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

Poster Abstracts (alphabetic order).

DEVELOPMENTAL LEAD EXPOSURE INCREASES ETHANOL-INDUCED LOCOMOTION IN *Caenorhabditis elegans*

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Previous studies have demonstrated that developmentally lead (Pb) exposure induces a higher susceptibility to several responses to ethanol. *Caenorhabditis elegans* has become an excellent model to study the neurobehavioral responses to drugs, including ethanol. Nematodes in the L3 stage were exposed to $Pb(NO_3)_2$ 5mg/L during 96 hs until their progenie reached the L1 stage. Thereafter, they were washed and transferred to a new plate free of Pb with food during 48 hs. The ethanol effects on motility were evaluated in L3 worms perinatally exposed to Pb, 2 hs after the ethanol concentration in the agar reached 200 or 400 mM. The average speed of ten worms was registered during 2 min either 10 min or 30 min after the onset of ethanol exposure to evaluate the initial depressor response that was followed by a recuperation effect characteristic of ethanol efects on motility. The results demonstrate that the perinatally-Pb exposed worms developed an increased motility both 10 and 30 min after 400 mM ethanol compared to their non-ethanol counterparts, an effect that was not evident in the control animals. No differences were observed in any group at the 200 mM ethanol concentration. Thus, the hypermotility observed in the perinatal Pb exposed worms implies a potentiation in the development of tolerance to the sedative effects of ethanol at the higher dose evaluated. It is concluded that the *C. elegans* model is suitable to reproduce the locomotor stimulants effect of ethanol that were previously reported by us in perinatally Pb-exposed rats.

ENVIRONMENTAL ENRICHMENT, BUT NOT CARBETOCIN, PREVENTS ETHANOL INTAKE IN C57BL/6 MICE

Almeida, M.G.; Rae, M.B.; Costa, G.A.; Silva, N.K.G.T.; Camarini, R.

Oxytocin (OT) is a hormone that plays a role in social experiences. Brain OT receptors (OTR) activation is associated to a diminished physiological response after stress, suggesting a relationship between social interaction and reduced stress levels. Environmental enrichment (EE) is an environmental model that combines several inanimate and social stimuli, which also favours social interaction. While stress is an important drug-use trigger, OT as well as EE, minimize drug-rewarding effects. This study aims to verify if OT or EE are protective against ethanol consumption before and after stress exposure. After an acquisition phase, male C57BL/6 mice received ethanol 20%/2 h/daily, following Drinking in the Dark (DID) protocol for 15 days. Afterwards, they were subdivided into 3 groups: standard housing (NE); EE; and carbetocin (CBT), an OT analogue. Mice were left in these conditions for 21 days and were reexposed to ethanol weekly for 2 weeks. On the 3rd week, all mice were exposed to a rat (predator stress-ST), with a net protecting the mice from the rat. Mice were given two-bottle choice between 20% ethanol and water for 24 hs, immediately after exposure to ST and at 7 and 14

days post ST. EE-ST drunk less ethanol (12,2 mg/kg) than NE-ST (21,6 mg/kg) 14 days post stress. In addition, CBT drunk less ethanol before stress (14,1 mg/kg), but increased their intake after stress (26,1 mg/kg). These results show that EE is protective against ethanol intake. However, CBT can induce an augmentation in ethanol drinking upon stress exposure.

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HUMAN NEONATES PRENATALLY EXPOSED TO ETHANOL EXHIBIT RESPIRATORY DEPRESSIONS WHEN EXPOSED TO THE OLFACTORY CUES OF THE DRUG

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Following maternal ethanol ingestion fetuses process the drug´s chemosensory cues present in utero. The unborn organism is also capable of associating these cues with physiological effects of the drug. In altricial mammals, ethanol suppresses fetal breathing movements. This effect appears to be associated with the drug´s sensory cues. Later exposure to ethanol odor elicits conditioned breathing depression. The present study analyzed whether different patterns of ethanol use or abuse during human pregnancy affects respiratory rates in neonates re-exposed to the odor of the drug. Mothers were classified as infrequent, moderate or excessive drinkers in accordance with ethanol ingestion patterns during pregnancy. Forty-three neonates were tested in terms of respiratory and cardiac frequencies when primarily exposed to ethanol or to lemon odor. No significant differences were observed across maternal groups in terms of neonatal body weights and sizes, head circumferences, Apgar scores, gestational ages, cardiac frequencies or oxygen saturation scores. When neonates were stimulated with lemon odor, respiratory frequencies were similar across maternal groups. Exposure to ethanol odor significantly affected breathing rates as a function of maternal drinking patterns. During the initial phase of the test, babies representative of excessive drinkers exhibited a significant decrease in breathing rates relative to neonates delivered by infrequent drinkers. The results indicate that neonatal breathing alterations are observed as a function of relatively high ethanol exposure during pregnancy and olfactory neonatal re-exposure to the drug´s chemosensory properties. This alteration suggests that ethanol-related learning processes are capable of disrupting the physiological well-being of human neonates.

ENANTIOMERIC SPECIFICITY OF SALSOLINOL ON THE μ-OPIOID RECEPTOR: A MOLECULAR MODELLING STUDY

Berríos-Cárcamo Pablo, Mario Rivera-Meza, Mario Herrera-Marschitz, Gerald Zapata-Torres

Salsolinol [(*S*) or (*R*)-1-methyl-5,6-dihydroxy-1,2,3,4-tetrahydroisoquinoline] is an endogenous dopamine-derived compound suggested to be involved in the pathogenesis of brain diseases like Parkinson's and alcohol abuse. Possible effects of salsolinol in Parkinson's disease due to (*R*)-salsolinol, which is a specific MAO-A inhibitor and a precursor of the neurotoxin N-methyl-(*R*)-salsolinol. On the other hand, (*S*)-salsolinol has been shown to be a μ-opioid receptor agonist, which could be related to the mechanism of ethanol abuse, while (*R*)-salsolinol is much less potent. Here we studied the interactions of the salsolinol enantiomers inserted by molecular docking on the binding pocket of the μ-opioid receptor. The aim was to determine the molecular interactions that promote enantiomeric specificity of (*S*) and (*R*)-salsolinol. In a 300 ns molecular dynamics simulation, (*S*)-salsolinol interacted with D147, Y148, M151, V236, W293, I296, H297 and V300, residues regarded as important for the binding of μ-opioid receptor agonists which stabilized (*S*)-salsolinol in the site. The chiral methyl group promoted the interaction of (*S*)-salsolinol with Y148. On the other hand, (*R*)-salsolinol was less stable in the binding site and its chiral methyl group did not promote a stable interaction. The results

suggest that the small methyl group of salsolinol can be impactful on its ability to bind a biomolecule, and, in the case of the μ-opioid receptor, promoted the binding of the (*S*) enantiomer.

ROLE OF ASTROCYTIC HEVIN ON ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE AND LOCOMOTOR SENSITIZATION IN MICE.

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Addiction is characterized by long-lasting changes in synaptic plasticity involving pre and postsynaptic element, astrocytes and elements of the extracellular matrix (ECM)1. Recently, a matricellular protein called hevin has been implicated in the nucleus accumbens (NAc) in resilience2 and structural plasticity3. We thus hypothesize that Hevin could play a role in alcohol response.
We used conditioned place preference (CPP) paradigm and locomotor sensitization to test the consequences of astrocyte hevin downregulation on alcohol reinforcing properties, sensitivity and locomotor reponse. Adult males C56BL6/J mice were used for the experiments. We inhibited Hevin expression in astrocytes using a viral construct expressing microRNAs against Hevin with an astrocytic promoter (AAV2.5-GFAP-EmGFP-miRHevin) and for control group we injected eGFP virus (AAV2.5-GFAP-eGFP). In the CPP experiment the virus was injected in the NAc and for locomotor sensitization the virus was injected in the dorsal striatum (CPu).
Our datas showed that the absence of Hevin in astrocyte decreased ethanol-induced CPP (time in ethanol-paired site in seconds: 291.7±18.5 GFP-pretest; 413.2±46.1 GFP-test; 254.9±17.2 miRHevin-pretest and 301.1±29,1 miRHevin-test, n=6-9) and locomotor sensitization (locomotion: 146.4±18.8 GFP-Saline; 322.3±36.9 GFP-Alcohol; 129.2±17.2 miRHevin-Saline and 226.2±27.2 2 miRHevin-Ethanol, n=7-9 per group) when compared to controls. In conclusion, this data shows that Hevin expression in astrocytes of the reward circuit play a role in alcohol addiction. Astrocytes control the activity of the medium spiny neurons (MSN), thereby regulating the global activity of the striatum. Given its role in synaptogenesis, Hevin might modulate reward by affecting structural plasticity of MSN.

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INVOLVEMENT OF AMYDALA ON ETHANOL CONTEXT-INDUCED REINSTATEMENT OF ALCOHOL SEEKING

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In human addicts and rat models, environmental stimuli in contexts associated with previous drug use can provoke relapse to drug seeking. Specific patterns of sparsely distributed neurons, called neuronal ensembles, have been recently hypothesized to encode learned associations between these drug-associated contexts and drug effects. We trained rats to self-administer ethanol in context A, extinguished drug-reinforced responding in a distinct context B and after extinction we assessed context-induced reinstatement in context A or B (control group). After reinstatement test, rats were anesthetized and perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde; brain were remove and coronal sections from Amygdala (Amg) were cut. In order to phenotype and quantify the different populations of Amg activated neurons in both contexts, were performed immunohistochemistry and molecular assays. On test day, reexposure to the ethanol-associated context (context A) reinstated ethanol seeking behavior in rats, but not reinstated the behavior when were exposed to the extinction context (context B). The expression of neural activity marker (FOS) in the basolateral amygdala (BLA) was increased in both contexts (A and B). However, in the central amygdala (CeA) it was only increased in Context B. The percentage of neurons activation accessed by double labeling for FOS and NeuN (neuron marker) during the reinstatement test in Context A and B were respectively 0.4% and 1.0% of neuron activation in CeA, 1.6% and 1.8% neuron activation in BLA. The main phenotype of neurons found in both (BLA and CeA) were cholinergic and GABAergic neurons and it seem to be related to the reinstatement to alcohol-seeking. Additionally, no significant differences of CRF and CRFR1 mRNA expression when rats were exposed to alcohol-associated context and extinction context during context-induced reinstatement of alcohol-seeking test. Our results demonstrate that context-induced reinstatement of ethanol seeking and indicate that cholinergic and GABAergic neurons in CeA may be involved in this behavior.

THE GOOD, THE BAD AND THE UGLY SIDE OF ENVIRONMENTAL ENRICHMENT ON ETHANOL'S EFFECTS

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Excessive consumption of alcohol, behavioral sensitization and conditioned place preference (CPP) to ethanol are parameters used to study key behavioral features underlying aspects of addiction. We have evaluated the role of environmental conditions (stress and environmental enrichment) on those ethanol-induced behavioral effects. Environmental enrichment (EE) involves inclusion of motor, sensory, social and cognitive stimuli to an environment and has been demonstrated to decrease psychostimulant-seeking behavior and their rewarding effects. In our laboratory, we have demonstrated that EE blocked and reversed the ethanol-induced behavioral sensitization (EBS). In line with psychostimulant studies, EE showed to have a positive effect on ethanol intake. After mice were exposed to an immobilization stress, EE group consumed less ethanol than standard condition (SC) group. The results suggest that EE may help the animal to cope better with stressful situations, resulting in blunted ethanol drinking. However, in a model of CPP, EE group exhibited higher ethanol preference, suggesting a sensitization of the reward system and/or strengthening of associative memory induced by EE. Moreover, although EE can decrease ethanol consumption in some circunstances, EE mice showed a higher voluntary ethanol intake than SC group after exposure to a chronic unpredictable stress (CUS) protocol. The results suggest that CUS, which is a model that induces enduring stress-related behavioral responses, interferes in ethanol intake in EE mice.

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THE ADOLESCENT SOCIAL ISOLATION OF RATS EXPOSED TO ETHANOL IN UTERUS AND LACTATION INCREASES ETHANOL CONSUMPTION IN THE ADULTHOOD.

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Chronic stress during adolescence and prenatal ethanol exposure are considered vulnerability factors for the establishment of alcohol abuse disorders (AUD). This study determined if PEE animals with a history of stress exposure increase their ethanol consumption at adulthood, when compared to control peers. Pregnant Sprague-Dawley rats were exposed to water or ethanol (10% v/v) both sweetened with 64 mg/l of sucralose during whole pregnancy period and 7 days post delivery. At adolescence (P21), control or developmental ethanol exposure (DEE) animals were housed in social isolation (SI) or in group conditions for 5 weeks. All animals were then tested for ethanol intake (5 or 10%, vs water) in a free choice and intermittent form. The percentage of ethanol preference and the grams of ethanol consumed were measured every two days for 4 weeks. Our results indicated that SI increased ethanol intake in DEE compared with SI control, rats. We suggest that PEE exacerbates stress-induced ethanol drinking. Follow-up studies will dissect the role of stress in the vulnerability of DEE animals to acquire alcohol abuse disorders. Funded by Fondecyt 1140855 and IBRO-PROLAB.

TOLERANCE AND SENSITIZATION INDUCED BY ETHANOL IN PREWEANLING RATS: SUBSEQUENT EFFECTS UPON ETHANOL CONSUMPTION AND TASTE AVERSION LEARNING.

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Chronic ethanol exposure can induce two opposite effects upon locomotion in preweanling rats: tolerance or sensitization. These effects depend on several factors, such as context pre-exposure. Prior experience in a salient context generates tolerance. On the contrary, sensitization develops as a function of the novelty of the testing context. The present study analyzed whether chronic ethanol exposure under conditions that promote tolerance or sensitization, affects ethanol consumption and ethanol-mediated taste aversion learning in preweanlings. Pups
were administered with water or 2.5 g/kg ethanol (postnatal days, PDs, 8-12) and placed in a salient novel context or a similar context to the maternal cage. During PDs 15-19 pups were tested in terms of ethanol or saccharin consumption. All tests were conducted in the mentioned salient context. Saccharin intake was followed by an i.g. administration of ethanol (1.5 or 2.5 g/kg) on PDs 15-17. On PDs 18-19, two extinction sessions took place. Pups trained with ethanol under conditions that favour tolerance development, exhibited heightened ethanol consumption. Ethanoltreated animals under conditions that favour sensitization showed lower ethanol intake patterns. Ethanol pre-exposure, independently from conditions that favour tolerance or sensitization, was a significant factor leading to the inhibition of conditioned taste aversions. These results suggest that ethanol pre-exposure influences ethanol intake in a context-dependent manner and that ethanolmediated taste aversions are reduced as a function of drug pre-exposure. The results suggest that ethanol-related early experiences sensitize the organism to the drug`s positive reinforcing effects and generates tolerance to its aversive unconditioned properties.

EARLY DEVELOPMENTAL ALCOHOL EXPOSURE ALTERS THE MOTOR ACTIVATING EFFECTS OF ALCOHOL DURING ADOLESCENCE

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It is important to analyze the motivational effects of early exposure to alcohol. The consumption of alcohol by the pregnant mother, even in moderate quantities, increases the possibilities of problematic consumption in the offspring. The present work

analyzed (Experiment 1) basal and alcohol-induced motor activity, a proxy of the appetitive motivational effects of this drug, in adolescent Wistar rats (females only) derived from mothers exposed to alcohol [10%, mixed in sucralose (64mg/l)] or sucralose only (control group), as the sole fluid for 22 h/day (plus a supplement of 2h/day of only water) throughout gestation and until postnatal day 7 (PD7). A second experiment analyzed basal or ethanol-induced activity in adult, Sprague Dawley male rats that had been or not exposed to alcohol, as described for Experiment 1. *Experiment 1:* the dams consumed an average of 6.0-7.0 g/kg/d of ethanol, approximately. On PDs 32 and 34 the adolescent offspring was habituated to an open field. On PDs 36, 38, 43 and 45, the animals received an administration of ethanol (0.0 or 2.0 g/kg, i.p.) and immediately after they were evaluated in an open field (OF) for 15 minutes. The results indicated the absence of significant differences between the groups during the habituation sessions. The rats of the prenatal control group did not exhibit motor activation by alcohol, in any of the tests. On the contrary, the rats derived from mothers exposed to alcohol showed a significant increase in motor activity after receiving alcohol, compared to control peers that had received vehicle. This stimulant effect of alcohol was particularly noticeable in the first part of the test and it was fairly similar across testing days. *Experiment 2:* On PD 80 the male offspring received an administration of ethanol (0.0 or 1.0 g/kg, i.p.) and immediately after they were evaluated in an OF for 10 minutes. The results indicated similar number of crossings and time in the center of the OF, across treatments. Animals exposed to ethanol, however, exhibited greater number of rearings – an indicator of exploratory activity -- than control counterparts. These results indicate that exposure to alcohol during the prenatal stage (and during the first days of the lactation) sensitizes the stimulant, motor activating, effects of alcohol, as measured during adolescence. Some effects of this developmental alcohol exposure persisted until adulthood, when adult rats with a history of early alcohol exposure exhibited alterations in ethanol-induced exploratory activity. Heightened sensibility to the rewarding effects of alcohol might be one of the mechanisms that underlie the increased risk for alcohol problems, exhibited by those that have been exposed to the drug early in life.

ENVIRONMENTAL ENRICHMENT ENHANCED ETHANOL CONSUMPTION AFTER CHRONIC UNPREDICTABLE STRESS

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Excessive consumption of alcohol is one of the key features in the process of addiction. Environmental conditions may affect craving and withdrawal-induced increase in ethanol intake. The objective of this study was to evaluate whether chronic unpredictable stress (CUS) would alter ethanol intake in animals maintained in an enriched environment (EE) during the whole experimente. The drinking paradigm was two-bottle choice in "drinking in the dark" (DID) with multiple deprivation periods and reexposures. After an acquisition phase (mice were given the two-bottle choice - 20% ethanol and water- during 2h/day for 15 days), male C57BL/6 mice were distributed into 2 groups: standard housing condition (SC) or EE and subsequently exposed to the DID protocol for 21 days. Afterwards, all mice were exposed to a protocol of chronic unpredictable stress (CUS) for 11 consecutive days, during which ethanol was not offered. Following this period, mice received weekly access to 20% ethanol for 3 weeks. On re-exposures to ethanol, EE mice showed a higher voluntary ethanol intake than SC group after CUS. The results suggest that CUS, which is a model that induces enduring stress-related behavioral responses, interferes in ethanol intake in EE mice.
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EFFECTS OF CENTRAL ADMINISTRATION OF ETHANOL OR ACETALDEHYDE UPON LATER ETHANOL INTAKE IN NEONATAL RATS

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Intracisternal administration of ethanol or acetaldehyde in neonatal rats exert positive reinforcing effects; a phenomenon revealed by appetitive responsiveness to a conditioned stimulus that signals the effects of these drugs. Interestingly, pre-exposure to ethanol or acetaldehyde later inhibits operant behaviors reinforced with an ethanol solution. In this study we analyzed whether central administrations of ethanol or acetaldehyde affect ethanol consumption following pairings of these drugs with the scent of alcohol. The study also analyzed if the state of central intoxication modulates ethanol ingestion. During postnatal days 2 and 4, Wistar pups were intracisternally administered with a buffer solution, ethanol (100 mg%) or acetaldehyde (0.35 or 0.52 μM). At postnatal day 6, ethanol intake was evaluated via an intraoral infusion procedure. Pups were either sober or under the central effects of the drug previously experienced. Motor activity was not affected by the state of central intoxication. Ethanol pre-exposed pups tested while intoxicated showed heightened ethanol preference as evidenced by body weight gains and latency to drip the solution. Pups pre-exposed to ethanol but tested under the effects of a buffer solution clearly rejected ethanol. The higher dose of acetaldehyde (0.52 μM) partially inhibited ethanol intake. The results indicate that central ethanol, but not acetaldehyde, acts as a positive interoceptive context favoring ethanol intake. The absence of this context in pups also pre-exposed to ethanol, appears to generate a negative emotional reaction that inhibits ethanol acceptance; a phenomenon comparable to a successive negative contrast effect.

NEONATAL EXPOSURE OF SEX HORMONES ON THE EXPRESSION OF GHRELIN RECEPTOR mRNA IN THE VENTRAL TEGMENTAL AREA OF ADULT SPRAGUE-DAWLEY RATS: IMPLICATIONS ON VOLUNTARY ETHANOL CONSUMPTION.

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The neonatal reprogramming with sex hormones during the postnatal day 1(PND-1) produces long-term functional changes on brain circuits related to reward and locomotion. Our laboratory has demonstrated that neonatal administration of Estradiol Valerate (EV) and Testosterone Propionate (TP) increases the dopamine content in Substantia Nigra-Ventral Tegmental Area (SN-VTA), associated to a greater expression of tyrosine hydroxylase, while the neonatal administration of Dihydrotestosterone (DHT), a non-aromatizable androgen, does not generate the same effects.
Ghrelin, a peptide hormone synthesized in stomach which regulates the energetic state and growth hormone release. Ghrelin increases the ethanol intake, while *antagonists of ghrelin receptor (GHSR-1a)* decrease the ethanol intake. In addition, circulating levels of ghrelin exhibit sexual dimorphism, suggesting that *Ghsr-1a* expression is modulated by sex hormones.
The aim of this study is to evaluate the effect of neonatal reprogramming with EV (0.1mg/50μL s.c.), TP (0.1mg/50μL s.c.), DHT (1mg/50μL s.c.) or sesame oil (50μL s.c.) on the expression of *Ghsr-1a* mRNA in the VTA of adult male and female Sprague-Dawley rats exposed or not to ethanol using the intermittent-access and two-bottle free-choice paradigm from PND-32 to PND-66.
EV-treated females and TP-DHT treated males, unexposed to ethanol, have lower expression of *Ghsr-1a* compared to controls. When animals exposed to ethanol are analyzed, EV-treated female rats shows higher expression of *Ghsr-1a*, while DHT-treated males have lower expression compared to controls. These results suggest that the neonatal exposition to sex hormones with estrogenic and androgenic activity alters the ghrelin system, dysregulating the dopaminergic neurotransmission, leading to decreased dopamine release; whereas the opposite occur in the EV-treated female rats that consume ethanol.

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EFFECTS OF PRENATAL ETHANOL EXPOSURE ON ALCOHOL INTAKE AND ANXIETY RESPONSES: MODULATION BY ENVIRONMENTAL ENRICHMENT OR SOCIAL ISOLATION

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Several studies indicate that prenatal ethanol exposure (PEE) alters ethanol intake at adolescence. At present, however, little is known on how this effect of PEE is modulated by exposure to protective (e.g., enriched) or adverse (e.g., with high stress load) environments. This work analyzed chronic ethanol intake (12 24-h tests, every other day save Sundays for 4 weeks), and anxiety responses in young adult Wistar rats, males and females, exposed or not to prenatal alcohol (continuous access to 10% ethanol, throughout gestation and during the first week of breastfeeding). As adolescents (postnatal day 21 to 42) the rats were housed under conditions of environmental enrichment, under stress (social deprivation) or under standard, animal-facility, conditions. Anxiety responses were evaluated through the light-dark box test.
Social isolated animals, particularly the males, showed increased consumption of alcohol during the initial tests, as compared to enriched or control animal, and display higher levels of anxiety. A paradoxical result was animals treated prenatally with alcohol exhibited reduced alcohol intake than vehicle-exposed controls. This effect was not significantly modified by housing conditions at adolescence. The paradoxical effect could be explained by the development of aconditioned aversion towards the taste or smell of alcohol, due to the association between these properties of the drug and maternal maltreatment or lack of appropriate maternal care. Future studies should assess this possibility.

ETHANOL ALTERS INTRACELLULAR PATHWAYS IN HUMAN NEURONS

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Excessive alcohol drinking promotes mTORC1 activation and protein translation in neurons. The synaptic protein translation is controlled by mTORC1 through downstream proteins phosphorylation and activation. However, the role of mTORC1 downstream effectors-dependent synaptic proteins - the p70 ribosomal S6 kinase, ULK1 and FMRP - in alcohol excessive drinking remains largely unknown. Thus, this study aims to determine whether alcohol treatment could alter mTORC1 and its downstream pathway in neuronal human cells. To do so, we treated during 30 minutes human neuroblastoma cells (SH-SY5Y) with ethanol in different concentrations (50, 100, 200, 400 and 600mM) and with rapamycin (as a positive control). The activation of mTORC1, p70 S6K, ULK1 and FMRP were measured by Western Blotting assay. Our preliminary results demonstrates that alcohol increased dose-dependent mTORC1 activation (CTL: 100, 50mM: 76.97, 100mM: 100.29, 200mM: 119.78, 400mM: 113.58, 600mM: 143.74 and Rapamycin: 86.98) and decreased phosphorylation of p70 S6K (CTL: 100, 50mM: 56.79, 100mM: 41.43, 200mM: 33.28, 400mM: 13.75, 600mM: 11.92 and Rapamycin: 3.98), ULK1 (CTL: 100, 50mM: 37.90, 100mM: 37.01, 200mM: 22.43, 400mM: 25.74, 600mM: 30.82 and Rapamycin: 69.40) and FMRP (CTL: 100, 50mM: 34.62, 100mM: 34.32, 200mM: 25.70, 400mM: 25.34, 600mM: 30.99 and Rapamycin: 35.70). As expected, alcohol increased dose-dependent mTORC1 activation (as observed in animal models) but decreased all its downstream pathways. In this way, we can hypothesize that, just

like animal models, alcohol increases mTORC1 and could be related to alcohol abuse and addiction. Further studies will be provided to elucidate the meaning in decreasing downstream mTORC1-dependent pathways. Financial Support: FAPESP (Process Number: 2016/18701-3).

REPEATED BINGE DRINKING EPISODES FROM ADOLESCENCE TO ADULTHOOD GENERATES ANXIETY-LIKE AND MEMORY OBJECT-RECOGNITION IMPAIRMENT ASSOCIATED TO LONG TERM DAMAGE IN BIOCHEMICAL PROCESSES

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Adolescence is characterized by intense neuroplasticity and high sensitivity to psycotropic drugs including Ethanol. Binge drinking, i.e. the consume of high ethanol doses in a limited period (2 h), is common in adolescents persisting to adulthood and can have long-term deleterious effects on brain maturation. Here, we submitted female Wistar rats to a binge drinking model consisting of ethanol intragastric administration (3.0 g/kg, 20% ethanol w/v; 3 days on/4 days off) from postnatal day (PND) 35 to PND 86, and addressed putative alterations on motor, cognitive and emotional behavior, as well as neurochemical changes on hippocampus at PND 37, PND 58, PND 86 and PND 100 (i.e., following 14 days of ethanol withdrawal). Binge drinking increased anxiety-like behaviors in the open field and elevated plus-maze tests and impaired the sort-term memory in the object-recognition task, without gross motor alterations. The observed behavioral impairments were accompanied by increase in plasmatic corticosterone levels, reduced hippocampal BDNF levels and changes on oxidative balance. These findings provide original evidence that repeated binge drinking episodes from adolescence to adulthood generates long-term changes on neurobehavioral and neurochemical in female rats that can persist even following a considerable period of ethanol withdrawal.

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CHRONIC RESTRAINT STRESS AND ACUTE BINGE ETHANOL INTOXICATION INDUCE APOPTOSIS IN THE ADOLESCENT BRAIN

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Restraint stress (RS) promotes release of corticosteroids and induces neurotoxicity in the hippocampus. Most of the studies analyzing this phenomenon have employed protracted days) exposure to restraint stress. RS also increases ethanol intake and exacerbates anxiety patterns in adolescent and adult rats, an effect that is reversed by ethanol administration only in adolescents. Binge ethanol administration can induce brain toxicity, analogous to that induced by stress. On this basis, it could be postulated that ethanol intoxication may facilitate stress-induced neurotoxicity. In the present study, we analyzed whether adolescent rats exposed to five episodes of RS exhibit neurodegeneration in the dorsal and ventral hippocampus [CA1, CA2, CA3 and dentate gyrus

(DG)]; and whether this is modulated by a binge, yet brief (two administrations of 2.5 g/kg ethanol, separated by 120 min), ethanol administration. The results indicated a synergistic, neurotoxic effect between RS and ethanol in dorsal DG; whereas RS induced neurodegeneration in CA1 of dorsal hippocampus and CA2 and CA3 of ventral hippocampus. Binge ethanol administration induced neurotoxic effects in DG and CA2 and CA3 of dorsal hippocampus and in CA1, CA2 and CA3 of ventral hippocampus. The study highlights the vulnerability of the developing brain to alcohol insult and stress exposure.

ASSESSMENT OF MATERNAL CARE BEHAVIORS IN RAT LINES SELECTED FOR HIGH OR LOW ALCOHOL INTAKE DURING ADOLESCENCE

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The literature suggests that 50% of alcohol use disorders (AUDs) is attributable to genetic factors. In our laboratory we have produced two lines of Wistar rats, short-term selected for high (STDRHI) or low (STDRLO) alcohol consumption during adolescence. The STDRHI animals exhibited, on filial generations 1 (F1), F2 or F3 and relative to STDRLO peers, exacerbated anxiety, increased sensitivity to the stimulatory effects of alcohol and insensitivity to the aversive effects of the drug alcohol. These results suggest that the genes responsible for modulating these behavioral traits are also responsible for modulating alcohol consumption during adolescence. It is possible, however, that these differences are consequences of other differences between the lines, for example, differences in maternal care. The objective of this study was to analyze maternal behaviors in STDRHI and STDRLO F1 litters, on postnatal days (DP) 7 and 12. We assessed time spent in the nest, grooming of the pups and time adopting a breastfeeding stance. Non-maternal behaviors, such as self-licking, exploration and resting out of the nest, were also evaluated. The mothers and their pups were accommodated in new, clean cages for 60 minutes and the behavior was analyzed in 5 min on, 15 minutes off fractions. STDRHI and STDRLO dams display fairly similar sets of behaviors, although STDRHI dams exhibited, when compared to STDRLO dams, significantly greater exploration and self-licking at PD 7. These findings indicate that the behaviors aimed at the pups were relatively similar in both lines. The behavioral and alcohol intake differences between STDRHI and STDRLO rats do not seem to be related to differences in maternal care.

EXPRESSION OF ETHANOL-SENSITIVE GLYCINE RECEPTORS IN BRAIN REGIONS OF THE REWARD SYSTEM

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Glycine Receptors (GlyR) are abundantly expressed in spinal cord and brain stem. However, currently it is recognized its presence in upper brain regions, but its role and properties are still unknown. The reward system is a neural circuitry involved in the process of rewarding natural stimuli and also during the consumption of addictive drugs, like ethanol. To begin the characterization of the role of GlyR in the regulation of the reward system and consequently ethanol consumption, we analyze three important regions of the reward system, such as Ventral Tegmental Area (VTA), nucleus Accumbens (nAc) and Prefrontal cortex (PFC).
With immunohistochemistry technics, we studied the presence of glycinergic transmission in these three regions. We used a transgenic mouse that expresses GFP under the promoter of the neuronal transporter of glycine (GlyT2), used as a glycinergic neuronal marker. Electrophysiological recordings using whole cell patch clamp in brain slices of C57BL/6J mice, we found the presence of spontaneous glycinergic synaptic activity, sensitive to strychnine (4 □M). We also analyze the evoked glycine currents on dissociated neurons of these regions. The concentration-response curve showed a different EC50 of glycine among the regions. And

the GlyR of neurons from VTA and nAc were sensitive to 100 mM of ethanol, but not PFC.
In conclusion, the presence of GlyRs in these three mesolimbic regions could be important for the regulation of the reward system and the rewarding properties of ethanol.
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MILD STRESS INDUCED BY MATERNAL MANIPULATION DURING LATE GESTATION AND INFANTILE ETHANOL CONSUMPTION INDUCE CHANGES IN MU AND KAPPA OPIOID RECEPTORS, BUT NOT IN OPIOID LIGANDS PRECURSORS.

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Infant rats are vulnerable to ethanol's motivational effects. Additionally, stress mediates sensitivity to ethanol's postabsortive effects and consumption. The impact of mild stressors (i.e. maternal intubation during late gestation) remains scarcely understood during early ontogeny. This work sought to characterize changes in opioid ligands precursors and receptors gene expression in hypothalamus, after prenatal manipulation and infantile ethanol intake. Dams received an intragastric administration (i.g) of ethanol (2g/kg), water or were undisturbed, during gestational days 17-20. At postnatal days (PDs) 14-15, pups were evaluated in terms of 0% or 5% ethanol consumption. A third untreated group was added. After intake test (PD15), mRNA levels of opioid ligands precursors and receptors were measured, by real time PCR. The mRNA expression of ligand precursors did not appear to be affected by either, prenatal manipulation or postnatal intake of the drug. However, the mRNA expression of mu and kappa receptors seems to be sensible to prenatal mild stress and ethanol intake. Infantile ethanol intake seemed to up-regulate the expression of mu and kappa receptors in naïve pups. Nevertheless, ethanol consumption (PDs14-15) resulted in a down-regulation of opioid receptors when pups were exposed to mild prenatal stress. Our results suggest that the induction of a mild stress during late gestation is enough to make relatively permanent changes in mu and kappa expression on hypothalamus, when pups have the opportunity to intake the drug. This work was supported by grants from ANPCyT (PICT 2011-0130) CONICET and SECyT-UNC.

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. Funded by FONDECYT grant N° 1140855.

BRIEF ETHANOL EXPOSURE INDUCES BREATHING DEPRESSION AND A MARKED INCREASE IN APNEAS IN NEONATAL RATS

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The effects of early ethanol exposure upon neonatal respiratory plasticity have received progressive attention given a multifactorial perspective related with the Sudden Infant Death Syndrome or hypoxia-associated syndromes. This preclinical study was performed in rat neonates during the brain growth spurt period (postnatal days 3-9) which is equivalent to the 3rd human gestational trimester. Breathing rates and apneas were examined in pups receiving vehicle or a relatively moderate ethanol dose utilizing a whole body plethysmograph. Ethanol exposure progressively exerted a detrimental effect upon breathing patterns. A test conducted at PD 9 when pups were sober confirmed ethanol´s detrimental effects upon respiratory plasticity (breathing depression). Pre-exposure to the drug also resulted in a highly disorganized respiratory response following a hypoxic event characterized by heightened apneic episodes during a recovery normoxia phase. In a 2nd test conducted at PD 9 while pups were intoxicated and undergoing hypoxia, an attenuated hyperventilatory response was observed. In this test there were also indications that prior ethanol exposure depressed breathing rates during hypoxia and a recovery normoxia phase, without an increase in apneas. These results indicate that brief ethanol experience significantly disorganizes respiratory patterns. It causes respiratory depressions during and after a hypoxic event. This breathing anomaly was accompanied by a marked increase in the number of apneas during the recovery normoxia phase when pups were tested sober. These results indicate serious deleterious effects of the drug upon breathing patterns and the emergence of apneic episodes during a critical stage where respiratory plasticity is being structured.

THE EFFECT OF MONO- OR BIPARENTAL CARE ON ETHANOL-INDUCED LOCOMOTOR STIMULATION IN C57BL/6 MICE.

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Social attachment plays an important role in progeny development. Different social experiences during early development and throughout life can affect ethanol use and abuse. In the present study we aimed to analyze if different rearing conditions (mono- or biparental care), in a non-monogamous mice strain, are able to modify ethanol-induced locomotor stimulation. This effect induced by psychoactive drugs has been considered an index of positive rewarding effects of the drug. C57BL/6 infant mice were reared in a monoparental (MP, only mother) or biparental (BP, father and mother) care condition. At postnatal days (PD) 16, 17, 18 and 20, infants were administered with a 0.0 or 2.0 g/kg ethanol dose and evaluated in an open field test. Results indicate that both groups are sensitive to ethanol stimulating effects. MP animals showed tolerance to ethanol-induced stimulation since PD17. Nevertheless, BP animals did not show tolerance to ethanol effect even after the four days of test. Moreover, the intensity of this ethanol effect was also more robust in BP than MP infants. These results indicate that presence of the father in a non-

monogamous strain alter ethanol rewarding effects. Further research is being conducted aimed to analyze parents' behavior during this sensitive period.

REPEATED MATERNAL SEPARATION, ALCOHOL INTAKE, HPA AXIS AND BEHAVIOUR: REVERSION BY ENRICHED ENVIRONMENT

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We assessed the long-term impact of colder maternal separation (RMS) and ethanol intake on both the hypothalamic-pituitary-adrenal (HPA) axis and emotional behavior (plus elevated maze, dark darkness, and open field tests) in adolescent and adult Wistar rats. RMS was applied from the postnatal day (PD) 2 in which the offspring were separated from their mothers and exposed to cold stress (4 ° C) 1 h per day for 20 days. After treatment, they were exposed to a voluntary intake of ethanol (6%) or dextrose (1%) for 7 days by the PD22-29 double bottle method. Then they were exposed to 30 days enriched environment (EE). Finally, the animals were exposed to a second voluntary intake for 7-days. In PD66 we did the behavioral and hormonal tests. The results obtained highlight the consequences of stress during early life. This was reflected in the vulnerability to ethanol consumption, behavior and hormonal response.

FEAR MEMORY RECALL INCREASES ANXIETY-LIKE RESPONSE AND ALCOHOL INTAKE IN ETHANOL DEPENDENT RATS: REVERSION BY RECONSOLIDATION INTERFERENCE .

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Withdrawal from chronic ethanol facilitates the formation of contextual fear memory with its retrieval promoting an increase in anxiety-like state and ethanol consumption. Considering that a fear memory can be attenuated by the interference with reconsolidation, we examined if these effects of fear recall in ethanol withdrawn rats (ETOH) can be reversed by reconsolidation disruption. Male Wistar rats made dependent via an ETOH containing liquid diet (6% v/v) for 14 days. Contextual fear conditioning was performed on day 3 of withdrawal. To interfere with reconsolidation process rats received d-cycloserine (DCS, 5 mg/kg, ip) 30 min before memory retrieval and then were injected with propranolol (PROP,10 mg/Kg, ip). Rats treated with saline (SAL) after retrieval and non-ethanol dependent animals were also evaluated. The beer (ethanol, 6%v/v) intake and performance in the elevated plus maze (EPM) were examined 30 min after recall of a memory interfered or not. ETOH animals treated with DCS/SAL showed a significant decrease in the percentage of time spent on the open arms and in the number of open arm entries in the EPM, and also exhibited elevated alcohol intake following fear memory retrieval. However, ETOH rats treated with DCS/PROP displayed beer consumption and EPM performance similar to control groups. Our results suggest that reconsolidation disruption prevents the increase in the anxiety-like response and alcohol intake promoted by fear memory recall in ETOH rats. Hence, fear memory reconsolidation interference acquires relevance in the context of alcoholism.

ROLE OF PRELIMBIC *"NEURONAL ENSEMBLES"* IN CONTEXT-INDUCED REINSTATEMENT OF ALCOHOL SEEKING IN RATS.

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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 pre-limbic cortex. We trained rats to self-administer alcohol in Context A and extinguished their lever-pressing in a distinct Context B. On test day, reexposure to the alcohol-associated Context A reinstated 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 Vehícle drug context vs 4.0±2.0 Daun02 drug context, $p<0.05$). Our data suggest that context-induced reinstatement of alcohol seeking is mediated by activation of context-selected prelimbic cortex neuronal ensembles.

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STUDIES OF REWARD AND AVERSION IN ALCOHOL AND NICOTINE CO-ABUSE

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Knowledge of the adverse effects of combined and excessive use of alcohol and nicotine has existed for decades. On the other hand, knowledge of the factors motivating co-abuse is relatively lacking. It has been hypothesized that the high rate of comorbid use is due to increased rewarding effects of the drugs in combination, compared to the effect of either drug alone. Alternatively, adverse effects that curb nicotine or alcohol use could be reduced by their combined use. The current status of research in these areas will be discussed, with an emphasis on existing animal models and current limitations. Unique interactive effects of nicotine and alcohol have been identified that are synergistic in nature for some traits. Some data suggest that specific nicotinic acetylcholine receptor subtypes may play a role in combined sedative effects of these drugs. However, the potential role of nicotinic acetylcholine receptors or other mechanisms in the synergistic stimulant effects of combined nicotine and alcohol have not been examined. More research is needed to determine mechanisms linked to the combined effects of nicotine and alcohol that increase risk for dependence in individuals who comorbidly use tobacco products and drink alcohol. Supported by the Department of Veterans Affairs and NIH NIAAA P60AA010760, R24AA020245, and T32AA07468.

DEVELOPMENTAL ETHANOL EXPOSURE DELAYS THE EXTINCTION OF A CONDITIONED FEAR MEMORY AND INCREASES ETHANOL CONSUMPTION, DIFFERENTIALLY IN MALES AND FEMALES.

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Repeated exposure to ethanol increases retrieval of fear-conditioned memory, which promotes posttraumatic stress disorder (PTSD). Individuals with PTSD are more likely to develop alcohol-related disorders. Also, it is known that in individuals suffering from disorders related to stress

and trauma can use alcohol to relieve symptoms of PTSD.
We determined if animals given developmental ethanol exposure (DEE) (a) exhibited heightened ethanol consumption, and (b) are more susceptible to retain a fear memory induced by a traumatic event.
Pregnant Sprague-Dawley rats were exposed to water or ethanol (10% v/v), both sweetened with 64 mg/l of sucralose. Auditory fear conditioning (FC) was performed in the male and female offspring, at postnatal day 45. Memory was measured by freezing behavior during acquisition, retention and extinction (days 1, 2 and 9, respectively). Ethanol intake (preference vs. water and grams of ethanol consumed) was measured every two days for 3 weeks after the extinction test.
Our results indicated that 1) The acquisition and retention of the aversive memory was similar in DEE and control animals, and in males and females. 2) DEE males and females, but not their control counterparts, exhibited persistence of the aversive memory during the extinction test. 3) Female rats, controls as well as DEE, exhibited significantly greater ethanol preference than male rats. 4) DEE males and females exhibited greater ethanol intake than their controls right after extinction test.
These results suggest that DEE animals are more vulnerable to exhibit long term retention of aversive memories, and to ingest significantly more ethanol. These results have implications for the understanding of the pathogenesis of posttraumatic stress disorders and comorbid alcohol intake. Funded Fondecyt 1140855 and IBRO-PROLAB.

NEONATAL EXPOSURE TO SEX HORMONES INCREASES VOLUNTARY ETHANOL INTAKE IN ADOLESCENT SPRAGUE-DAWLEY RATS: PHARMACOLOGICAL EVALUATION OF THE ENDOGENOUS OPIOIDERGIC SYSTEM.

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The aim of this research was to evaluate if the increased ethanol intake observed in adult rats treated during first hours of post-natal life (PND1) with estradiol valerate (EV) is due to the activation of the endogenous opioidergic system (EOS).
Methods: Sprague-Dawley rats treated at PND1 with a single dose of EV (0.1mg/50µL s.c.), Testosterone Propionate (TP 0.1mg/50µL s.c.) or sesame oil (50µL s.c.) were exposed from PND 32 to 60 to two-bottles free choice paradigm. At PND 60, we evaluate dopamine (DA) and DOPAC content in Nucleus Accumbens (NAcc) of these rats. In addition, in other group of rats, the ethanol intake was measured during the first 2 hours of access to ethanol, after the administration of Naloxone (2 mg/Kg s.c.), a mu-opioid antagonist.
Results: In EV and TP rats the ethanol intake was higher than control rats, showing at neurochemical level a reduction in DA content and an increase in DOPAC content in NAcc. On the other hand, naloxone administration reduce ethanol intake in EV rats (baseline: 1.12 ± 0.15 g/kg/120min in female rats and 0.94 ± 0.05 g/kg/120min in male rats to 0.66 ± 0.10 g/kg/120min in female rats and 0.62 ± 0.13 g/kg/120min in male rats). In the same rats, saline administration (1 mL/Kg s.c.) does not affect ethanol intake (1.36 ± 0.05 g/kg/120min in female rats and 1.37 ± 0.20 g/kg/120min in male rats) regard to baseline in EV rats.
Conclusion: Rats treated with sex hormones have high levels of ethanol intake in the adulthood and they are associated with a reduction in NAcc DA content. In addition, the ethanol intake in EV rats is sensitive to the modulation for EOS. Therefore, neonatal exposure to sex hormones is a vulnerability factor to promote alcohol dependence.

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INSULAR DOPAMINE AND ALCOHOL CONSUMPTION IN THREE ANIMALS MODELS OF MOOD DISORDERS AT ADOLESCENCE.

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It is well known that monoamine systems are altered in mood (i.e., depression and mania) disorders, and that individuals, including adolescents, with this psychopathology exhibit enhanced alcohol consumption. This work assessed the relationship between insular dopamine levels and alcohol consumption in three pharmacological animal models of mood disorders. Thirty-day old, male and female, Wistar rats were used in three experiments. Depressive-like states were induced by reserpine (0.0 or 1.0 mg/kg/day, for 4 days), whereas mania-like states were induced by amphetamine (0.0 or 4.0 mg/kg/day, for 5 days) or methylphenidate (0.0 or 10.0 mg/kg/day, for 4 days). The animals were tested for motor activity in an open field test (OFT) after each administration. At termination of the chronic drug exposure, half of the animals were sacrificed to dissect the insular cortex and measure dopamine levels by HPLC. The other half was tested for ethanol intake in a two-bottle test (concentration: 5%, v/v; three 24 h tests, separated by 48 h). The results indicated that reserpine significantly decreased motor activity in the OFT, whereas amphetamine and methylphenidate significantly increased motor activity in the OFT. Dopamine levels were significantly decreased after reserpine and amphetamine, but not after methylphenidate. Reserpine treated females and amphetamine treated males exhibited increased alcohol consumption relative to control counterparts, whereas methylphenidate did not signficantly alter alcohol intake levels. The results suggest that decreased levels of monoamines at the insula may be one the factors associated with increased alcohol consumption in adolescents with mood disorders.

ALCOHOL USE, CONSEQUENCES, AND THEIR ASSOCIATION WITH PSYCHOLOGICAL DISCOMFORT IN YOUNG URUGUAYANS.

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Little is known about patterns of alcohol consumption among youth and young adults from Uruguay, and even less is known about the consequences of, and emotional predisposition for, alcohol use in this population. This study described the ocurrence of alcohol use, and alcohol-related consequences in a large sample of Uruguayans (*n*=1527, 27% men, mean age=23,5±3,5 years). The association between these behaviors and psychological discomfort and sensitivity to emotional contagion were also assesed. Participants completed an online survey that measured alcohol use [alcohol use disorders identification test (AUDIT), and ad-hoc consumption questionnaire), consequences of alcohol use [young adult alcohol consequences questionnarie (YAACQ)], psychological discomfort (Kessler scale) and proclivity to emotional contagion (Emotional contagion scale). The results indicated a 91% prevalence of alcohol use in the last 12 months, with 46% of these subjects (50% in men) engaging in heavy episodic drinking (drinking 4/5 standard drinks in a single ocassion). Seventy percent of heavy drinking episodes were done in a 2-hr timeframe. There was a significant ($p<0.05$) correlation among psychological discomfort and AUDIT and YAACQ scores, and between psychological discomfort and emotional contagion, but not between the latter scale and the AUDIT or YAACQ scores. These results indicate a relatively high ocurrence of alcohol use behaviors among late adolescents and young adult Uruguayan. The results also support the hypothesis postulating mood variations, but not emotional contagion, as a predisposing factor for alcohol consumption

NEONATAL REPROGRAMMING WITH SEX HORMONES ON THE EXPRESSION OF DOPAMINE TRANSPORTER IN MESOLIMBIC AND NIGROESTRIATAL PATHWAYS OF ADULT RATS.

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Neonatal reprogramming with sex hormones produces long-term functional changes in the brain. Neonatal exposure to estradiol valerate (EV) has been shown to increase dopamine (DA) content in Substantia Nigra- Ventral Tegmental Area (SN-VTA), Nucleus Accumbens (NAcc), and Striatum (S) of adult rats. This increase in the content and release of DA is associated with an increase in tyrosine hydroxylase immunoreactivity in SN-VTA. However, the effects of neonatal reprogramming with sex hormones on the expression of DA transporter (DAT) in mesolimbic and nigroestriatal pathways of adult rats have not been described.
In this study we evaluated the effect of neonatal administration of EV (0.1 mg/50uL), Testosterone Propionate (TP, 1 mg/50uL) or Dihydrotestosterone (DHT, 1 mg/50uL) on the expression of DAT in SN, VTA, S and NAcc from adult rats by immunoblot and RT-qPCR.
The results demonstrate in females treated with EV a decrease of DAT mRNA in SN-VTA and an increase in VTA of rats DHT. Regarding protein levels, it was observed that glycosylated DAT (glyco-DAT) decreased in S-NAcc in EV, TP and DHT rats. Regarding non-glycosylated DAT (non-glyco-DAT) expression decreased only in S. In males treated with EV and DHT there is an increase in DAT mRNA in SN and a decrease in VTA expression of TP-DHT rats. Regarding the protein levels, males treated with EV showed a decrease of glyco-DAT in NAcc.
These results demonstrate that neonatal exposure to sex hormones modifies DAT expression differentially in the major nuclei of the mesolimbic system. Further studies are needed to determine the functionality of changes in levels of this protein.

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BEHAVIORAL ASPECTS OF ENVIRONMENTAL ENRICHMENT IN RESPONSE TO NICOTINE ADDICTION AND MEMORY FUNCTION.

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Addiction is a familiar phenomenon around the world. Drug addiction is a chronic, relapsing disorder in which there is a compulsive and drug-seeking and drug-taking behavior with negative consequences. Majority of people are found addicted to the legal substances, such as nicotine, caffeine and alcohol. Nicotine is an alkaloid found in tobacco and is the main cause leading to addictive tobacco smoking. Along with known genetic and psychological factors leading to nicotine addiction, some environmental factors are also known to influence its addiction like social isolation [1, 2]. Environmental enrichment (EE) is a means of improving human welfare by providing opportunity to interact socially. Enriched environment improves physical or psychological health, increase neurogenesis during neurodevelopment of brain. In our present investigation we have shown that the enriched environment has protective effects against nicotine addiction. In addition to this we have also assessed the effects of enriched environment on learning and memory in rats [3, 4]. Here, the Male Albino Wister rats were divided into 2 groups. Rats in first group were provided isolated environment while in second group were socially combined and were placed in an enriched environment 2 weeks before performing the test. Rats in both groups were further divided into control and test rats; that were treated with intraperitoneal (I.P) injections of nicotine at a dose of 0.5mg/ml/kg. Addiction parameter was evaluated by conditioned place preference (CPP) test [5]. Learning and memory was assessed by elevated plus maze test. Behavioral studies statistics were analyzed by ANOVA, which showed significant effects of enriched environment in reducing nicotine addiction. Furthermore, it also reduced the drug seeking behavior and improved learning and memory function in rats. Our findings showed that positive environmental conditions play

a beneficial role in reducing or treating the adverse effects of nicotine addiction and enhances memory acquisition in rats. Thus, we could show that enriched environment induces increase in neurogenesis which may also play part in improved memory performance.

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EVALUATION OF THE ETHANOL INTAKE IN ENRICHED ANIMALS DURING AND AFTER A CHRONIC UNPREDICTABLE STRESS PROTOCOL

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Alcohol use disorder is one the main risk factors that lead to sickness, disability and death worldwide, according to WHO. Environmental conditions (housing, stress) can alter ethanol consumption in laboratory animals and humans. The aim of this study was to evaluate whether EE would alter ethanol intake during and after a chronic unpredictable stress protocol. The drinking paradigm was two-bottle choice in "drinking in the dark" (DID). After an acquisition phase (2h/day to ethanol 20%), male C57BL/6 mice were distributed into 2 groups: standard housing condition (SC) or enriched environment (for 21 days). Afterwards, mice were exposed to a chronic unpredictable stress (CUS) for 11 consecutive days. Two-bottle choice (20% ethanol vs. water) was offered to all mice before and during CUS and for more 7 additional days after CUS. Following this period, mice were weekly re-exposed to the two-bottle choice twice. During the CUS period and also during re-exposures, EE mice showed greater ethanol intake than SC mice. These results suggest that environmental enrichment interact with CUS enhancing ethanol intake in C57BL/6 mice.

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NEONATAL PROGRAMMING WITH ESTRADIOL INCREASES THE NEUROCHEMICAL AND REWARDING EFFECTS INDUCED BY MORPHINE IN ADULT RATS.

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Neonatal programming with sex hormones produces long-term functional changes in various tissues, including the brain. For example, neonatal exposure to estradiol valerate (EV) increases the catecholamine content in brain circuits related to reward and locomotion. Moreover, sex hormones have been shown to increase the expression of the opioid receptor and beta-endorphins. Therefore, neonatal reprogramming with hormones could alter morphine response during adulthood in rats and predispose to addiction.
The aim of this work was to evaluate the behavior of conditioned place preference (CPP) and the morphine-induced dopamine (DA) release in nucleus accumbens (NAcc) in Sprague-Dawley adult rats exposed during the first postnatal day (PND) to oil of sesame (50 µL sc) or EV (0.1 mg / 50 µL sc). The CPP protocol was performed from the PND-56 with a duration of 5 days and injected during the conditioning morphine (3 mg/kg s.c.) or saline (1 mL/kg s.c.). Microdialysis studies were performed at PND-60, measuring the basal and morphine-stimulated DA release (5 mg/kg i.v.) in NAcc.

The results show that morphine-induced place preference is significantly higher that produced by saline. In addition, in animals treated with EV the morphine-induced place preference was higher than that observed in control animals. Consistent with these results, a higher NAcc DA release was observed in EV rats compared to control rats.
The results demonstrate that neonatal administration of EV increases the pharmacological effects of morphine, possibly through increased expression of mu-opioid receptors in GABAergic interneurons of the ventral tegmental area.

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PRESENCIA DE SUBUNIDAD ALFA 1 DEL RECEPTOR DE GLICINA EN REGIONES DEL SISTEMA DE RECOMPENSA

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El receptor de glicina (GlyR) esta descrito primariamente en neuronas inhibitorias de la medula espinal y tronco encefálico, donde se ha demostrado que es potenciado por etanol sin embargo, poco se conoce de estos receptores en regiones supraespinales. Actualmente, se reconoce la existencia de cuatro subunidades alfa y una beta que forman canales pentaméricos homoméricos o heteroméricos con distintas propiedades. Recientemente, utilizando ingeniería genética se generó un ratón KI con una mutación en alfa 1 (KK385-386AA), en el cual el GlyR alfa 1 presenta una menor sensibilidad a modulación por etanol.
Utilizando este ratón nos propusimos comparar la sensibilidad a etanol de los GlyR en regiones del sistema de recompensa como, corteza prefrontal (PFC), núcleo Accumbens (nAc) y área tegmental ventral (VTA) en ratones wildtype (WT) y KI para caracterizar diferencias fenotípicas. Estas son áreas del sistema mesocorticolímbico relacionado por numerosos estudios a las adicciones. Postulamos que las diferencias entre ambos ratones indicarían la presencia de alfa 1 en la región estudiada.
Experimentos de western blot utilizando un anticuerpo que detecta alfa 1 en forma selectiva mostro expresión de esta subunidad en las tres regiones estudiadas. Estudios de inmunohistoquímica utilizando rebanadas de 60 m de grosor también mostraron la expresión de la subunidad en las tres regiones, aunque con distintas distribuciones celulares. Finalmente, registros de célula completa usando patch clamp en neuronas disociadas, mostraron la que neuronas de PFC fueron insensibles al etanol en animales WT y KI. En VTA y nAc, etanol potenció el GlyR en ratones WT, pero no en KI sugiriendo presencia de alfa1 en estas regiones.

ALTERED GENE EXPRESSION OF KAPPA OPIOID SYSTEM, BDNF AND NOCICEPTIN SYSTEM ON MESOLIMBIC BRAIN AREAS AFTER PRENATAL ALCOHOL EXPOSURE.

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Several experiments indicated that moderate prenatal alcohol exposure (PEE, 1-2 g/kg, gestational days 17 to 20) induces a significant, facilitatory effect on subsequent ethanol consumption in infant or adolescent rats. This effect may be the consequence of PEE enhancing or reducing the appetitive and aversive motivational effects of ethanol, respectively. The mechanisms underlying PEE effects are, however, still elusive. The endogenous opioid system has been proposed as an important target of alcohol's actions and ethanol exposure seems to alter the developmental trajectory of opioid systems, possibly affecting the hedonic effect of ethanol. The aim of this study was to describe the effect of PEE on gene expression of mesocorticolimbic areas. Pregnant Wistar rats received daily intragastric administration of alcohol (0.0 or 2.0 g/kg). Female and male offspring were analyzed on gene expression levels of predynorphin (PDYN) and kappa opioid receptors (KOR), Brain-Derived Neurotrophic Factor (BDNF), nociceptin (NOC) and nociceptin receptor (NOP) in

mesocorticolimbic areas of the brain (infants and dolescents).
PEE induced enhanced gene expression levels of PDYN in Ventral Tegmental Area (VTA) during infancy and KOR in VTA and Prefrontal Cortex (PC) in both ages. Moreover, gene expression of NOC was significantly elevated in Nucleus Accumbens (NA). PEE induced elevated levels of NOP in PC, but reduced levels in VTA. Finally, VTA exhibited lower levels of BDNF in PEE rats. These results confirm that a moderate exposure to alcohol during the last days of pregnancy causes alterations on mesocorticolimbic system, that could lead to a vulnerability state and alcohol consumption.

VENTRAL STRIATUM DIRECT PATHWAY POSSIBLY MEDIATES ETHANOL CONTEXT-INDUCED REINSTATEMENT: PRELIMINARY DATA

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Alcohol addiction is a complex behavioral phenomenon and considered a world health problem. High rates of relapse are observed in alcohol addiction and one of the main problems for the treatment is the high rates of relapse. In human addicts, environmental stimuli previously associated with drug use can evoke relapse to drug seeking after prolonged period of abstinence. Learned associations play a significant role in addiction and relapse. These drug-related cues are complex combinations of different stimuli and complex neuronal pathways. Studies suggest the participation of ventral striatum direct and indirect pathways on learning associative behaviors and drug seeking. We hypothesized that D1 medium spine neurons in nucleus acumbens (NAc) encode learned associations between alcohol effects and environmental cues. Here, we investigated, by disconnection procedure, whether context-induced reinstatement of alcohol seeking is mediated by projections from NAc (D1 medium spine neurons) to substantia nigra reticulate (SNr) (termed, direct pathway). For that, male Long Evans rats (n=6) were trained to self-administrate ethanol in context A and extinguished lever pressing in a distinct context B. On the test day, the context-induced reinstatement of ethanol seeking was tested in the ethanol context (A). To assess the role of direct pathway, subjects were ipsilateral cannulated into NAc and SNr and 10 minutes before the test, rats received microinjections of saline or $CoCl_2$ on SNr and SCH23390 (D2 antagonist) on NAc. Results demonstrate that rats with ipsilateral direct pathway blocked did not reinstate ethanol seeking (Mean±SEM - Active bar press SCH23390/$CoCl_2$: 0.6667 ± 0.3333 x Active bar press Saline: 44.00 ± 9.866; t=4,390 p<0,05), suggesting that context-induced reinstatement of alcohol seeking is possibly mediated by the activation of striatal direct pathway.