

VIII LASBRA INTERNATIONAL MEETING: “Neurobiological basis of alcoholism: from molecules to behavior”

Oral Presentations (alphabetic order).

INTERACTION BETWEEN A BDNF POLYMORPHISM AND DEVELOPMENTAL ETHANOL EXPOSURE

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Exposure to alcohol during embryonic development causes alterations in many organs and systems, a condition known as Fetal Alcohol Spectrum Disorders (FASD). Studies indicate that genetic factors determine the severity of FASD. In this study, we investigated the modulation of FASD severity by a prevalent single nucleotide polymorphism in the human brain-derived neurotrophic factor (BDNF) gene (val66met), which disrupts intracellular trafficking and activity dependent secretion of BDNF. A number of studies have linked the Val66Met genotype with increased incidence of neuropsychiatric conditions commonly associated with FASD, such as mood disorders. We hypothesized that mice carrying the BDNFmet/met polymorphism would be more severely affected by developmental ethanol exposure. We used a transgenic mouse model of this polymorphism (Warnault et al., *Biological Psychiatry*, 79: 463-73, 2016). Dams were subjected to binge-like ethanol exposure in vapor chambers during gestational days 12-19 and pups during postnatal days 2-9. We found that ethanol exposure reduced volumes in the dentate gyrus and the CA1 hippocampal regions of BDNFmet/met but not BDNFval/val mice.

Moreover, ethanol-exposed BDNFmet/met mice displayed complex alterations in anxiety-like behavior and trace fear conditioning, which could be a consequence of disruptions in adult neurogenesis in the dentate gyrus. This study demonstrates a novel gene x environment interaction between the BDNF val66met polymorphism and developmental EtOH exposure, suggesting that individuals carrying this prevalent polymorphism could be at higher risk of developing more severe forms of FASD. Therefore, screening for this polymorphism could allow for earlier, more aggressive interventions against these disorders.

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Keywords: ethanol, fetal, hippocampus, BDNF

THE PRELIMBIC CORTEX NEURONAL ENSEMBLES ENCODE ASSOCIATIVE MEMORIES BETWEEN ALCOHOL REWARD EFFECTS AND CONTEXTUAL CUES.

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Environmental contexts previously associated with drug use provoke relapse to drug use in humans and reinstatement of drug seeking in animal models of drug relapse. We examined, whether context-induced reinstatement of alcohol seeking is mediated by activation of neuronal ensembles of the prelimbic cortex. We also evaluated changes in gene expression related to alcohol seeking. To assess a causal role for the prelimbic neuronal ensembles in context-induced reinstatement of alcohol seeking, we used the Daun02 inactivation procedure to selectively inactivate these neurons. We trained c-fos-lacZ transgenic rats to self-administer alcohol in Context A and extinguished their lever-pressing in Context B. On induction day, we exposed rats to either Context A or a novel Context C for 30 min and injected Daun02 or vehicle into prelimbic cortex 60 min later. On test day, 3 d after induction day, the ability of Context A to reinstate alcohol seeking was attenuated when Daun02 was previously injected after exposure to Context A (active lever presses: 16.0 ± 4.0 Vehicle drug context vs 4.0 ± 2.0 Daun02 drug context; $p < 0.05$). In addition, we assessed whether context-induced reinstatement was associated with molecular alterations selectively induced within context-activated Fos-expressing neurons. We used fluorescence-activated cell sorting to isolate reinstatement-activated Fos-positive neurons from Fos-negative neurons in prelimbic cortex and used quantitative PCR to assess gene expression within these two populations of neurons. Context-induced reinstatement was associated with increased expression of GABA α 5 GABAergic receptor subunit (3.04 ± 1.09 , and 1 ± 1.15 ; respectively) and a decrease in GluR1 (0.16 ± 0.65 and 1 ± 0.33 ; respectively) and GluR2 (-0.3 ± 1.41 and 0.99 ± 0.89 ; respectively) glutamatergic AMPA receptor subunit in only Fos-positive neurons. Our Our results demonstrate an important role of the prelimbic cortex neuronal ensembles in context-induced reinstatement of alcohol seeking and that this reinstatement is associated with unique gene alterations in Fos-expressing neurons.

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THE DEVELOPMENTAL ETHANOL EXPOSURE MODIFIES SPATIAL MEMORY AND ADDICTIVE BEHAVIOR: ROLE OF REACTIVE OXYGEN SPECIES

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Ethanol intake during pregnancy may generate severe effects in the cognitive development of the offspring. Prenatal ethanol exposure (PEE) in both human as well as animal models alters cognitive behavior including memory learning and abuse alcohol and other drugs disorders. It has been described that PEE increases oxidative stress in developing organs, including the brain. Indeed, even a brief exposure to ethanol during gestation can produce perdurable imbalance between the levels of intracellular reactive oxygen species (ROS) and brain antioxidants that can be correlated with cognitive deficits. However, the impact of the general antioxidant treatment in the adult age of the exposed offspring, and the specific ROS-dependent mechanism, has still not been fully studied. We quantified the levels of antioxidant gene mRNA in mesocorticolimbic brain regions and tested the particular role NADPH oxidase 2 (NOX2) (postsynaptic superoxide generator) on impairment of spatial memory acquisition as well as in the increased ethanol seeking behavior of animal developmental exposure to ethanol (DEE). We observed that DEE adult offspring expressed low levels of antioxidant mRNAs in VTA and low levels of NOX2 mRNA in prefrontal cortex and hippocampus. In vivo inhibition of NOX2, rescued the cognitive alterations of DEE animals. Moreover, inhibition of NOX2 into ventral tegmental area (VTA) blocked alcohol-seeking behavior. We are currently performing studies to understand the specific mechanism by which NOX2 is contributing in the memory impairments and vulnerability to alcohol consumption and seeking behavior.

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Keywords: Oxidative stress, Hippocampus, Fetal Programming, Drug addiction.

ALCOHOL INTAKE: FROM INITIATION TO BINGE-DRINKING. MECHANISMS AND TRANSLATIONAL OPPORTUNITIES.

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The progression from; (i) initial ethanol intake, to (ii) chronic ethanol intake (without overt intoxication and negative reinforcement) and (iii) relapse-like binge drinking, can be distinguished as separate entities in preclinical studies: (i) Drug studies have shown that ethanol metabolism into acetaldehyde by brain catalase is required to elicit the early intake of ethanol. Further, early ethanol intake and dopamine release were fully abolished by blockade (shRNA) of catalase synthesis at the ventral-tegmental-area. Acetaldehyde trapping agents or gene-induced (Aldh2) degradation of acetaldehyde also inhibited early ethanol intake up to 90%. (ii) Conversely, after chronic voluntary ethanol intake, a reduction of acetaldehyde generation or of acetaldehyde levels no longer influence chronic ethanol intake, whether determined in operant conditions or by voluntary intake. Alcohol odor appears to be a conditioned cue that appears responsible for the protracted ethanol intake during the chronic maintenance phase, likely due to cue induced increases in glutamate in amygdala and the nucleus accumbens. Neuroinflammation induced by ethanol has been shown to reduce the levels of the astrocyte glutamate transporter (GLT-1), to also markedly increase extracellular glutamate levels. On the chronic ethanol maintenance phase, intake is inhibited by 70-75% by (a) the administration of

an anti-inflammatory drug glutathione precursor (N-acetyl cysteine) or (ib) the injection of anti-inflammatory mesenchymal stem cells. (c) Following chronic ethanol intake, if animals are deprived of ethanol for a long period (often 14 days), are subsequently exposed to ethanol cues and are allowed ethanol re-access, a marked relapse-like intoxicating (“binge”) intake occurs (termed the Alcohol-Deprivation-Effect: ADE). After ethanol deprivation, brain acetaldehyde is again required to elicit the ADE, while neuroinflammation is perpetuated. The ADE is inhibited by 70-85% by anti-catalase shRNA, the intracerebral administration anti-inflammatory mesenchymal stem cells or orally administered N-Acetyl Cysteine. Overall, studies indicate that the administration of (i) oral N-Acetyl Cysteine and (ii) anti-inflammatory mesenchymal stem cells afford translational opportunities for the treatment of alcohol-use disorders.

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CAN ALCOHOL DEPENDENT PATIENTS REALLY REDUCE THEIR DRINKING: WHAT IS THE EVIDENCE?

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Abstinence from any alcohol remains the safest treatment option for patients with alcohol dependence. Individuals who set an abstinence goal during treatment are most likely to achieve abstinence following treatment. However, there is also evidence that the requirement of abstinence is an extremely challenging threshold which deters a large number of people from seeking treatment (Mann et al., World Psychiatry, 2017). Many individuals with AUD do not wish to seek treatment

because they are unwilling or feel unable to engage in abstinence. Thus, allowing for alternative treatment options that offer drinking reduction goals is an important step to decrease the treatment gap associated with alcohol use disorder.

Observational studies show that remission from alcohol use disorders (AUD) is frequent and that many individuals with AUD - including those with alcohol dependence - reduce alcohol consumption to non-problematic levels (i.e., remit) even in the absence of treatment (Mann et al., *European Addiction Research*, 2017). Controlled studies have tested reduced alcohol consumption and show sustained improvements in drinking reductions for many patients following behavioral treatments and pharmacotherapy.

The guidance papers of European and United States authorities have taken note of these research findings and accept “intermediate harm reduction” or “low-risk drinking limits” as indicators of treatment success. The FDA recommends a low risk drinking outcome of no heavy drinking days (defined as no more than 70 grams of alcohol for men and no more than 56 grams of alcohol for women). The EMA allows several harm reduction goals, including change from baseline in mean daily consumption of alcohol and by reduction in number of heavy drinking days

Based on the compelling scientific evidence, there is growing recognition that harm reduction outcomes including alcohol reduction need to be considered in addition to abstinence for defining treatment success, even among alcohol dependent patients. This holds the potential to increase the appeal of treatment for many of those underdiagnosed and untreated patients around the world.

Keywords: Alcohol dependence, Harm reduction, Reduced drinking, Pharmacotherapy, Controlled drinking.

FEAR MEMORY RECONSOLIDATION IN ETHANOL WITHDRAWN RATS.

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Fear memories induced by stress and drugs of abuse are less vulnerable to reconsolidation interference indicating a resistance to the occurrence of the destabilization phase after recall. However, the mechanisms and the impact of this resistance still require elucidation. A contextual fear memory formed following withdrawal from chronic ethanol consumption is resistant to destabilization-reconsolidation process, with its recall increasing alcohol intake. Moreover, this resistant memory becomes vulnerable to disruption by pre-retrieval d-cycloserine administration. Here, we investigated the effects of fear memory recall and d-cycloserine on the destabilization mechanism in ethanol withdrawn (ETOH) animals. Next, we examined whether fear memory recall could promote a negative affective-like state with the resistance to memory destabilization being implicated in this effect. To address this issue, ETOH rats were evaluated in the elevated plus-maze and in a novel context after fear recall. Finally, the effects of destabilization blockade by nimodipine in control animals and destabilization induction by d-cycloserine in ETOH rats were examined in these tests. Although fear memory recall did not enhance the destabilization markers (GluN2B subunit and polyubiquitinated proteins) in the basolateral amygdala complex of ETOH rats, pre-retrieval d-cycloserine facilitated these molecular events. An elevated freezing response in a novel context and anxiety-like behavior were observed in ETOH rats after retrieval, which were prevented by d-cycloserine and replicated in control rats treated with nimodipine. In conclusion, resistance to the destabilization is involved in the aversive-like state induced by fear memory retrieval in ETOH animals, suggesting its role in the maintenance of alcohol dependence.

RAT EXPOSURE TEST: A STUDY OF DIFFERENT BRAIN AREAS ACTIVATION, EVALUATION OF CRFERGIC NEUROTRANSMISSION INFLUENCE AND EFFECT UPON ETHANOL CONSUMPTION.

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Exposure to stressful events has been identified as one of the risk factors for alcohol abuse. Individuals who have high levels of anxiety or depression are more prone to consume alcohol which one is known by its anxiolytic effects. Anxiety and fear would have their origin in animal defense behavior that occurs in response to the dangers and threats found in their environment. Rodents have played a key role in the study of the neurobiology of emotional states. The Rat Exposure Test (RET) is a model that allows evaluating the anxiety-like behaviors in mice due to the fact of rat is an aversive stimulus to mouse who can be predate by rat. RET apparatus is divided in two equal-sized compartments (the surface and the predator compartment) separated by a wire mesh screen. Home chamber is a small box made of black Plexiglas on three sides and clear Plexiglas on the fourth side to facilitate videotaping. The home chamber is connected to the exposure cage by a clear Plexiglas tube tunnel. Once exposed to RET mouse will exhibit anxiety-like behaviors such as risk assessment and prefers to spend more time in the home cage (called protected area because it is farther from the rat) compared to surface area, closer the rat (called unprotected area). Anxiolytic drugs have the characteristic of increase time spent on the surface, the most aversive area, showing fear decrease besides of decrease behaviors related risk assessment. The aim of the present study was (i) to evaluate the activity of brain area through Fos-positive cells counting, after immunohistochemistry protocol, in brain areas involved in the stress response such as periaqueductal grey (PAG), basolateral amygdala nuclei (BLA) and ventral portion of hippocampus (VH) in mice exposed to rat, toy rat once or repeatedly and its controls (naïve mice), (ii) to evaluate the effect of corticotropin-releasing factor (CRF) antagonist in the same brain area described above upon the anxiety-like behaviors induced by RET exposition and (iii) the evaluation of ethanol consumption in mice exposed to RET as a model of stressor once or repeatedly. Results showed: an increase of fos-immunoreactive cells into the dorsal-PAG, ventral-Hippocampus, but not into the BLA, in mice exposed to rat alive

but not to toy rat, compared to control not stressed, showing an increase of activity of these two brain areas in response to RET exposition. Results also showed anxiolytic-like effect in animal microinjected with CP 376395, a CRF type 1 receptor antagonist into the three brain areas, showing a participation of this peptide in the modulation of RET-induced behavioral response. Moreover, it was observed an increase in the ethanol intake in mice exposed to RET demonstrating that this kind of stress can influence the ethanol consumption. Taken together, results can classify rat exposure test as a model of stress and anxiety activating brain areas involved in stress response and that the CRF peptide is involved in these responses and, finally, that the stress underwent in this model can increase the consumption of ethanol.

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SHORT-TERM SELECTION FOR HIGH- AND LOW ETHANOL INTAKE DURING ADOLESCENCE

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The majority of diagnoses of alcohol abuse and dependence take place in the age range of adolescence and young adulthood. The mechanisms underlying the rapid transition from adolescent onset of alcohol intake to alcohol abuse problems are yet not understood. Some theories postulate that the adolescents may exhibit enhanced sensitivity to alcohol's appetitive yet blunted responsivity to ethanol's aversive effects. Yet this pattern does not help explain why only a minor fraction of adolescents progress to problematic patterns of ethanol intake, despite being exposed to similar levels of alcohol. Preclinical, animal models may help dissect the mechanisms underlying this phenomenon. In the present study we produced rodent strains short-term, selectively, bred for high or low ethanol intake during adolescence. To our knowledge, selective breeding program for low and high levels of ethanol drinking have been performed in adulthood but not during adolescence. We short-

term produced two lines of rat, as a function of high (STDRHI) or low (STDRLO) ethanol consumption during adolescence. These lines were derived from a founding population of approximately 120 heterogeneous Wistar rats. Besides the expected differences in the selected trait, STDRHI adolescent rats had, in comparison with STDRLO peers, greater innate anxiety in the open field and light-dark transition tests, as well as significantly greater ethanol-induced behavioral stimulation and a reduced conditioned taste aversion by ethanol. STDRHI rats also exhibit heightened baseline neural activation, as measured via Fos immunoreactivity (Fos-ir), in nucleus accumbens core (AcbC) and ventral tegmental area (VTA), and in central, basolateral and medial amygdaloid nucleus (Bla, Cem and Me, respectively). Ethanol-induced Fos-ir in Cem was exhibited by STDRLO, but not by STDRHI. It seems that those adolescent with greater likelihood of exhibiting ethanol intake also exhibit an anxiety-prone phenotype, greater responsivity to ethanol's appetitive yet reduced sensitivity to ethanol's aversive effects, and an altered pattern of baseline and ethanol-induced neural response to the drug.

Keywords: short-term selection, alcohol, adolescence, rat, motivational effects

ROLE OF ETHANOL METABOLITES IN ALCOHOL ABUSE AND DEPENDENCE

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Ethanol is metabolized in the brain by catalase to acetaldehyde, which is subsequently eliminated by aldehyde dehydrogenase (ALDH2). Despite its peripheral aversive effects, animal studies have shown that acetaldehyde displays rewarding effects at central level, since it can be self-administered

intracranially by rats at micromolar concentrations, suggesting that acetaldehyde would be the active effector of ethanol. The development of ALDA-1, a small organic molecule that acts as a chaperone of ALDH2 and increasing its catalytic activity, had provided a pharmacological tool to reduce acetaldehyde levels in the brain, and thus, to reduce the rewarding effects of ethanol. Additionally, acetaldehyde can condense with dopamine in the brain to form salsolinol, a potent reinforcing compound. Animal studies have shown that salsolinol administration can sensitize animals for ethanol drinking. This effect of salsolinol is blocked by the administration of opioid antagonists, which is in agreement with in vitro studies showing that salsolinol is an agonist of the mu-opioid receptor. Regarding the aversive effects of peripheral acetaldehyde, recent studies have shown that fenofibrate, a PPAR alpha agonist, is able to increase the levels of catalase in the liver and to elevate blood levels of acetaldehyde in the presence of ethanol. Fenofibrate administration to alcohol-preferring UChB rats results in 70% of reduction of voluntary ethanol intake and a marked reduction of the ethanol-induced conditioned place preference. These results highlight the dual nature of acetaldehyde in ethanol effects, being rewarding at central level but useful as a deterrent of ethanol consumption at the periphery.

Keywords: acetaldehyde, salsolinol, ALDA-1, fenofibrate