

Speaker's Oral presentations (by alphabetic order)

INVOLVEMENT OF L-TYPE CHANNELS IN THE BEHAVIORAL EFFECTS INDUCED BY ETHANOL

Carlos M.G. Aragón. Area de Psicobiología.
Universtitat Jaume I. Campus de Riu Sec. Apartat.
8029 AP. 12071 Castelló. aragon@psb.uji.es

Brain homogenates and astrocytes in culture incubated with ethanol are able to produce significant levels of acetaldehyde. The main system of this central ethanol oxidation is mediated by the enzyme catalase. By reacting with H₂O₂, brain catalase forms compound I (the catalase-H₂O₂ system), which is able to oxidize ethanol to acetaldehyde in the brain. The brain catalase-H₂O₂ (compound I) system has been proven to be involved in the regulation of some of the behavioral effects of ethanol. Specifically, the role of central catalase in ethanol-induced behavioral effects has been soundly established. Data suggesting the contribution of compound I in the control of the effects produced by ethanol in rodents have been derived from two different approaches. Several reports have demonstrated that it is possible to decrease or increase the stimulating effects of ethanol in mice by reducing or increasing

brain catalase activity and thereby altering central ethanol metabolism. It has also been shown that increasing or reducing the rate of central H₂O₂ production (which is necessary to form compound I) boosts or blocks the ethanol-induced behavioural effects. Altogether, these behavioral studies suggest that the activity of the brain catalase-H₂O₂ system may be mediating some of the effects of ethanol. Moreover, administration of D-penicillamine to animals, a thiol amino acid that inactivates acetaldehyde, is able to prevent behavioral effects triggered by ethanol. These data support the notion that acetaldehyde formed centrally by the activity of the cerebral catalase-H₂O₂ system plays a key role in some of ethanol's behavioral effects. The mechanism of action by which acetaldehyde exerts its effects in the Central Nervous System remains unclear. However, given that the behavioral effects observed after alcohol administration occur almost immediately, we propose a possible mechanism of action throughout a calcium-dependent signalling pathway. Central formed acetaldehyde would trigger a cytoplasmatic Ca²⁺-i release mediating the activation of several kinases responsible for the necessary neural actions that result in relevant behavioural changes produced by ethanol.

USE OF IN VIVO MICRODIALYSIS AND IMMUNOHISTOCHEMISTRY METHODS IN STUDIES OF ADDICTION DRUGS.

Camarini, Rosana. Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, 05508-900, Brasil, camarini@icb.usp.br

Studies have indicated that adolescents (ADL) respond to drugs of addiction differently than from adults (ADU). Repeated administration of low doses of ethanol or cocaine gradually increases locomotor activity in ADU Swiss mice, a phenomenon known as behavioral sensitization, which has been implicated in drug craving. We have demonstrated that ADL mice show either tolerance or no sensitization after repeated ethanol injections. In contrast, ADL displayed higher levels of locomotor activity compared to ADU in response to repeated cocaine. We have also investigated whether ethanol or cocaine pretreatment in either adolescence or adulthood would alter the dopaminergic/glutamatergic response to a subsequent drug challenge. Microdialysis procedure revealed that ADU mice tolerated to the capacity of ethanol enhance NAc levels of glutamate (GLU). On the other hand, ethanol-treated ADL mice displayed greater peak increases in accumbal GLU in response to a subsequent ethanol challenge as compared to saline-treated ADL mice. In general, cocaine-treated mice displayed lower peak increases in

extracellular GLU when compared to the saline-treated mice. ADU exhibited greater peaks in extracellular GLU in response to cocaine than ADL. These data provide evidences for the role of the glutamatergic system on behavioral sensitization to ethanol and cocaine and suggest an inverse effect between behavioral sensitization and glutamatergic response. There is a wealth of data showing the contribution of c-fos and pCREB in the development of addiction. We have also examined potential differences in c-fos and pCREB immunoreactivity (IR) following ethanol treatment between ADL and ADU mice. Repeated ethanol significantly decreased c-Fos expression in prefrontal cortex (PFC) of ADL mice pretreated with ethanol as compared to the saline control. While acute ethanol decreased pCREB IR in limbic PFC of ADO mice, this effect was reversed following repeated treatment. The results suggest age-linked differences in drug experience-dependent plasticity, what may influence the effects of addictive drugs in ADL.

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DISSOCIATION OF PSYCHOMOTOR SENSITIZATION AND LOSS OF CONTROL OVER ETHANOL CONSUMPTION IN MICE.

Roseli Boerngen-Lacerda, Ph.D. Universidade Federal do Paraná, Departamento de Farmacologia, Centro Politécnico, Caixa Postal, nº19031, CEP: 81540-970. e-mail: boerngen@ufpr.br

The key question in addiction research is what is responsible for the transition from a controlled drug use to a compulsive drug taking that culminates in loss of control over drug consumption in some susceptible individuals. Vulnerability for relapse in addicted individuals persisted for years after abstinence suggesting long-term neuroadaptations. Among the proposed theories the “incentive sensitization theory” has many evidences. This theory states that “repeated exposure to addictive drugs can, in susceptible individuals and under particular circumstances, persistently change brain cells and circuits that normally regulate the attribution of incentive salience to stimuli, a psychological process involved in motivated behavior”. Sensitization research in animals has been mainly studied with respect to locomotor activity which reflects the engagement of brain incentive systems, including mesocorticolimbic dopamine system. For ethanol, locomotor sensitization was firstly described by Masur & Boerngen in 1980 and was characterized in mice as a robust and persistent phenomenon with great variability among individuals. Some

studies using voluntary ethanol consumption showed negative correlation between ethanol intake and the locomotor sensitizing effects of ethanol while others showed positive correlation. Then, we decided to study the possible correlation of ethanol-induced locomotor sensitization and ethanol intake in mice submitted to an addiction model adapted and validated in our laboratory. The main characteristics of this model are: extended access to free choice of ethanol; loss of control over ethanol intake when solutions are adulterated with quinine (less palatable); face, construct and predictive validities; reliability; restricted number of mice developed addiction-like behavior (like in humans). Then, using this paradigm, we tested if mice characterized as addicted and non-addicted on ethanol would express more intense psychomotor sensitization and conversely, if mice previously characterized as sensitized and non-sensitized for ethanol-induced psychomotor sensitization would have different vulnerability to develop addiction. In both experiments we observed that no correlation exists between ethanol-induced psychomotor sensitization and predisposition of mice to become addicted to ethanol. It seems that although psychomotor sensitization involves a striking and persistent nervous system adaptation, the neuroplastic changes induced by continued voluntary drug exposure may be different from those related to psychomotor sensitization. The neuroadaptations that could promote addictive behavior and psychomotor sensitization are independent processes.

**PRENATAL ETHANOL EFFECTS ON
CORTICAL NEURONAL PLASTICITY**

Alexandre E Medina, PhD. Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA – USA, amedina@vcu.edu

Fetal Alcohol Spectrum Disorder (FASD) is characterized by a constellation of behavioral and physiological abnormalities including learning and sensory deficits. There is growing evidence showing that many of these deficits may be a consequence of a disruption on neuronal plasticity (see for review (Medina, 2011)). Much of our knowledge on the basic mechanisms of neuronal plasticity came from studies in the visual cortex. Classic experiments showed that if the eyelid of one eye is closed during a period of development (monocular deprivation), neurons in the ocular dominance (OD) column that should receive information from the deprived eye become instead wired to the experienced eye. As a result, OD columns representing the deprived eye shrink while the ones representing the experienced (open) eye expand. OD plasticity has been widely used to study the mechanisms that underlie neuronal plasticity in general and shares common mechanisms with learning and memory. Using a

combination of electrophysiology and optical imaging of intrinsic signals we showed that OD plasticity is permanently impaired in a ferret model of third trimester alcohol exposure (Medina et al., 2003,2005). These findings provided us with a novel approach to explore the mechanisms that underlie neuronal plasticity deficits in FASD and how it can be restored. In a series of studies we demonstrated that targeting specific transcription factors (CREB and SRF) we could successfully restore OD plasticity and visual cortex functional organization even when the intervention was done long after the period of alcohol insult. These interventions were done a) Pharmacologically – With the use of a Phosphodiesterase type 1 inhibitor (Vinpocetine) (Krahe et al., 2009; Medina et al., 2006) and b) Molecularly – With the use of Viral mediated gene transfer (Paul et al., 2010) or ex-vivo gene delivery (implantation of modified cells) (unpublished results) . Recently, we have demonstrated that Vinpocetine could also improve morris maze performance (a different type of neuronal plasticity) after early alcohol exposure (Filgueiras et al., 2010). Our findings may be potentially significant from a clinical standpoint and suggest that neuronal plasticity enhancement have a role to play as a clinical intervention in FASD.

ETHANOL'S EFFECTS ON OPIOIDERGIC SYSTEMS IN MESOLIMBIC AND NIGROSTRIATAL REGIONS

Méndez, Milagros, Instituto Nacional de Psiquiatría Ramón de la Fuente. Calzada México Xochimilco 101, Col. San Lorenzo Huipulco, 14370 México D.F., México. ubach@imp.edu.mx

The dopaminergic mesocorticolimbic system plays a key role in the reinforcing properties of ethanol and other drugs of abuse. The dopaminergic activity of the nigrostriatal pathway has also been involved in ethanol addictive processes. Ethanol reinforcement and high alcohol drinking behaviour may be partially mediated by the ethanol-induced activation of endogenous opioid systems. Activation of opioidergic transmission by ethanol may involve alterations in the expression, processing, release, and degradation of opioid peptides, as well as binding of endogenous opioids to their receptors. Thus, alterations in opioidergic activity in specific brain areas may be critical in the mechanisms of ethanol reinforcement and dependence. In this work, we investigated the acute and chronic ethanol effects on β -endorphin and Methionin-enkephalin (Met-enk) expression in brain areas of the reward circuit and in the nigrostriatal pathway. We also studied ethanol's effects on ligand binding to mu (μ) and delta (δ) opioid receptors. Male Wistar rats (250 g) received a single ethanol dose (2.5 g/kg) by intragastric administration and were sacrificed at

different time points after drug exposure. For chronic experiments, rats were treated with ethanol (10 %) for 30 days. Ethanol-paired control groups were made of sucrose or water; an *ad libitum* group was also included. Beta-endorphin and Met-enk content was measured by radioimmunoassay in different brain structures. Ligand binding studies were performed by receptor autoradiography in frozen brain sections, using 8 nM of [3 H]-(D-Ala², MePhe⁴, Gly-ol⁵)-enkephalin ([3 H]-DAMGO) or [3 H]-(2-D-Penicillamine, 5-D-Penicillamine)-enkephalin ([3 H]-DPDPE) as μ and δ radioligands, respectively. Acute ethanol administration decreased β -endorphin content in the hypothalamus 1 h post-treatment, but did not alter peptide content in the ventral tegmental area (VTA), nucleus accumbens (NAcc), prefrontal cortex (PFC) or *substantia nigra* (SN). In contrast, acute ethanol decreased Met-enk content in the caudate-putamen (CP) and NAcc, with no effect in the PFC. Chronic ethanol exposure did not modify β -endorphin levels in these brain areas, but sucrose treatment significantly increased peptide content in the NAcc and SN. Chronic ethanol treatment increased and decreased Met-enk content in the PFC and CP, respectively. Chronic sucrose exposure increased and decreased Met-enk levels in the PFC and SN, respectively. Ligand binding studies revealed that acute ethanol treatment selectively modified [3 H]-DAMGO and [3 H]-DPDPE binding to μ and δ receptors in mesolimbic and nigrostriatal areas. In contrast, chronic ethanol exposure did not change [3 H]-DAMGO binding, but decreased [3 H]-DPDPE

binding in the CP, PFC and SN *pars reticulata* (SNr). Our findings suggest that the endorphinergic and enkephalinergic systems participate in ethanol reinforcement and dependence. Different neural mechanisms, opioidergic systems and specific brain regions may be involved in these processes. Sucrose reinforcement may also involve activation of distinct opioidergic pathways.

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ALCOHOL EXPOSURE DURING PREGNANCY AND LACTATION AFFECTS RESPONSIVENESS TO ALCOHOL'S SENSORY AND PHARMACOLOGICAL PROPERTIES.

Molina, Juan. Instituto de Investigación Médica M. y M. Ferreyra (INIMEC - CONICET). Friuli 2434, Córdoba, CP 5000, 351-481465, Argentina. Córdoba, CP 5000, 351-4334064, juancmolina2003@yahoo.com

Adolescent ethanol consumption greatly affects later ethanol consumption: the earlier adolescents use ethanol, the more likely they will abuse ethanol later in life (DeWitt et al., 2000). Our suggestion (Spear and Molina, 2005), however, is that still earlier exposure to ethanol is responsible for – partially -- shaping adolescent ethanol consumption. In the present talk I will review an extensive set of early experiments with rats and mice showing that fetal or infantile ethanol exposure increases later ethanol ingestion (e.g., Chotro et al. 2007). Then, I will focus on experiments that have assessed not only classical consequences of fetal and infantile ethanol exposure (including growth deficiency, brain damage or craniofacial malformation), but also consequences for subsequent ethanol ingestion and noningestive measures of responsiveness to ethanol that might promote ethanol abuse (Abate et al., 2000, 2001, 2002). We suggest that, in addition to simple passive pre-exposure effects, associative

conditioning is likely to occur in the fetus or infant when it is exposed to ethanol. This is, the young organism learns that chemosensory properties of ethanol (e.g., taste, flavor) or other stimuli present in the context of ethanol administration predict the positive rewarding postabsorptive effects of the drug. This process may result in increased seeking or consumption of the drug later in life. Recent experiments testing this intriguing hypothesis are described.

TECHNIQUES FOR ASSESSING ETHANOL-MEDIATED LEARNING: FIRST- AND SECOND ORDER CONDITIONED PLACE PREFERENCE, CONDITIONED TASTE AVERSION, OPERANT SCHEDULES AND ASSESSMENT OF ANXIETY-LIKE BEHAVIORS IN ANIMAL MODELS.

Ricardo Pautassi. Instituto de Investigación Médica M. y M. Ferreyra (INIMEC - CONICET). Friuli 2434, Córdoba, CP 5000, 351-481465, Argentina. Córdoba, CP 5000, 351-4334064, rpautassi@gmail.com

Adult rats and adult mice differ in their sensitivity to ethanol's effects when assessed through conditioned place preference (CPP) and ethanol-induced motor activation, common tests for ethanol's motivational effects. Adult mice express robust CPP and behavioral activation when treated with ethanol. In contrast, adult rats avoid locations that signal the drug and usually fail to exhibit ethanol-induced motor activation. In terms of conditioned taste aversion (CTA) and ethanol-induced anxiolysis both species seem to be fairly and similarly sensitive to ethanol both effects.

Recent data suggests that this typical species-related pattern of responsiveness to ethanol may be less obvious when testing takes place at adolescence. Adolescent mice exhibited CPP by ethanol when given a high ethanol dose (4 g/kg) but not with a more moderate dose (2.0 g/kg) and only after extensive training (Dickinson et al.,

2008). Another study found that that adult mice expressed CPP by ethanol (2 g/kg); adolescent mice did so only after being exposed to substantial stress before conditioning (Song et al., 2007). Unlike mature mice, adolescent mice given repeated ethanol treatment did not show behavioral sensitization; instead, they exhibited gradually less ethanol-induced motor activity (Farias et al., 2009; also see Stevenson et al., 2008).

On the other hand, Philpot et al. (2003) found CPP by ethanol in young (postnatal day 25, PD25, 0.2 g/kg) and late adolescent rats (PD45, 0.5 and 1 g/kg), whereas young adults (PD60) exhibited signs of conditioned aversion. We have recently found CPP by ethanol (1.0 g/kg) in preweanling (PD 13-14) and adolescent (PD 30-33) rats (Nizhnikov, Pautassi et al., 2009; unpublished data). Appetitive conditioning by ethanol at adolescence can also be obtained by a modified, second-order version of the CPP test (Pautassi et al. 2008; dose: 2.0 g/kg). Furthermore, adolescent rats exhibit ethanol-induced motor activation (Acevedo, Pautassi et al., in press). These activating effects were specific for high (2.5 g/kg) but not low (0.5 g/kg) ethanol dose, were similar across males and females, and emerged when testing occurred during the rising limb of the blood ethanol curve (5-11 min). There was a relationship between predisposition to these ethanol's motor stimulating effects and ethanol affinity. Specifically, female rats selected for their high sensitivity to ethanol stimulation exhibited heightened ethanol intake when assessed in a three-

bottle, forced access, intake test.

Overall, these studies highlight the importance of assessing ethanol's motivational effects across different developmental stages and species. Perhaps the apparent insensitivity of rats -- as compared with mice -- to ethanol's rewarding effects changes when testing is shifted from adulthood to adolescence. This is, however, just a hypothesis. These species have not been yet tested in an equivalent mode during adolescence. The differences in sensitivity to ethanol's hedonics may relate to differences in equipment, breeding protocols, or temporal or stimuli parameters across the studies.

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**THE UChA (ALCOHOL ABSTAINER) AND
UChB (ALCOHOL DRINKER) LINE OF
RATS**

effect of ethanol and for their capacity to develop dependence after a prolonged use of alcohol.

Tampier, Lutske, Program of Molecular and Clinical Pharmacology. ICBM. Faculty of Medicine. University of Chile. Independencia 1027, Santiago. Chile. CP 70000, 56-2-9786908
ltampier@med.uchile.cl

Alcohol abuse and alcoholism is serious social problem. Population studies have shown that the predisposition of alcoholism is determined largely by genetic factors. Due to the cost and the ethical problems involved with studies in humans, most experiments must be carried in animal models of alcoholism. The UChA and UChB rat lines developed at the University of Chile are one of these models of rats that can be used to study alcohol problems. The selection began in 1949 from a group of Wistar rats. UChA now generation 94, drink less than 1g/kg/day of ethanol under a free choice condition between 10% ethanol solution and water. These rats are characterized by their low capacity to eliminate acetaldehyde formed during ethanol metabolism. UChA rats would be an animal analogue of some Eastern race individuals that have a point mutation in the gene that encodes the mitochondrial aldehyde dehydrogenase. The UChB rats line, now generation 84, drink more than 4g/kg/day under a free choice condition. They are characterized by its ability to develop acute tolerance to the depressant

USE OF MOLECULAR BIOLOGY FOR SCREENING THE PLASTICITY OF NEUROTRANSMITTER RECEPTORS biology of neurotransmitter receptors.

Elizabeth Teodorov, Mathematics, Computing and Cognition Center, Universidade Federal do ABC
eteodorov@usp.br

Molecular biology has been increasing in the last decades and is becoming a routine tool in both academic research and in clinical analysis. Thus, the use of molecular techniques for early diagnosis of diseases, paternity testing, forensic medicine and criminalistics, as well as physiological studies of transcription and translation (transcriptome and proteome projects) grow exponentially and require large financial investments. The expression of a specific gene or its protein product (e.g. membrane receptors) in healthy cells and in certain diseases has attracted much interest in the scientific community, particularly due to the fact that they can also be used on a population scale in clinical laboratories (human and animal). Many molecular techniques are currently offered and the choice of the most appropriate is directly linked to the object of study. Among the noteworthy are gene sequencing, immunohistochemistry, real-time PCR, southern blot, northern blot, western blot, laser microdissection, and RNAi. This seminar will present some techniques that are commonly used in clinical and academic research, as well as the main advantages and disadvantages inherent in each of them, exemplified by studies that focus on the