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Self-estimation of blood alcohol concentration in patients admitted in emergency department

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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 0.6 g/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±0.3 g/L, n=20). Interestingly, moderate AUD patients over-estimated BAC (0.5±0.5 g/L, n=3), mild AUD patients correctly estimated BAC (0.0±0.3 g/L, n=5), and severe AUD patients clearly under-estimated BAC (-1.1±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.

The role of the gut-brain axis in alcohol dependence: design of the Gut2Brain study

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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

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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.

Gene expression changes associated with stress-induced alcohol escalation

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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

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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

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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

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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

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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

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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 ($p_{(hypergeometric)}$ < 1.02e-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%; $p_{(hypergeometric)}$ = 7.10e-1152), followed by open sea (30%; $p_{(hypergeometric)}$ = 7.40e-502) and CpG island shores (18%; $p_{(hypergeometric)}$ = 2.10e-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=8.9e-10), endothelial cells (Endot, n=61; p=4.7e-12), microglia (Microg, n=54; p=6.3e-92), oligodendrocytes (Oligod, n=57; p=3.2e-10) and neurons (Neur, n=66; p=1.6e-14). Using Ingenuity pathway analysis, the following pathways were enriched: axonal guidance signaling (19 genes, p= 5.5e-3), Wnt/B-catenin signaling (19 genes, p=1.4e-6), synaptic long term depression (18 genes, p=1.9e-5), CREB signaling in neurons (18 genes, p=1.5e-4), gap junction signaling (17 genes, p=2.6e-4), corticotropin releasing hormone signaling (13 genes, p=4.2e-4), dopamine cAMP signaling (14 genes, p=4.6e-4), opioid signaling pathway (18 genes, p=6.6e-4) and synaptogenesis signaling pathway (19 genes, p=5.5e-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_2O_2, a major reactive oxygen species of alcohol metabolism induces autophagy without involving mTOR

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Background and aims: Alcohol-mediated reactive oxygen species (ROS) formation in the liver, mainly H_2O_2, 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_2O_2 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_2O_2 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_2O_2 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_2O_2, the m-TOR expression was not suppressed as normally expected. Co-treatment of hepatoma cells with H_2O_2 and the mTOR inhibitor Rapamycin led to an increased autophagic flux as compared to single H_2O_2 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_2O_2 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_2O_2-induced signaling pathways that regulate autophagy.

Prevalence and demographic characteristics of alcohol use disorder in Chinese Shandong provincial adultresidents: A cross-section epidemiologic survey

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Background and 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 27,917 enrolled, and 27,489 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.

Effect of trauma and family alcohol using situations in childhood onthe occurrence of alcohol dependence in Chinese male patients

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Background and 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.

Cross-modal processing of emotions in Severe alcohol use disorders: Impaired discrimination of anger and fear under dynamic audiovisual conditions

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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.

A new animal model of pain-induced alcohol relapse: Involvement of mu and kappa opioid receptors in the mesocorticolimbic system

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Several clinical studies have undercovered 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.

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Adipokines (adipocytokines), selected clinical and nutritional variables in the patients with alcohol dependence

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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

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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, 3 g/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 (45 mg/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

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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 4,000 students and 2,000 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, neurocognitive-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-γ agonists in an animal model of fasd

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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-γ agonist pioglitazone can attenuate ethanol-induced cellular toxicity, microglial morphological change, and expression of inflammatory molecules. This suggests that PPAR-γ agonists may hold therapeutic potential for those affected by FASD. We also evaluated the effect of the PPAR-γ agonist docosahexaenoic acid (DHA) on ethanol-induced neuroinflammation. DHA is an ω-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-1β and TNF-α in the brain. Thus, PPAR-γ 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.
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Alcohol-related abnormalities in the early postnatal period can be corrected by multitarget low-affinity agonist of sigma-1, MT1 and MT3 receptors

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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.

"Alcohol and you?" A two day assessment program with a multidisciplinary team

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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

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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 erythropiesis 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. Hepcidin, 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 histoligically 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 progentors (MEPs) was drastically reduced in acute group suggesting impaired early stages of erythropoiesis. However, in chronic ethanol exposure a high number of proeryhtroblast (Ter119neg CD71high) and low number of late erythroblasts (Ter119high CD71med) was detected. Acute as well as chronic alcohol exposure led to significant hepcidin suppression accompnied by suppression of SMAD6 and 7 mRNA and an induction in HO-1 levels suggesting heme degradation. Ballooned hepatocytes and a large number of eryhthrocytes 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

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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

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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 ΔFos B expression in the dorsolateral striatum of long-term ethanol-experienced rats during the abstinence period

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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 ΔFos 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

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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

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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

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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. Psychoeducation (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.

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Incident neoplasia among heavy alcoholics: Relationship with body composition

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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 (χ^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

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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

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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-1β, frantalkine), 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.
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Ocimum sanctum *suppresses alcohol abstinence-induced depression-like behavior through regulation of biochemical and* GRIN2A, GRIN2B *gene expression of NMDA receptor signaling in rats*

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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 16th, 17th & 18th 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.

Nucleus reuniens of ventral midline thalamus is highly susceptible to permanent neuron loss in the rat model of binge drinking during third trimester

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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

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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, 2 h 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

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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

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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

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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

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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 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

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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 (1-3), and gut and immune alterations especially impact brain regions involved in executive function and inhibition (4, 5). 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=.56) and AUDIT (d=.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

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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 (?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 (ß=-3.186, P<0.01). Increased severity of depression, resulting from weak social support system, increased the severity of alcohol problem (ß=0.152, P<0.01). Increases in the duration in AA decreased severity of alcohol problem without the mediation of depression (ß=-0.039, P<0.05). Among the control variables, the alcohol problem severity from the 1st survey showed positive auto-regression effect (ß=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) receptoragonist reduce alcohol intake in patients with alcohol dependence?

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Aims: Glucagon-like peptide-1 (GLP-1) receptorstimulation 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 ourknowledge,not yet been investigated.
Methods: The effect of the once-weekly GLP-1-receptor-agonist, exenatide will be investigated in adouble-blinded, placebo-controlled, randomized clinical trial. 114 outpatients, age 18-70 years will be randomized to eitherplacebo orexenatide once-weekly for 26 weeks as a supplement to cognitivebehaviouraltherapy. The primary endpoint is reduction in numberof 'heavy drinking days', measured by the Time Line Follow Back (TFLB) 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 biomarkerof alcohol consumption from baselineto follow-up at week 26.
In addition to these clinical outcomeparameters, we willexplore 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 anew revolutionary treatment foralcohol dependence.
Financial support: The study is financed by Region Hovedstadens Forskningsfond, Region Hovedstadens Psykiatri and Fonden Novavì. The manufacturerof Bydureon®, 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

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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 (≈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.

Tuftsin peptide analog prevents ethanol-induced cognitive impairment in aged alcohol-withdrawn rats through modulation BDNF signaling pathway

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Background. Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), as tuftsin 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-OT and reduced turnover 5-HIAA/5-OT 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±0.2 R.D.U., "EtOH" 3.4±0.6 R.D.U.) and frontal cortex ("Control" 1.1±0.2 R.D.U., "EtOH" 1.8±0.3 R.D.U.). Selank prevented BDNF increase in hippocampus and frontal cortex.
Conclusions. The obtained results indicate pronounced effect of taftsin 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

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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 may 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

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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?

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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

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Background: Early in abstinence, patients with Alcohol Use Disorder (AUD) frequently present brain alterations, cognitive deficits and sleep disturbances (1, 2). 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 (3) 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

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Baclofen modulates psychophysiological responses to appetitive cues in treatment-seeking alcohol use disorder individuals

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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.

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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 $GABA_B$ receptor polymorphism rs29220

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Introduction: We have previously shown that the *GABBR1* rs29220 polymorphism is associated with response to baclofen, a GABAb 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 Apartate levels were measured in N=25 alcohol dependent patients using in vivo proton magnetic resonance spectroscopy (1H-MRS) 120 minutes following administration of PL or BAC. Blood samples were obtained for analysis of the single nucleotide polymorphism (rs29220) in the GABAB 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*

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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 GABAB 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?

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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 retrospec-

tive 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

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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

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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±3.18 in alcoholic patients and 6.28±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

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In France, 9.7% of the population drinks alcohol every day. Futhermore, 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

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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)

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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

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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-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

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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 1st year class of every faculty of the Universidade de Santiago de Compostela. The Alcohol Use of Dissorder Identification Test was used for measuring both RAC, – total score ≥5 for women and ≥6 for men –, and BD – drinking ≥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% vs12.3% 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 asses and that can offer more information that we expected to. At this study we do not only confirm that the amount of alcohol consumption that a student who practices alcohol asses 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

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Background: 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

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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[1], Jean-Jacques Yonga[1], Murielle Girard[1], Philippe Nubukpo[1] ([1] 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 de 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.

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[1], Jean-Jacques Yonga[1], Murielle Girard[1], Philippe Nubukpo[1] ([1] 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 tehmselves (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.

Clinical and biological monitoring of subjects with Alcohol Use Disorder after alcohol withdrawal treatment

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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 dependance. 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.

Impact of addictology expert patients on medical students: A qualitative study

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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.

Alcohol-Related Brain Injury in alcohol use disorder patients attending a UK secondary care hospital

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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 ≤23. Participants were all in-patients aged ≥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 ≤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 ≤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 (95% CI: 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

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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

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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

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Introduction: 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}=0.604; p=0.008), melatonin (r_{16}=0.509; p=0.031), alpha-MSH (r_{13}=0.645; p=0.009), Substance P (r_{16}=0.470; p=0.049), oxytocin (r_{16}=0.667; p =0.002), orexin (r_{14}=0.742; p=0.001).

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)

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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.

Materials and methods: 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.

Acute IP administration of N-acetylcysteine prevents the activation of the mesocorticolimbic system triggered by intra-VTA ethanol administration in rats

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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 inmunoreactivity 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

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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

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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 longitudi-

nal 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

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Background. Early Identification and Brief Interventions (EIBI) in primary care is a proven (cost-)effective method for reducing harmful drinking in society (1-5). Unfortunately, integration in daily practice is low (6-8). It is suggested that a more integrated EIBI-culture is necessary to fully utilise its effect (9-11) and that community involvement might provide a tool to stimulate that (10, 12). 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

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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 24 h after 2 binge-like ethanol exposure (3 g/kg, i.p.) applied during the different phases of the oestrus cycle. We also used exogenous E2 (180 µg/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 24 h delay. However, LTD was abolished at 24 h 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 24 h delay when ethanol was injected with a higher dose of ethanol (3.75 g/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.

Alcohol consumption and gender gap in cardiovascular mortality in Europe

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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

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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 1 h 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

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We included 255 alcoholic patients (aged 59±11; drinkers of 200±150 g/day during 32±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 (x2=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 stength 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

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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

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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)

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Chronic alcohol exposure in alcohol-preferring (sP) rats provokes mild brain and liver inflammatory responses and a specific atrophy of the corpus callosum

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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.
Materials and methods: 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 Kuppfer 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.

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Nalmefene alleviates alcohol-induced neuroinflammation: A PET imaging study with [^{18}F]DPA-714 in an adolescent rats

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Introduction. Alcohol exposure during adolescence induces important and long-lasting brain damages, which are assumed to involve neuroinflammatory processes. [^{18}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) (1). In another group, nalmefene (0.4 mg/kg, s.c) was injected 1 hour prior to ethanol. MicroPET imaging with [^{18}F]DPA-714 (~37 MBq, i.v.) was performed 24 h after the last alcohol/saline injection. In each experiment, the brain distribution of [^{18}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 an blood alcohol concentration of 2.40±0.5 g/L at 5 min after injection. The regional V_{Ts} of [^{18}F]DPA-714 in alcohol exposed animals ($V_{T\ hippocampus}$=21.1±2.7 and $V_{T\ accumbens}$= 25.3±6.1) were significantly increased when compared to control animals ($V_{T\ hippocampus}$=5.9±0.8 and $V_{T\ accumbens}$= 7.07±1.3). Nalmefene significantly alleviated the alcohol-induced increase in [^{18}F]DPA-714 binding ($V_{T\ hippocampus}$= 14.02±5.22 and $V_{T\ accumbens}$=18.5±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 neuroinflamation. PET imaging using [^{18}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

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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

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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 disregulated 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.

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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

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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.

Measuring alcohol craving after virtual reality exposure: A method comparison in social drinkers

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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

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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

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Impact of alcohol exposure on the development and maturation of the cerebral cortex

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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

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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-β among alcoholics

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Transforming growth factor beta-1 (TGF-β1) is a pleiotropic cytokine. Its relationship with atherosclerosis is debatable, protective or deleterious effects having been described. It has been reported that TGF-β1 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-β1, tumor necrosis factor (TNF)-α, interleukin (IL)-4, IL-6 and interferon-γ (IFNG). Ankle-brachial index was recorded in 48 patients. Serum TGF-β1 levels were significantly higher among patients (t=2.73; p=0.008), no differences existing among cirrhotic (17246±11021 pg/ml) and non-cirrhotic (21340±12442 pg/ml). TGF-β1 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; ρ=-0.37; p=0.004), IL-4 (ρ=-0.31; p=0.009), INF-γ (ρ=-0.28; p=0.001) or IL-6

(ρ=-0.38; p=0.001), but not with TNF-α (ρ=-0.02) or C-reactive protein (ρ=-0.22, 0.06>p>0.05). By multivariate analysis only BMI, IL-6 and HDL-cholesterol showed independent relationships with TGF-β1. No relationships were observed with ankle-brachial index or calcium in the aortic arch, hypertension, diabetes, left ventricular hypertrophy or atrial fibrillation. Therefore TGF-β1 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

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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±153 g ethanol/day during 33±14 years), aged 58.62±11.21 years and 27 age-matched (54.52±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 (ρ=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

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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

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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_2) as well as hypoxic conditions (1% O_2) 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 and 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)

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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

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Many studies have reported that heavy substance use is associated with impaired response inhibition. Studies typically focused on associations with a single substance, while poly-substance 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

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Background and aims: Liver-secreted hepcidin is the systemic masterswitch of iron homeostasis and its dysregulation leads to iron accumulation in most of chronic liver diseases. Hepcidin is regulated by iron, inflammation or H_2O_2, but the role of NOX1 and its products ROS/H_2O_2 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 (10 ng/ml and 100ng/ml) under normoxia (21% O2) or hypoxia (1% O_2). The exposure of Huh7 cells to macrophage-conditioned medium with LPS was also investigated. Hepcidin, IL-1β, IL-6, C/EBPδ 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-1βand IL-6 levels in THP-1 macrophages, but decreased STAT3 expression in a concentration-dependent manner under 21% and 1% O_2. Interestingly, 10ng/ml LPS increased the expression of hepcidin whereas 100ng/ml LPS decreased the expression of hepcidin under 21% O_2. In contrast, both LPS concentrations decreased the expression of hepcidin under low oxygen conditions (1% O_2) in THP-1 macrophages. In addition, LPS decreased SMAD6, p-SMAD1/5/8 and CEBPδin THP-1 macrophages under normoxia (21% O_2).
Conclusion: Our findings underscore a possible role of NOX1 and subsequent ROS/H_2O_2 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/EBPδ.