sensitization induced in mice by an extended period of daily ethanol administrations (45 days). The second aim of the present study is to test whether ethanol sensitization results in a conditioned increase in locomotor activity when sensitized mice are confronted to a placebo test (saline injection) in the testing environment. This phenomenon, called conditioned response, has been well established with psychostimulants such as cocaine. However, the occurrence of an excitatory conditioned response after repeated exposure to a stimulant dose of alcohol is still being debated.

For these purposes, Swiss and DBA/2J, the two most popular mouse strains in the field, received 45 consecutive daily ethanol administrations (respectively 2.5 and 2.0 g/kg) and their locomotor activity was daily recorded to test the development of ethanol sensitization. At the end of the procedure, a placebo test and a challenge test were conducted to assess respectively the conditioned response and the inter-group ethanol sensitization.

The results of the present study show that ethanol sensitization continues to develop beyond the usual duration of ethanol exposure used in the previous studies. Thus, ethanol sensitization reach maximal levels after about 25 injections in DBA2/j mice and 40 injections in Swiss mice. However, it may be noted that the core phase of the development of ethanol sensitization occurred in both strains during the first 20 days. Remarkably, ethanol sensitization after such a long daily ethanol treatment resulted in both an upward shift of the magnitude of ethanol stimulant effects and a prolongation of these effects in time (up to 30 minutes). Finally, the results of the placebo test clearly indicated an absence of conditioned response in both strain of mice.

## ARTERIAL STIFFNESS AND TGF- $\beta$ AMONG ALCOHOLICS

Vera-Delgado Víctor, González-Navarrete Lourdes, Aguilera-García Selena, Melchor, Martín-González Candelaria, Romero-Acevedo Lucía, Martínez-Martínez Daniel, González-Reimers Emilio.

Servicio de Medicina Interna. Hospital Universitario de Canarias. Tenerife, Canary Islands (Spain)

Transforming growth factor beta-1 (TGF- $\beta$ 1) is a pleiotropic cytokine. Its relationship with atherosclerosis is debatable, protective or deleterious effects having been described. It has been reported that TGF- $\beta$ 1 is increased in alcoholics and heavily involved in liver fibrogenesis. However, its role on vascular risk factors in these patients has not been analyzed. This is the objective of this study. We included 79 heavy alcoholics and 34 controls. Calcium deposition in the aortic arch was assessed in the plain thorax X-ray film. All the patients underwent complete laboratory evaluation, including cholesterol fractions and serum levels of TGF- $\beta$ 1, tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-4, IL-6 and interferon- $\gamma$  (IFNG). Ankle-brachial index was recorded in 48 patients. Serum TGF- $\beta$ 1 levels were significantly higher among patients ( $t=2.73$ ;  $p=0.008$ ), no differences existing among cirrhotic ( $17246 \pm 11021$  pg/ml) and non-cirrhotic ( $21340 \pm 12442$  pg/ml). TGF- $\beta$ 1 showed significant correlations with total cholesterol ( $r=0.28$ ;  $p=0.017$ ) and HDL-cholesterol ( $r=0.25$ ;  $p=0.042$ ), and an inverse correlation with body mass index (BMI;  $\rho=-0.37$ ;  $p=0.004$ ), IL-4 ( $\rho=-0.31$ ;  $p=0.009$ ), INF- $\gamma$  ( $\rho=-0.28$ ;  $p=0.001$ ) or IL-6 ( $\rho=-0.38$ ;  $p=0.001$ ), but not with TNF- $\alpha$  ( $\rho=-0.02$ ) or C-reactive protein ( $\rho=-0.22$ ,  $0.06 > p > 0.05$ ). By multivariate analysis only BMI, IL-6 and HDL-cholesterol showed independent relationships with TGF- $\beta$ 1. No relationships were observed with ankle-brachial index or calcium in the aortic arch, hypertension, diabetes, left ventricular hypertrophy or atrial fibrillation. Therefore TGF- $\beta$ 1 levels are increased in alcoholics, but they are not related to vessel wall calcification or arterial stiffness.

## **BRAIN-DERIVED NEUROTROPHIC FACTOR AND HANDGRIP STRENGTH AMONG ALCOHOLICS.**

Víctor E. Vera Delgado; Lourdes Gonzalez Navarrete; Lucía Romero Acevedo; Iván Ribot Hernández; Candelaria Martín González; Elisa Espelosín-Ortega\*; Melchor Rodríguez Gaspar; Emilio González Reimers.

Servicio de Medicina Interna. \* Servicio de Laboratorio. Hospital Universitario de Canarias. Tenerife, Canary Islands (Spain)

Brain derived neurotrophic factor (BDNF) is involved in neurogenesis and in the protection against oxidative damage and neuronal apoptosis. After exercise there is an increased expression of this myokine, especially in skeletal muscle and brain. Low BDNF levels have been described in neurodegenerative diseases. Alcoholics show both muscle atrophy and brain atrophy. Thus, this study was performed in order to analyse the behavior of BDNF among alcoholics and its association with brain atrophy and muscle mass and strength. Serum BDNF values were prospectively determined to 82 male alcoholics (drinkers of  $197 \pm 153$  g ethanol/day during  $33 \pm 14$  years), aged  $58.62 \pm 11.21$  years and 27 age-matched ( $54.52 \pm 7.78$  years,  $Z=1.77$ ;  $p=0.11$ ) controls, and compared with handgrip strength, with the presence of brain atrophy, assessed by computed tomography (CT), and with the intensity of alcoholism and liver function derangement. BDNF values were significantly lower among patients (median=6320, interquartile range=2404-12080 vs 19660(16228-27182 pg/ml,  $Z=6.36$ ;  $p<0.001$ ). Handgrip strength (significantly reduced among patients) was correlated with BDNF values, both in the whole population ( $\rho=0.25$ ;  $p<0.05$ ), and, especially, in patients over 59 (median value) years ( $r=0.57$ ,  $p<0.001$ ). BDNF was poorly related to liver function, but showed no relation at all with CT assessed brain atrophy. We conclude that chronic alcoholics show decreased BDNF values that are related to muscle function impairment rather than to age, brain atrophy, liver dysfunction, or the amount of ethanol consumed.

## **Thiamine substitution in alcohol use disorder**

*Nathalie Pruckner<sup>1</sup>, Sandra Vyssoki<sup>2</sup>, Benjamin Vyssoki<sup>1</sup>*

<sup>1</sup> Clinical Division of Social Psychiatry, Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria

<sup>2</sup> St. Pölten University of Applied Sciences, Austria

### **Introduction:**

Patients with alcohol use disorder (AUD) frequently suffer from cognitive deficits ranging from mild symptoms to most severe forms like Wernicke encephalopathy (WE). WE is caused by thiamine deficiency and, if left untreated, can progress to Korsakoff syndrome, which constitutes severe anterograde amnesia, confabulation and behavioral abnormalities. We conducted a review of the current medical treatment guidelines for AUD in order to identify recommendations for the use of thiamine.

### **Methods**

Three different keyword combinations (“alcohol treatment guideline”, “alcohol withdrawal guideline” and “alcohol treatment recommendation”) were entered in Pubmed and Scopus, additional guidelines were searched screening the online sites of the respective agencies or societies. In total, 14 guidelines were included.

### **Results**

Thiamine was mentioned in all but one of the reviewed publications. Specifications on application modalities and indications varied considerably. While the majority of reviewed guidelines recommended parenteral thiamine only for patients at high risk for WE, some gave no information regarding the application form or dosage.

### **Conclusions:**

Substitution of parenteral thiamine in suspected WE is a well-established treatment regimen and high-dose treatment with parenteral thiamine in several daily doses should be considered a state-of-the-art procedure. Yet, hardly any evidence-based recommendations exist on a more general use of thiamine as a preventative measure. Suggestions according to medical guidelines vary widely. Further research is of utmost importance to better define and implement recommendations on use of thiamine in patients with alcohol use disorder.

## **Hepatic iron overload in alcoholic liver disease: The role of sinusoidal endothelial cells in iron sensing**

Wang, Shijin<sup>1</sup>; Peccerella, Teresa<sup>1</sup>; Mueller, Sebastian<sup>1</sup> and Rausch, Vanessa<sup>1</sup>

<sup>1</sup>Center for Alcohol Research and Salem Medical Center, University of Heidelberg,  
Heidelberg, Germany

**Background and Aims:** So far, hepatic iron overload in patients with alcoholic liver disease is poorly understood. Hepcidin, the master switch of systemic iron homeostasis and is regulated by the BMP signaling pathway. Recent data showed that liver sinusoidal endothelial cells (LSECs) express the highest amount of BMP6 among different hepatic cell types and are able to regulate iron homeostasis *in vivo*. However, the exact mechanisms, how iron levels are sensed by ECs and how BMP signaling is involved in the regulation of systemic iron metabolism as well as which cells are involved is still not completely known. The aim of this study is to establish an *in vitro* co-culture model to mimic the crosstalk between LSECs and hepatocytes for the investigation of the exact role of LSECs in regulating iron metabolism.

**Methods:** Huh7 cells (hepatocytes) and SK hep cells (endothelial cells) were cultured alone and treated with increasing concentrations of ferric iron and with two iron chelators (desferal and SIH) under normoxic (21% O<sub>2</sub>) as well as hypoxic conditions (1% O<sub>2</sub>) for 24 hours. Next, direct co-cultures with SK hep and Huh7 cells were established by inserts as well as indirect co-cultures by using the supernatant of SK hep cells treat Huh7 cells. Hepcidin, BMP6, BMP2, TFR1 and ferritin were assessed by qRT-PCR and the Bmp6 concentration in medium was determined by ELISA.

**Results:** Treatment of SK hep cells with ferric iron led to significantly increased Bmp6 and hepcidin mRNA expression under hypoxia, whereas no effect on Huh7 cells was detected. In direct co-cultures the mRNA expression levels have similar trends as found in SK hep and Huh7 single cultures. Of note, BMP6 expression in SK hep was extreme low and may not be sufficient to maintain adequate levels in the medium to induce signaling in hepatocytes.

**Conclusion & Outlook:** SK hep cells are able to sense iron changes (iron supplementation or chelation) but no adequate response of hepatocytes towards BMP6-induced signaling could be observed. In the future, other EC lines (HUVECs), co-culture systems and 3D *in vitro* models will be explored to better understand the crosstalk on iron regulation.

## **GDNF / Glia cell line derived neurotrophic factor – a promising new treatment target in Alcohol use disorder (AUD)**

Andreas Wippel<sup>1</sup>, Stephan Listabarth<sup>1</sup>, Andrea Gmeiner<sup>1</sup>, Daniel König<sup>1</sup>, Vid Velikic<sup>1</sup>, Benjamin Vyssoki<sup>1</sup>

<sup>1</sup>*Clinical Division of Social Psychiatry, Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria*

### Background:

According to the World Health organization (WHO) alcohol accounted for 5,3 % of all deaths worldwide in 2018. Alcohol is consumed globally but only a small part of consumers transit from social drinking habits to uncontrolled, compulsive drinking. Craving is a hallmark symptom of alcohol dependence. Currently available anti-craving treatment options are limited in number, lack in efficacy and face non-compliance in clinical use. The dopaminergic system plays a key role in the reward circuit in dependence as well as craving behavior. Therefore, new treatment options should target neuronal reward pathways to normalize adaptations to chronic alcohol exposure and reduce craving.

### Methods:

Two different keyword combinations ("alcohol use disorder AND Gdnf" "addiction AND Gdnf") were entered in PubMed. Relevant findings are presented as a narrative review.

### Results:

GDNF acts as a regulator of dopamine release and firing rates in the midbrain, especially in ventral tegmental area (VTA) and nucleus accumbens. Several studies in rodents suggest that GDNF is an alcohol responsive gene, which is upregulated in short term alcohol intake and downregulated during withdrawal after excessive alcohol intake. These results were confirmed for humans in two independent studies investigating GDNF serum levels and alcohol intake. Furthermore, elevated GDNF produced suppression of alcohol-drinking behaviours in rats and reduced GDNF facilitated the escalation of alcohol drinking.

### Discussion:

The escalation from moderate to excessive drinking could be a result of a breakdown of endogenous GDNF systems, therefore, GDNF could be a marker for AUD and may serve as a treatment target to reduce craving.

# Is (poly-) substance use associated with impaired inhibitory control?

## A mega-analysis controlling for confounders

Yang Liu<sup>1,2\*</sup>, Wery P.M. van den Wildenberg<sup>1,3</sup>, Ysanne de Graaf<sup>4</sup>, Susan L. Ames<sup>5</sup>, Alexander Baldacchino<sup>6</sup>, Ragnhild Bø<sup>7</sup>, Fernando Cadaveira<sup>8</sup>, Salvatore Campanella<sup>9</sup>, Paul Christiansen<sup>10</sup>, Eric D. Claus<sup>11</sup>, Lorenza S. Colzato<sup>12</sup>, Francesca M. Filbey<sup>13</sup>, John J. Foxe<sup>14</sup>, Hugh Garavan<sup>15</sup>, Christian S. Hendershot<sup>16</sup>, Robert Hester<sup>17</sup>, Jennifer M. Jester<sup>18</sup>, Hollis C. Karoly<sup>19</sup>, Anja Kräplin<sup>20</sup>, Fanny Kreusch<sup>21</sup>, Nils Inge Landrø<sup>7</sup>, Marianne Littel<sup>22</sup>, Sabine Steins-Loeber<sup>23</sup>, Edythe D. London<sup>24</sup>, Eduardo López-Caneda<sup>25</sup>, Dan I. Lubman<sup>26</sup>, Maartje Luijten<sup>27</sup>, Cecile A. Marczinski<sup>28</sup>, Jane Metrik<sup>29</sup>, Catharine Montgomery<sup>30</sup>, Harilaos Papachristou<sup>31</sup>, Su Mi Park<sup>32,33</sup>, Andres L. Paz<sup>34</sup>, Géraldine Petit<sup>10</sup>, James J. Prisciandaro<sup>35</sup>, Boris B. Quednow<sup>36</sup>, Lara A. Ray<sup>37</sup>, Carl A. Roberts<sup>10</sup>, Gloria M.P. Roberts<sup>38</sup>, Michiel B. de Ruiter<sup>39</sup>, Claudia I. Rupp<sup>40</sup>, Vaughn R. Steele<sup>11</sup>, Delin Sun<sup>41,42</sup>, Michael Takagi<sup>43,44</sup>, Susan F. Tapert<sup>45</sup>, Ruth J. van Holst<sup>46</sup>, Antonio Verdejo-Garcia<sup>47</sup>, Matthias Vonmoos<sup>36</sup>, Marcin Wojnar<sup>48</sup>, Yuanwei Yao<sup>49</sup>, Murat Yücel<sup>50</sup>, Martin Zack<sup>51</sup>, Robert A. Zucker<sup>18</sup>, Hilde M. Huizenga<sup>1,3,52\*\*</sup> & Reinout W. Wiers<sup>1,2,\*\*</sup>

### Affiliations

<sup>1</sup>Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands;

<sup>2</sup>Addiction, Development, and Psychopathology (ADAPT) Lab, Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands;

<sup>3</sup>Amsterdam Brain and Cognition Center, University of Amsterdam, Amsterdam, The Netherlands;

<sup>4</sup>Faculty of Science (FNWI), University of Amsterdam, Amsterdam, The Netherlands;

<sup>5</sup>School of Community and Global Health, Claremont Graduate University, Claremont, CA, USA;

<sup>6</sup>Division of Population and Behavioural Sciences, St Andrews University Medical School, University of St Andrews, St Andrews, Scotland, UK;

<sup>7</sup>Clinical Neuroscience Research Group, Department of Psychology, University of Oslo, Oslo, Norway;

<sup>8</sup>Department of Clinical Psychology and Psychobiology, University of Santiago de Compostela, Galicia, Spain;

<sup>9</sup>Laboratoire de Psychologie Médicale et d'Addictologie, ULB Neuroscience Institute (UNI), CHU Brugmann-Université Libre de Bruxelles (U.L.B.), Brussels, Belgium;

<sup>10</sup>University of Cyprus, Nicosia, Cyprus;

<sup>11</sup>The Mind Research Network and Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico;

<sup>12</sup>Leiden University, Cognitive Psychology Unit & Leiden Institute for Brain and Cognition, Leiden, the Netherlands;

<sup>13</sup>The Mind Research Network, The University of Texas at Dallas, Texas, USA;

<sup>14</sup>University of Rochester Medical Center, School of Medicine and Dentistry, Rochester, USA;

<sup>15</sup>Department of Psychiatry, University of Vermont, Burlington, USA;

- <sup>16</sup>Centre for Addiction and Mental Health, Campbell Family Mental Health Research Institute and Institute for Mental Health Policy Research, Toronto, Canada;
- <sup>17</sup>School of Psychological Sciences, University of Melbourne, Melbourne, Australia;
- <sup>18</sup>Department of Psychiatry, University of Michigan, Michigan, USA;
- <sup>19</sup>Institute of Cognitive Science, University of Colorado Boulder, Colorado, USA;
- <sup>20</sup>Work Group Addictive Behaviours, Risk Analyses and Risk Management, Faculty of Psychologie, Technische Universität Dresden, Germany;
- <sup>21</sup>Department of Psychology, University of Liège, Belgium;
- <sup>22</sup>Department of Psychology, Erasmus University Rotterdam, Rotterdam, The Netherlands;
- <sup>23</sup>University of Bamberg, Department of Clinical Psychology and Psychotherapy, Bamberg, Germany;
- <sup>24</sup>Department of Psychiatry and Biobehavioral Sciences at the University of California, Los Angeles, USA;
- <sup>25</sup>Psychological Neuroscience Lab, Research Center in Psychology (CIPsi), School of Psychology, University of Minho, Braga, Portugal;
- <sup>26</sup>Turning Point, Eastern Health and Eastern Health Clinical School, Monash University, Melbourne, Australia;
- <sup>27</sup>Behavioural Science Institute, Radboud University, Nijmegen, The Netherlands;
- <sup>28</sup>Northern Kentucky University, Highland Heights, USA;
- <sup>29</sup>Center for Alcohol and Addiction Studies, Brown University School of Public Health, Providence, USA;
- <sup>30</sup>School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, UK;
- <sup>31</sup>Maastricht University, Faculty of Psychology and Neuroscience, The Netherlands;
- <sup>32</sup>Department of Psychiatry, SMG-SNU Boramae Medical Center, Seoul, Republic of Korea;
- <sup>33</sup>Department of Clinical Medical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea;
- <sup>34</sup>Department of Psychology, Charles Schmidt College of Science, Florida Atlantic University, USA;
- <sup>35</sup>Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston SC, USA;
- <sup>36</sup>Experimental and Clinical Pharmacopsychology, Department of Psychiatry, Psychotherapy, and Psychosomatics, Psychiatric Hospital, University of Zürich, Zürich, Switzerland;
- <sup>37</sup>University of California Los Angeles, Department of Psychology, Los Angeles, CA, USA;
- <sup>38</sup>School of Psychiatry, University of New South Wales, Sydney, Australia;
- <sup>39</sup>Division of Psychosocial Research and Epidemiology, Netherlands Cancer Institute, Amsterdam, The Netherlands;
- <sup>40</sup>Department of Psychiatry, Psychotherapy and Psychosomatic, Medical University Innsbruck, Austria;
- <sup>41</sup>Duke-UNC Brain Imaging and Analysis Center, Duke University, Durham, NC, USA;
- <sup>42</sup>VA Mid-Atlantic Mental Illness Research, Education and Clinical Center (MIRECC), Durham, NC, USA;
- <sup>43</sup>Child Neuropsychology Research Group, Murdoch Children's Research Institute, Melbourne Australia;
- <sup>44</sup>Melbourne School of Psychological Sciences, University of Melbourne, Melbourne, Australia;

<sup>45</sup>Department of Psychiatry, University of California, San Diego, USA;

<sup>46</sup>Amsterdam UMC, University of Amsterdam, Department of Psychiatry, Amsterdam Institute for Addiction Research, Amsterdam, The Netherlands;

<sup>47</sup>School of Psychological Sciences, Monash Institute of Cognitive and Clinical Neurosciences (MICCN), Monash University, Australia;

<sup>48</sup>Department of Psychiatry, Medical University of Warsaw, Warsaw, Poland;

<sup>49</sup>State Key Laboratory of Cognitive Neuroscience and Learning and IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing, China;

<sup>50</sup>School of Psychological Sciences, Turner Institute for Brain and Mental Health, and Monash Biomedical Imaging Facility, Monash University, Melbourne, Victoria, Australia;

<sup>51</sup>Molecular Brain Science Research Section Centre for Addiction and Mental Health, Toronto, Canada;

<sup>52</sup>Research Priority Area Yield, Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands;

### **\*Corresponding author**

Department of Psychology, University of Amsterdam, Amsterdam, Nieuwe Achtergracht 129B, 1018 WS Amsterdam, The Netherlands.

Email address: [y.liu3@uva.nl](mailto:y.liu3@uva.nl)

### **\*\*Shared senior authorship**

### **Abstract**

Many studies have reported that heavy substance use is associated with impaired response inhibition. Studies typically focused on associations with a single substance, while polysubstance use is common. Further, most studies compared heavy users with light/non-users, though substance use occurs along a continuum. The current mega-analysis accounted for these issues by aggregating individual data from 43 studies (3610 adult participants) that used the Go/No-Go (GNG) or Stop-signal task (SST) to assess inhibition among mostly “recreational” substance users (i.e., the rate of substance use disorders was low). Main and interaction effects of substance use, demographics, and task-characteristics were entered in a linear mixed model. Contrary to many studies and reviews in the field, we found that only lifetime cannabis use was associated with impaired response inhibition in the SST. An interaction effect was also observed: the relationship between tobacco use and response inhibition (in the SST) differed between cannabis users and non-users, with a negative association between tobacco use and inhibition in the cannabis non-users. In addition, participants’ age, education level, and some task characteristics influenced inhibition outcomes. Overall, we found limited support for impaired inhibition among substance users when controlling for demographics and task-characteristics.

# Role of NOX1 on hepcidin signaling in the crosstalk between macrophages and hepatocytes

Yu, Linna<sup>1</sup>, Peccerella, Teresa<sup>1</sup>; Mueller, Sebastian<sup>1</sup> and Rausch, Vanessa<sup>1</sup>

<sup>1</sup> Center for Alcohol Research and Salem Medical Center, University of Heidelberg, Heidelberg, Germany

**Background and Aims:** Liver-secreted hepcidin is the systemic master switch of iron homeostasis and its dysregulation leads to iron accumulation in most of chronic liver diseases. Hepcidin is regulated by iron, inflammation or H<sub>2</sub>O<sub>2</sub>, but the role of NOX1 and its products ROS/H<sub>2</sub>O<sub>2</sub> in monocyte-derived macrophages on hepcidin regulation under (patho)physiological conditions is poorly understood. We here investigate the role of NOX1 on regulating hepcidin and cytokines in inflammatory macrophages and subsequent effects on hepatocytes mimicking (patho)physiological conditions (cell ratios, oxygen levels and inflammation).

**Methods:** THP-1 monocytes were differentiated into macrophages and co-cultured with Huh7 cells at physiological cell ratio (4:1) and treated with different LPS concentrations (10ng/ml and 100ng/ml) under normoxia (21% O<sub>2</sub>) or hypoxia (1% O<sub>2</sub>). The exposure of Huh7 cells to macrophage-conditioned medium with LPS was also investigated. Hepcidin, IL-1 $\beta$ , IL-6, C/EBP $\delta$  and SMAD6 mRNA levels were assessed by qRT-PCR and the expression of NOX1, p-STAT3, STAT3 and p-SMAD1/5/8 proteins was analyzed by western blot.

**Results:** LPS significantly increased NOX1, p-STAT3, IL-1 $\beta$  and IL-6 levels in THP-1 macrophages, but decreased STAT3 expression in a concentration-dependent manner under 21% and 1% O<sub>2</sub>. Interestingly, 10ng/ml LPS increased the expression of hepcidin whereas 100ng/ml LPS decreased the expression of hepcidin under 21% O<sub>2</sub>. In contrast, both LPS concentrations decreased the expression of hepcidin under low oxygen conditions (1% O<sub>2</sub>) in THP-1 macrophages. In addition, LPS decreased SMAD6, p-SMAD1/5/8 and CEBP  $\delta$  in THP-1 macrophages under normoxia (21% O<sub>2</sub>).

**Conclusion:** Our findings underscore a possible role of NOX1 and subsequent ROS/H<sub>2</sub>O<sub>2</sub> concentrations on hepcidin regulation and induction of cytokine production in inflammatory macrophages involving the STAT3 signaling pathway. In the future, we aim at studying in detail hepcidin signaling by using WT and truncated hepcidin promoter constructs and siRNA-mediated knockdown of TLR4, NOX1, STAT3 or C/EBP $\delta$ .

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