

**Alcoholism and Stress: A Framework for Future Treatment Strategies**

Volterra, Italy  
May 6 – 9, 2014

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## PROGRAM

**Tuesday, May 6, 2014**

- 8:15am – 8:30am      **Introduction**  
Marisa Roberto, Giovanni Manghetti, Augusto Mugellini, Rocco Damone, and Marco Buselli
- 8:30am – 9:00am      **Opening Remarks**  
George F. Koob
- 9:00am – 10:30am     **Symposium I**  
STRESS INTERACTIONS AND INFLUENCES ON DEPENDENCE AND ALCOHOL CONSUMPTION  
Chairs: Kathleen A. Grant, Giovanni Biggio  
Discussant: Giovanni Biggio
- S3    Stress Interactions with Alcohol Dependence and Drinking in Mice  
Howard Becker
- S4    Viral-vector-induced steroidogenesis in the VTA increases 3 $\alpha$ ,5 $\alpha$ -THP and reduces long-term operant ethanol self-administration  
A. Leslie Morrow
- S5    Pituitary and adrenal response to corticotropin-release hormone during chronic ethanol self-administration in male cynomolgus monkeys  
Christa Helms
- S6    The impact of binge drinking on brain structure and associated behaviours; the role of stress  
Dora Duka
- 10:30am – 11:00am    Coffee Break
- 11:00am – 12:30pm   **Symposium II**  
TRANSLATIONAL STUDIES ON EARLY LIFE STRESS AND ALCOHOL ADDICTION  
Chair: Jeff Weiner  
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Rajita Sinha
- S8    Neurobiological and behavioural studies on early life stress and adolescent alcohol drinking in a translational initiative  
Ingrid Nylander
- S9    Chronic social stress persistently facilitates the NMDAR-LTP induction mechanism in VTA dopaminergic neurons  
Claire Stelly
- S10   Early life stress increases behavioral and neurobiological risk factors of alcohol addiction in male Long Evans rats  
Jeff Weiner
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- 1:30pm – 3:00pm      **Symposium III**  
CENTRAL VERSUS SYSTEMIC STRESS SYSTEMS IN ALCOHOL DEPENDENCE  
Chair: Scott Edwards  
Discussant: Edie Sullivan
- S11   Functional recruitment of central amygdala glucocorticoid receptor signaling in alcohol-dependent rats  
Scott Edwards

- S12 Comparison of effects of four glucocorticoid receptor (GR) antagonists on compulsive alcohol drinking in rats  
Leandro Vendruscolo
- S13 Alcohol drinking, stress hormones, and addiction vulnerability  
Heather Richardson
- S14 Brain glucocorticoids and effects of glucocorticoid receptor antagonists  
Hilary Little

3:00pm – 4:00pm **POSTER SESSION**

4:00pm – 5:30pm **Symposium IV**

NEW RESULTS FROM AN OLD FRIEND: TRANSLATIONAL STUDIES OF NPY

Chair: Thomas Kash

- S15 NPY signaling inhibits extended amygdala CRF neurons to suppress binge alcohol drinking  
Thomas Kash
- S16 Epigenetic and signaling mechanisms of Neuropeptide Y regulation in the amygdala: role in anxiety and alcoholism  
Subhash C. Pandey
- S17 Functional genetic variants on NPY, stress response and relapse of substance abuse  
Ke Xu
- S18 A novel, brain penetrant NPY-Y2R antagonist in animal models of alcohol-intake and anxiety  
Annika Thorsell

**Wednesday, May 7, 2014**

8:30am – 9:30am **Plenary Lecture**

- S1 Opioid systems: probing molecular processes of brain function  
Brigitte Kieffer

9:30am – 11:00am

**Symposium V**

MAINTAINING BALANCE: THE ROLE OF ENDOGENOUS CANNABINOID SIGNALING IN ALCOHOL-RELATED REWARD, AVERSION PROCESSING AND STRESS RESPONSIVITY

Chair: Loren H. Parsons

- S19 Acetaldehyde as a drug of abuse: Involvement of endocannabinoid- and dopamine neurotransmission  
Carla Cannizzaro
- S20 Role of 2-arachidonoylglycerol-mediated plasticity at inhibitory synapses onto midbrain dopamine neurons in increased consumption of and preference for ethanol in Sardinian alcohol-preferring rats  
Miriam Melis
- S21 Functional interplay between CRHR1 and endocannabinoid signaling in the regulation of stress and anxiety  
Matthew Hill
- S22 Increased alcohol consumption in rats after subchronic antidepressant treatment  
Fernando Rodriguez de Fonseca

11:00am – 11:30am Coffee Break

- 11:30am – 1:00pm     **Symposium VI**  
HOW DOES STRESS INFLUENCE ALCOHOL DRINKING: MOLECULAR, NEUROIMAGING AND BEHAVIOURAL MEDIATORS  
Chair: Gunter Schumann
- S23    Effect of psychosocial stress on brain activity and its consequence for psychopathology and alcohol drinking: a project from the IMAGEN study  
Gunter Shumann
- S24    Genome-wide identification of methylation profiles associated with alcohol dependence, impulsiveness and brain activation  
Sylvane Desrivieres
- S25    Stress and the development of excessive alcohol intake  
Andreas Heinz
- S26    The role of glucocorticoid receptor function in stress-induced alcohol consumption  
Rainer Spanagel
- 1:00pm – 2:00pm     Lunch
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Chair: Candice Contet
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Candice Contet
- S28    SK channel adaptations promote maladaptive alcohol intake and relapse  
Fredric Hopf
- S29    Repeated stress and emotion: The role of KCa channels in the amygdala  
Amiel J. Rosenkranz
- S30    Large conductance calcium-and voltage- activated potassium (BK) channels and the control of the neuroendocrine stress axis  
Mike Shipson
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EXPLORING STRESS AS A MULTI-SYSTEM, MULTI-DIMENSIONAL RESPONSE: SEX SPECIFICITY AND TREATMENT IMPLICATIONS  
Chair: Sara Jo Nixon  
Introduction: Howard Becker
- S31    Alcohol, stress resilience, and neurocardiac signaling: Gender differences and implications for addiction treatment  
Marsha Bates
- S32    Amygdala mechanisms underlying escalation of alcohol use  
Heidi Lesscher
- S33    Targeting the noradrenergic system for stress-precipitated smoking relapse: An examination of gender differences  
Sherry McKee
- S34    Nicotine use in alcoholics: Normalizing stress and performance?  
Sara Jo Nixon
- 3:30pm – 4:00pm     Coffee Break



4:00pm – 5:30pm

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Chair: Mark Brodie

Discussant: Michela Marinelli

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Mark Brodie

S36 Profound and selective decrease of dendritic spines in the nucleus accumbens of ethanol dependent rats.  
Marco Diana

S37 Differential Effects of Ethanol and Inhalants on Ventral Tegmental DA Neurons.  
John Woodward

S38 Food and alcohol: common neuroadaptations in the VTA  
Hitoshi Morikowa

4:00pm – 5:30pm

**Symposium X**

UNDERSTANDING THE ROLE OF STRESS AND DISTRESS TOLERANCE ON ALCOHOL USE IN YOUTHS

Chair: Paola Pedrelli

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Stacey Daughters

S40 Distress tolerance, alcohol use and alcohol-related problems among female and male college students  
Paola Pedrelli

S41 Distress tolerance over time predicts increases in alcohol use across adolescence  
Laura MacPherson

S42 A randomized controlled trial of a behavioral economic intervention for substance abuse in a diverse college sample  
Ali Yurasek

**Thursday, May 8, 2014**

8:30am – 9:30am

**Plenary Lecture**

S2 Mechanisms through which alcohol stimulates the hypothalamic-pituitary-adrenal axis: acute and long-term consequences  
Catherine Rivier

9:30am – 11:00am

**Symposium XI**

APPLYING THE NEW GENOMICS TO ALCOHOL DEPENDENCE

Chair: R. Adron Harris

Introduction: R. Adron Harris

Discussant: Samir Zakhari

S43 Biological networks underlying lifetime consumption of alcohol within human prefrontal cortex  
Sean Farris

S44 microRNA contribution to the development of alcohol dependence  
Andrzej Pietrzykoski

S45 Synaptomics: Using genomic expression profiling to identify chronic ethanol-induced alterations in the synaptic transcriptome  
Michael Miles

- S46 Reverse engineering transcriptional networks to identify critical neuroadaptations during protracted withdrawal from alcohol dependence  
Pietro P. Sanna

11:00am – 11:30am Coffee Break

11:30am – 1:00pm

**Symposium XII**

**YOUNG INVESTIGATOR AWARD**

Chairs: Antonio Noronha, Fulton Crews

- S47 Excessive alcohol drinking in the context of sensitized stress signaling  
Scott Edwards
- S48 Chronic alcohol exposure disrupts cognitive function and D2/D4 receptor modulation of prefrontal cortical function  
Heather Trantham-Davidson
- S49 Ethanol produces CRF dependent regulation of glutamatergic transmission in the Central Amygdala  
Yuval Silberman
- S50 Phosphodiesterase 10A inhibition reduces alcohol self-administration even in stress history and alcohol-dependent rats  
Marian Logrip

1:00pm – 2:00pm Lunch

2:00pm – 3:30pm

**Symposium XIII**

**CORTICOSTRIATAL CIRCUITRY AND HABITUAL ETHANOL-SEEKING BEHAVIOR**

Chair: L. Judson Chandler

Discussant: Ruben Gonzalez

- S51 Firing patterns of dorsal striatal neurons to alcohol-associated cues  
Donita Robinson
- S52 Innate and acquired differences in the nucleus accumbens in the acquisition and expression of habitual alcohol seeking.  
Jacqueline M. Barker
- S53 Endocannabinoid signaling in orbitofrontal cortex modulates habit formation  
Christina Gremel
- S54 Changes in the behavioral and neural control in the transition from goal-directed to habitual alcohol-seeking  
Laura Corbit

3:30pm – 4:30pm

**POSTER SESSION**

4:30pm – 6:00pm

**Symposium XIV**

**TRANSLATIONAL STUDIES ON GENETIC AND GENOMIC ANALYSES OF STRESS, ALCOHOL DEPENDENCE AND DRINKING**

Chair: Howard C. Becker

Introduction: Robert Williams

Discussant: Wolfgang Sommer

- S55 Chronic Intermittent Ethanol Exposure Influences Voluntary Ethanol Intake in BXD Strains of Mice  
Marcelo Lopez
- S56 Identification and validation of cross-species conserved gene networks associated with progressive ethanol consumption  
Angela Batman
- S57 Epigenetic signatures associated with chronic alcohol use

- Lucia Carbone  
S58 Sequencing the transcriptome in ethanol consuming rhesus macaques  
Robert Hitzemann

**Friday, May 9, 2014**

- 8:30am – 10:00am     **Symposium XV**  
STRESS AND ALCOHOL: A LIFESPAN PERSPECTIVE  
Chair: C. Fernando Valenzuela  
Discussant: Lindsey Grandison  
S59 Transgenerational Epigenetic Effects of Fetal Alcohol Exposure on Hypothalamic Gene Expression in Rats  
Dipak Sarkar  
S60 Third Trimester Ethanol Exposure Impairs Modulation of Synaptic Transmission by Dopamine in the Basolateral Amygdala  
C. Fernando Valenzuela  
S61 Early-life stress, HPA axis and addiction neurocircuitry  
Delia Beelli  
S62 Withdrawn
- 10:00am – 10:30am    Coffee Break
- 10:30am – 12:30pm   **Symposium XVI**  
CLINICAL TREATMENTS ROUNDTABLE  
Chair: Barbara J. Mason  
Discussant: Kenneth R. Warren  
S63 A selective NOP receptor agonist MT-7716 with efficacy in animal models of alcoholism  
Koji Teshima  
S64 Effects of CRF1 antagonists on overactive anxiety and stress circuit in humans disorders: effects on social anxiety and potential relevance for alcoholism  
Emilio Merlo-Pich  
S65 A Glucocorticoid Antagonist Shows Therapeutic Potential for Alcohol Dependence in a POC Human Laboratory Study  
Barbara J. Mason  
S66 A Double-Blind, Placebo Controlled Trial Assessing the Efficacy and Safety of Varenicline Tartrate for Alcohol Dependence  
Joanne Fertig  
S67 An extension of the current treatment paradigm for alcohol dependence: Results from nalmefene phase III trials  
Karl Mann
- 12:30pm – 1:30pm    Lunch
- 1:30pm – 3:00pm     **Symposium XVII**  
STRESS, MEMORY AND ALCOHOL ABUSE DISORDER  
Chairs: Segev Barak, Dorit Ron  
S68 Mammalian target of rapamycin complex 1 (mTORC1) protein translation and alcohol drinking - recent findings  
Dorit Ron  
S69 The way stress remaps memory systems in our brain  
Gal Richter-Levin

- S70 Erasure of alcohol-associated memories by mTORC1 inhibition prevents relapse  
Segev Barak
- S71 microRNA-206 in rat medial prefrontal cortex regulates BDNF expression and alcohol drinking  
Markus Heilig

3:00pm – 4:00pm

**POSTER SESSION**

4:00pm – 5:30pm

**Symposium XVIII**

**BRAIN REWARD AND STRESS SYSTEMS IN EXCESSIVE ALCOHOL DRINKING**

Chair: Nicholas W. Gilpin

Discussant: Adolf Pfefferbaum

- S72 Lateral hypothalamus is critical for context-induced relapse to alcohol seeking after punishment  
Nathan Marchant
- S73 The role of midbrain dopamine neurons in controlling variable alcohol drinking behaviors  
Ming-Hu Han
- S74 Individual Differences in Stress-Induced Behavioral Dysregulation Mediated by Corticotropin-Releasing Factor (CRF) in Central Amygdala (CeA)  
Nicholas Gilpin
- S75 Mechanisms of stress-enhanced fear learning in a rat model of post-traumatic stress disorder  
Igor Spigelman

ABSTRACTS FOR  
**Alcoholism and Stress: A Framework for Future Treatment Strategies**  
VOLTERRA, ITALY  
MAY 6 – 9, 2014

**PLENARY LECTURES**

**SYMPOSIA**

**S1**

**Opioid systems: probing molecular processes of brain function**

Brigitte L. Kieffer, Claire Gavériaux-Ruff

IGBMC, INSERM/CNRS/Université de Strasbourg, Strasbourg France

Molecular processes underlying brain function, plasticity and disease are highly complex. These operate within specific compartments of neuronal cells, and translate at systems level through neuronal connectivity in highly organized neural circuits. Probing molecular events in high-order responses, and understanding their relevance to brain activity requires the development of targeted manipulations that ultimately impact on neuronal function in vivo, and possibly behavior. Genetic approaches have been instrumental towards these aims, and the opioid system has proved to be a pioneer model system in the development of gene targeting approaches in mice. Opiates have been used since thousand years for their pain-relieving and rewarding properties. These compounds produce their potent effects by activating opioid receptors in the brain, thereby hijacking a complex neuromodulatory system that includes three receptors ( $\mu$ ,  $\delta$  and  $\kappa$ ) normally stimulated by endogenous peptides. The presentation will focus on this fascinating and complex neuromodulatory system, which controls sensory modalities (pain) and emotional state or responses (euphoria, dysphoria, stress). Targeted mutagenesis in mice has allowed demonstrating the distinct role of each receptor in behavioral responses, identifying yet unreported roles for these “old” receptors, discovering novel gene targets for psychiatric research and visualizing receptor trafficking in live neurons for the first time. These rapidly evolving experimental approaches are now broadly applied to track molecules at work in the brain, and their use has both fundamental and therapeutic implications in neuroscience.

**S2**

**Mechanisms through which alcohol stimulates the hypothalamic-pituitary-adrenal axis: acute and long-term consequences**

Catherine Rivier

The Clayton Foundation for Peptide Biology, the Salk Institute, La Jolla, CA 92037, USA

In rodents, alcohol stimulates the hypothalamic-pituitary-adrenal (HPA) axis through mechanisms that depend on corticotropin-releasing factor (CRF) and vasopressin synthesized in, and released from, the paraventricular nucleus (PVN) of the hypothalamus. Alcohol can also increase CRF synthesis and secretion in isolated hypothalamic cells, and upregulate CRF promoter activity in an immortalized cell line, which indicates a direct action of the drug on the CRF gene. However in intact animals, the stimulatory effect of alcohol involves nitric oxide (NO) and brain stem catecholamines, neurotransmitters known for their ability to activate the HPA axis. It is interesting as well as potentially clinically relevant that prior exposure to alcohol modifies the response of this axis to many stimuli. In adult rats, 3 consecutive daily alcohol injections will blunt the normal activation of PVN CRF neurons by this drug for at least 3 weeks, while adolescent rats exposed to alcohol vapors on postnatal days 28-42 exhibit a long-term loss of HPA axis response to various stressors. In contrast, rodents exposed to alcohol during embryonic development will present an hyperactive axis throughout their adult life, a phenomenon due at least in part to upregulation of NO signals to the PVN. As CRF and glucocorticoids exert potent effects on many brain areas, as well as on the gastrointestinal tract, the immune system and reproductive functions, the ability of alcohol to alter their synthesis and normal response to homeostatic threats, will likely exert a deleterious influence on the overall health of the organism.

**Symposium I**

**STRESS INTERACTIONS AND INFLUENCES ON DEPENDENCE AND ALCOHOL CONSUMPTION**

Chairs: Kathleen A. Grant, Giovanni Biggio

Discussant: Giovanni Biggio

**S3**

**Stress Interactions with Alcohol Dependence and Drinking in Mice**

H.C. Becker\*, M.F. Lopez, W.C. Griffin, and R.I. Anderson

Charleston Alcohol Research Center, Medical University of South Carolina, Charleston, SC 29425 USA

Repeated cycles of chronic intermittent ethanol (CIE) exposure results in escalation of voluntary ethanol drinking in C57BL/6J mice. Studies were conducted to address the following questions: does CIE exposure alter response to subsequent stress challenge? And, does stress experience concurrent with CIE exposure further exacerbate increased drinking? CIE-exposed (dependent) mice exhibited altered response to forced swim (FS) stress (more struggling behavior) compared to nondependent mice. This effect was observed after 4 (but not 2) cycles of CIE exposure and was evident at least 7 days post-withdrawal. Similar results were obtained in ethanol-naïve mice after central (icv) administration of CRF (50-200 ng), suggesting that CRF activation plays a role in altered stress responsiveness following CIE exposure. Results will be described from studies aimed at examining the role of CRF1R and kappa opiate receptors in this effect of CIE exposure. In a separate series of studies, the effect of FS stress exposure during ethanol access in the CIE model was examined. CIE-exposed and control mice received either no stress, FS stress during each of 4 cycles of drinking or only during the last cycle of drinking. CIE-exposed mice that received FS stress during every test cycle (but not just the last cycle) showed a greater increase in ethanol intake (~4.0 g/kg) compared to CIE no-stress mice (~3.3 g/kg) and control no-stress mice (~2.5 g/kg). FS stress did not alter ethanol intake in nondependent controls. Collectively, these data indicate a reciprocal interaction between stress and alcohol dependence, with CIE exposure altered stress responsiveness and stress exposure further increasing CIE-induced escalation of drinking. Supported by NIAAA grants U01 AA014095, U01 AA020929, and P50 AA010761, and VA Medical Research.

**S4**

**Viral-vector-induced steroidogenesis in the VTA increases 3 $\alpha$ ,5 $\alpha$ -THP and reduces long-term operant ethanol self-administration**

A. Leslie Morrow, Jason B. Cook, David F. Werner, Antoniette M. Maldonado-Devincci, Maggie N. Leonard, Kristen R. Fisher, Todd K. O’Buckley, Patrizia Porcu, Thomas J. McCown, Clyde W. Hodge, Joyce Besheer

Psychiatry, Pharmacology and Bowles Ctr for Alcohol Studies, UNC School of Medicine, Chapel Hill, NC 27599, USA

The GABAergic neuroactive steroid (3 $\alpha$ ,5 $\alpha$ )-3-hydroxypregnan-20-one (3 $\alpha$ ,5 $\alpha$ -THP) alters ethanol consumption with bi-directional actions. To optimize the therapeutic potential for neuroactive steroids, we employed vector-mediated gene delivery to alter neurosteroids at specific brain sites. We developed a recombinant adeno-associated-viral-vector (rAAV) to drive neurosteroidogenesis by delivery of cytochrome P450 side chain cleavage (P450scc), the rate-limiting enzymatic reaction in steroidogenesis. Following vector characterization, we studied the effect of rAAV2-P450scc vector delivery to VTA and NAc on ethanol reinforcement and consumption in alcohol-preferring (P) rats. rAAV2-P450scc or control green fluorescent protein expressing vector (rAAV2-GFP) was injected bilaterally into the VTA or NAc of alcohol preferring (P) rats previously trained to self-

administer ethanol. rAAV2-P450scc vector delivery to the VTA specifically reduced ethanol responding by 20% (two-way ANOVA,  $p < 0.005$ ) and ethanol intake by 14% (two-way ANOVA,  $p < 0.01$ ) compared to control rats over 3 weeks. In contrast, P450scc overexpression in the NAc did not alter ethanol self-administration. General locomotor activity and thigmotaxis were not changed. P450scc overexpression in the VTA produced a 36% increase in  $3\alpha,5\alpha$ -THP positive cells in the VTA, however, transduction of NAc did not increase local  $3\alpha,5\alpha$ -THP immunoreactivity. VTA neurons were co-labeled with  $3\alpha,5\alpha$ -THP and tyrosine hydroxylase, or solely labeled by  $3\alpha,5\alpha$ -THP. Thus, P450scc gene delivery increased  $3\alpha,5\alpha$ -THP positive cells in the VTA, causing a persistent reduction of ethanol reinforcement and consumption. rAAV2-P450scc is a useful tool for studying the role of neuroactive steroids in ethanol reinforcement and consumption, and may provide new therapeutic strategies for treatment of alcoholism. Supported by NIAAA and the Bowles Center for Alcohol Studies.

## S5

### **Pituitary and adrenal response to corticotropin-release hormone during chronic ethanol self-administration in male cynomolgus monkeys**

Christa M. Helms, Kathleen A. Grant

Division of Neuroscience, Oregon Health & Science University, Beaverton, OR 97006, USA

The hypothalamic-pituitary-adrenal (HPA) axis output reflects homeostatic challenges and is disrupted in alcoholics. Macaques are an excellent species to study HPA axis adaptation to chronic alcohol due to their similar endocrine system, long lifespan and propensity to chronically drink alcohol. Male cynomolgus monkeys ( $n = 12$ , 7-9.5 years) were induced to drink ethanol using schedule-induced polydipsia and then given 22 h/d access to 4% ethanol for 14 months. Pituitary responsiveness to corticotropin-releasing hormone (1  $\mu\text{g}/\text{kg}$  ovine CRH, i.v.) was determined by assay of plasma adrenocorticotrophic hormone (ACTH) and cortisol at baseline (-15 min) and 15, 30, 45, 60 min. Across all time points, CRH increased ACTH and cortisol by (mean  $\pm$  SD)  $135 \pm 35\%$  and  $163.5 \pm 48\%$  from baseline to peak, respectively. During the induction of ethanol, however, the ACTH response to CRH was blunted. Notably, 4/12 monkeys had a suppression of ACTH following CRH in induction and these monkeys became the heavier drinkers in the cohort. Cortisol increased after CRH similarly before and during ethanol access. These data appear to have captured a homeostatic adaptation of the pituitary response of the HPA axis early in the establishment of drinking to intoxication. Continued access to ethanol (22 h/d) restored the relative ACTH response to CRH but basal levels of ACTH fell and remained low throughout the chronic daily self-administration. The results suggest that early in the course of drinking to intoxication, ACTH homeostasis is disrupted and with continued drinking is reset and defended at low circulating levels.

## S6

### **The impact of binge drinking on brain structure and associated behaviours; the role of stress**

Theodora Duka

School of Psychology, University of Sussex, Brighton, BN1 9QH UK

Stress plays an important role in the initiation and maintenance of alcohol abuse and may promote binge drinking. Binge drinking in turn is associated with changes in brain structure and impaired cognition and emotion. Acute stress may therefore have a differential impact on alcohol related behaviours in binge drinkers and non-binge drinkers. We have performed a series of studies with young adults to examine the consequences of binge drinking on brain structure and the impact of stress on alcohol related behaviours, which may lead to binge drinking. We found that binge drinking scores (derived from the Alcohol Use Questionnaire) were positively correlated with the number of early stress events experienced ( $r=0.567$ ;  $p<0.05$ ). We also found that acute stress increases strong desires to drink among social drinkers who are bingers compared to non-bingers ( $p<0.05$ ). Binge drinking was associated with reduced white matter integrity (functional anisotropy) in the corpus callosum (CC) as measured by Diffusion Tension Imaging (DTI); reduced gray matter in areas associated with sensory input (middle temporal pole) and behavioural control (superior frontal gyrus) as measured by Voxel Based Morphometry (VBM) was also found in bingers compared to non-

bingers. Functional anisotropy in prefrontal as well as parietal/temporal/occipital CC regions was negatively correlated with binge score ( $r_s = -.595$ ,  $p = .009$  and  $r = -.573$ ,  $p = .013$  respectively). These data highlight the impact of binge drinking on brain structures and provide an initial insight into the ways that stress may interact with binge drinking.

## Symposium II

### **TRANSLATIONAL STUDIES ON EARLY LIFE STRESS AND ALCOHOL ADDICTION**

Chair: Jeff Weiner

Discussant: Klaus Miczek

## S7

### **Neurobiological mechanisms linking early life stress to risk of alcoholism in humans**

Rajita Sinha,

Yale University School of Medicine, New Haven, CT 06519, USA

Epidemiologic research clearly indicates that early life stress and cumulative adversity increases risk of developing alcoholism and also perpetuates the chronic relapsing nature of alcoholism. Early life stress is known to have long-lasting changes to the hypothalamic-pituitary-adrenal (HPA) axis and in extrahypothalamic cortico-striatal limbic pathways that regulate stress, emotions and reward responses. Dr. Sinha will present data from studies in children and at-risk non-dependent social drinkers using human laboratory, neuroimaging and behavioral assessments to show neural, neuroendocrine and behavioral changes related to early trauma and cumulative stress that predict greater reactivity to reward, alcohol as well as increased alcohol intake and alcohol related problems. Results will focus on specific trauma related peripheral neuroendocrine profiles including blunted cortisol awakening levels and lower HPA axis response to stress and increased sympathetic arousal that relate to stress-related hyperactivity in limbic regions, including the hypothalamus, amygdala and hippocampus, in vulnerable individuals. Findings will also show that high trauma and cumulative adversity sensitizes responses of striatal regions to alcohol and high calorie food cues and increased craving for these stimuli. Finally, evidence of trauma-related blunted medial prefrontal responses to stress predicting high alcohol craving and intake in high risk individuals will be shown. Implications of these findings for addressing trauma-related alcoholism vulnerability with specific interventions that may harness neuroplasticity in these stress-vulnerable systems will be discussed.

## S8

### **Neurobiological and behavioural studies on early life stress and adolescent alcohol drinking in a translational initiative**

Nylander <sup>1</sup>, Palm <sup>1</sup>, Daoura <sup>1</sup>, Granholm <sup>1</sup>, Rowley <sup>1</sup>, Todkar <sup>1</sup>, Comasco <sup>2</sup>, Roman <sup>1</sup>

<sup>1</sup>Neuropharmacology, Addiction & Behaviour, Department of Pharmaceutical Biosciences, <sup>2</sup>Dept of Neuroscience; Uppsala University, Uppsala, Sweden

Early life stress and adolescent alcohol consumption can cause long-term alterations in gene expression, change brain function and behaviour and thereby determine the individual response to alcohol and the liability for compulsive intake. We examine the impact of early life stress, i.e. disturbance of early social interactions and adolescent alcohol exposure, on opioid and monoaminergic targets and on behavioural development. In a translational initiative we use animal models to follow up clinical findings of childhood stress and adolescent drug exposure to further investigate gene expression and epigenetic profiles in specific brain areas. Early life stress alters endogenous opioids, affects adult voluntary alcohol consumption and alcohol-induced effects. Proopiomelanocortin expression is reduced in the hypothalamus and beta-endorphin is lower in the pituitary gland after early life stress. Individual behavioural profiling reveals that the risk-taking trajectory is affected by disturbed early social interactions. Adolescent drug intake attenuates the protective effects of a beneficial rearing environment; the alcohol intake is the same and the effects on opioids are similar, e.g. increased dynorphin in the hippocampus, regardless of early life history. In vivo studies reveal that adolescent alcohol exposure also affects adult drug-

induced effects on dopamine networks, e.g. by altered dopamine release after a challenge with amphetamine. Ongoing studies point out the importance of discriminating effects of housing conditions and alcohol in voluntary drinking models to avoid confounding effects of individual housing. The studies show the impact of early life events on end points of relevance to addiction; behavioural development and opioid and dopamine function. Financial support: Swedish Medical Research Council, SRA, ERAB - The European Foundation for Alcohol Research; the Swedish Council for Working Life and Social Research (2011-0627) and Fredrik and Ingrid Thuring Foundation.

### S9

#### **Chronic social stress persistently facilitates NMDAR-LTP induction mechanism in VTA dopaminergic neurons**

Claire Stelly, Hitoshi Morikawa

Waggoner Center for Alcohol and Addiction Research, Section of Neurobiology, University of Texas at Austin, Austin, TX 78712, USA

Stressful experience heightens addiction risk in humans and promotes learning of drug-associated stimuli in rodents. These effects of stress persist after acute hypothalamic-pituitary-adrenal activation has subsided, suggesting that stress induces enduring cellular adaptations that promote later learning. Ventral tegmental area (VTA) dopaminergic (DA) neurons are critical for learning the motivational valence of reward-paired cues. Synaptic plasticity in DA neurons may contribute to learning driven by natural rewards or addictive drugs. Long-term potentiation of NMDA receptor signaling (NMDAR-LTP) in DA neurons is a cellular mechanism that may underlie the conditioned DA burst response to reward-paired cues. NMDAR-LTP induction depends upon coincident activation of inositol 1,4,5-triphosphate (IP<sub>3</sub>) receptors by IP<sub>3</sub> and calcium (Ca<sup>2+</sup>) influx. NMDAR-LTP induction is enhanced by repeated drug treatment or social isolation via a PKA-mediated increase in IP<sub>3</sub>R sensitivity. NMDAR-LTP may also be modulated by chronic stress. To test this idea, male rats were subjected to repeated episodes of social defeat. Whole-cell patch clamp recordings were made from DA neurons identified by electrophysiological hallmarks. G<sub>q</sub>-mediated Ca<sup>2+</sup> signaling is significantly enhanced following 5 or 10 days of stress, but not after a single stress episode. When animals were killed at varying intervals after stress, the effect of stress was shown to decay gradually over the course of 30 days. As facilitation of G<sub>q</sub>-mediated Ca<sup>2+</sup> signaling correlates strongly with NMDAR-LTP magnitude, these results suggest that NMDAR-LTP will be increased in stressed animals, providing a potential cellular mechanism contributing to the enhancement of drug conditioning by prior stressful experience.

### S10

#### **Early life stress increases behavioral and neurobiological risk factors of alcohol addiction in male Long Evans rats**

Anushree Karkhanis, Mary Jane Skelly, Andrew Rau, Jordan Yorgason, Ann Chappell, Eugenia Carter, Sara Jones, Brian McCool, Jeff Weiner  
Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27151, USA

Epidemiological studies have established a clear link between chronic early life stress (ELS) and increased vulnerability to alcoholism. Unfortunately, little is known about the neurobiological mechanisms responsible for the increased risk of addiction associated with ELS. Here, we employed a rodent ELS model to identify neural substrates that may contribute to increased addiction vulnerability. In this model, male Long Evans rats are raised in cohorts of 4 in large cages (Group Housed, GH) or are housed individually in smaller cages (Socially Isolated, SI) during adolescence (postnatal day 28-70). Following the adolescent housing manipulation, all subjects are then housed individually in adulthood. As observed in many prior studies, SI rats displayed significant and enduring increases in anxiety-like behaviors (e.g. elevated plus-maze). SI rats also exhibited impaired extinction of fear learning. Social isolation also significantly increased measures of home-cage and operant ethanol self-administration. Neurobiological studies have identified several enduring effects of SI on dopamine signaling in the nucleus accumbens, including increased electrically-stimulated dopamine release and uptake and elevated expression of the dopamine transporter. SI also led to

increased ethanol-stimulated accumbal dopamine release. Electrophysiological studies in the basolateral amygdala revealed SI-associated increases in glutamatergic transmission and metaplastic changes synaptic plasticity. Interestingly, some of these adaptations mirror those seen in animal models of ethanol dependence. Together, these data provide further evidence that ELS increases many behavioral risk factors for alcoholism. These findings also suggest that ELS may increase addiction vulnerability, in part, via a disruption of mesolimbic dopaminergic signaling and glutamatergic plasticity. Supported by AA 17531, AA 21099, and AA 10422.

### Symposium III

#### **CENTRAL VERSUS SYSTEMIC STRESS SYSTEMS IN ALCOHOL DEPENDENCE**

Chair: Scott Edwards

Discussant: Edie Sullivan

### S11

#### **Functional recruitment of central amygdala glucocorticoid receptor signaling in alcohol-dependent rats**

Scott Edwards<sup>1</sup>, Eva R. Zamora-Martinez<sup>2</sup>, Joel E. Schlosburg<sup>2</sup>, Marian L. Logrip<sup>2</sup>, George F. Koob<sup>2</sup>, and Leandro F. Vendruscolo<sup>2</sup>

<sup>1</sup>Department of Physiology, LSU Health Sciences Center, New Orleans, LA, USA; <sup>2</sup>Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA 80237, USA

Alcohol dependence is a chronic, relapsing disorder characterized by a persistent desire to seek alcohol, loss of control over drinking, and emergence of a negative emotional state during withdrawal. Both central and peripheral stress systems have been implicated in the establishment and maintenance of dependence. Our hypothesis is that sustained corticosteroid release via the hypothalamic-pituitary-adrenal (HPA) axis following repeated episodes of alcohol intoxication/withdrawal drive compulsive alcohol drinking via potentiated glucocorticoid receptor (GR) function in central reinforcement circuitry. We have previously reported that GR mRNA levels are dysregulated across multiple stress/reward-related brain regions (prefrontal cortex, nucleus accumbens, central amygdala) in rats exposed to alcohol vapor to the point of dependence compared with non-dependent animals. Moreover, chronic GR antagonism with mifepristone (RU38486) prevented the development of compulsive alcohol drinking produced by chronic intermittent alcohol vapor exposure. Recently, we have discovered that alcohol-dependent rats display significant increases in GR phosphorylation at Ser211 in the central amygdala (CeA) compared with non-dependent rats. This neuroadaptation is consistent with our hypothesis of central stress system sensitization during alcohol dependence since phosphorylation at Ser211 is a biomarker of transcriptionally active GR. In concert with neuroadaptation, mifepristone injected directly into the CeA reduces alcohol self-administration in dependent rats. Ongoing studies are determining whether alterations in GR-affiliated chaperone proteins and co-regulators are associated with the development of excessive alcohol drinking. These studies provide strong evidence for a sensitization of CeA GR signaling in alcohol dependence and will greatly assist the development of new treatment strategies for this disease. This work was generously supported by the following grants from NIAAA: AA020839 (SE), AA008459, AA006420, AA007456 (GFK), and by the Pearson Center for Alcoholism and Addiction Research.

### S12

#### **Selective glucocorticoid receptor (GR) antagonism blocks compulsive alcohol drinking in rats**

Leandro F. Vendruscolo, Lauren G. Macshane, Joel E. Schlosburg, Cara L. Buck, Jordan C. Malavar, Joseph K. Belanoff, Hazel J. Hunt, Scott Edwards, and George F. Koob

Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA 92037, USA

Compulsive and uncontrolled drinking and the appearance of negative affective symptoms during abstinence characterize alcoholism. We have reported that alcohol dependence is associated with altered expression of glucocorticoid receptor (GR) mRNA levels and sensitized brain reward/stress

systems. Chronic GR blockade with mifepristone reduces compulsive alcohol drinking specifically in dependent rats. A caveat for mifepristone use is its action in inhibiting progesterone receptors, which may affect behavior and produce side effects. Additionally, GRs interact at multiple levels to activate and repress transcription factors/gene transcription, causing various physiological effects. Thus, the synthesis and characterization of GR-specific antagonists may assist with the development of novel and more specific medications for alcoholism. We compared the effects of mifepristone and three brain-penetrant, GR-selective antagonists on alcohol self-administration in rats made dependent on alcohol by vapor exposure versus nondependent rats. Rats were injected with the GR antagonists CORT108297 (0-60 mg/kg), CORT118335 (0-10 mg/kg), CORT113176 (0-100 mg/kg), or mifepristone (0-60 mg/kg) 90 min prior to operant alcohol self-administration. CORT108297 produced no effect. CORT118335 dose-dependently reduced alcohol intake in both dependent and nondependent rats. CORT113176 dose-dependently reduced alcohol intake in both groups, but the drug was more effective in attenuating drinking in dependent rats. Mifepristone reduced alcohol drinking in dependent rats only. These results emphasize the critical role of GRs in alcohol dependence. Understanding the specific pharmacological mechanisms of different GR antagonists will assist with the development of more specific drugs for the treatment of alcoholism.

### S13

#### **Alcohol drinking, stress hormones, and addiction vulnerability**

Heather N. Richardson

Psychology-Neuroscience and Developmental Sciences Divisions, University of Massachusetts at Amhurst, Amhurst, MA 01003, USA

Stress hormones have been increasingly implicated in addiction vulnerability, possibly playing an important role at every stage of the pathway from use - to abuse - to dependence. We have been exploring how stress hormones and alcohol interact at these different stages of addiction to gain a better understanding of this dynamic relationship, which will help advance diagnostic and therapeutic intervention. Using rodent models, we have found that alcohol drinking activates the hypothalamic pituitary adrenal (HPA) axis, releasing corticosterone into the blood where it can act on receptors within the periphery and the brain. Over time, there is notable habituation to alcohol; an effect that others have shown contributes functionally to increased drinking. Alcohol exposure down-regulates neuroendocrine function through mechanisms within the HPA axis itself, and possibly, through mechanistic changes upstream of the hypothalamus. We have found changes in corticotropin releasing factor (CRF) populations within cortico-limbic circuits that are predictive of elevated drinking behavior. Altogether the findings indicate that the bi-directional relationship between alcohol and stress hormones could promote escalations in alcohol intake and further neuroadaptive changes in circuits that impact stress hormone production, a feed-forward loop that may push individuals along the trajectory from non-dependent drinking to alcohol dependence.

### S14

#### **Brain glucocorticoids and effects of glucocorticoid receptor antagonists**

Little, H.J.<sup>1</sup>, Shaw, S.G.<sup>2</sup>, Drummond, C.<sup>1</sup>, Prendergast, M.A.<sup>3</sup>

<sup>1</sup>Institute of Psychiatry, King's College, London, UK; <sup>2</sup>Department of Clinical Research, Vasoactive Peptide Group, Bern University, Switzerland; and <sup>3</sup>Department of Psychology, Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY 40536, USA

Our previous work showed that cognitive deficits in rodents caused by prolonged alcohol consumption can be prevented by administration of single doses of drugs during the acute withdrawal phase (Brooks et al., 2008). This suggested a window of opportunity for alcohol dependence treatment at the start of detoxification. We have also demonstrated that long-term alcohol consumption increases the brain concentrations of corticosterone, in both mice and rats (Little et al., 2008) and that corticosterone increases alcohol withdrawal neurotoxicity and calcium accumulation in CNS neurones (Mulholland et al., 2005). Our current work focuses on the ability of glucocorticoid antagonist drugs to reduce the behavioural consequences of alcohol withdrawal. Administration of one dose of the Type 2 glucocorticoid receptor antagonist, mifepristone, immediately after alcohol withdrawal, prevented the subsequent memory deficits in mice and significantly lowered

withdrawal signs (Jacquot et al., 2008). Repeated doses of this drug, given to rats during a 4-day binge alcohol treatment, substantially reduced the acute signs of alcohol withdrawal (Sharrett-Field et al., 2013). We have also studied the effects of the mifepristone analogue, ORG34517, that is more selective and lacks progesterone antagonist action. This compound significantly reduced acute withdrawal signs in rats and memory deficits in mice when given prior to withdrawal from chronic alcohol treatment. We are currently running a clinical trial of mifepristone in alcohol-dependent inpatients undergoing alcohol detoxification. The outcomes focus on cognitive function and indices of depression. Effects of mifepristone on severity of alcohol withdrawal, craving and relapse are also being evaluated.

### Symposium IV

#### **NEW RESULTS FROM AN OLD FRIEND: TRANSLATIONAL STUDIES OF NPY**

Chair: Thomas Kash

### S15

#### **NPY signaling inhibits extended amygdala CRF neurons to suppress binge alcohol drinking**

Thomas Kash

Dept. of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Binge alcohol drinking is the most common form of excessive alcohol consumption; however, the neurophysiological processes that control this risky behavior are unclear. While the neuropeptides corticotropin-releasing factor (CRF) and neuropeptide Y (NPY) have been identified as playing key, but opposing, roles in alcohol-drinking behaviors, the cellular basis of their interaction has proven elusive. We found that NPY Y1 receptor (Y1R) activation in the bed nucleus of the stria terminalis (BNST) of mice reduced binge alcohol drinking and enhanced inhibitory synaptic transmission through a novel post-synaptic mechanism. Further, chronic binge drinking led to common, persistent adaptations in Y1R function in the BNST of both rodents and monkeys. Finally, BNST CRF neurons were exceptionally sensitive to Y1R modulation of inhibition, and genetic inhibition of these neurons suppressed binge drinking. Thus Y1R may be an effective pharmacotherapeutic target for the treatment of alcohol use disorders.

### S16

#### **Epigenetic and signaling mechanisms of neuropeptide Y regulation in the amygdala: Role in Anxiety and Alcoholism**

Subhash C. Pandey

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The neuropeptide Y (NPY) system has been implicated in the process of alcoholism. However, the molecular mechanisms by which NPY signaling is regulated and plays a role in genetic predisposition to and development of alcoholism is currently less clear. The study of epigenetic mechanisms such as chromatin remodeling, is an emerging field and these mechanisms have been implicated in the pathophysiology of brain disorders. Animal lines, such as alcohol-preferring (P) and -nonpreferring (NP) genetic rats appear to be suitable models to study the neurobiological basis for the genetic predisposition to alcohol-drinking behaviors. P rats also display higher baseline anxiety-like behaviors than NP rats. Recently we found that amygdaloid chromatin remodeling may be involved in the process of alcohol dependence and decreased NPY expression in the central (CeA) and medial amygdala (MeA) of Sprague-Dawley rats during ethanol withdrawal after chronic ethanol exposure is involved in the development of anxiety-like behaviors. Both deficits in NPY expression in the CeA and MeA and development of anxiety-like behaviors during ethanol withdrawal is attenuated by treatment with trichostatin A (TSA), an HDAC inhibitor. In addition, the infusion of NPY into CeA during alcohol withdrawal attenuated the development of anxiety-like behaviors, which is prevented by co-infusion with an NPY-Y1 receptor antagonist. Adolescent intermittent ethanol (AIE) exposure produced deficits in NPY levels in the CeA and MeA and histone acetylation in the promoter of the NPY gene at adulthood. AIE also produced anxiety-like and alcohol drinking behaviors at adulthood. We also



investigated the role of chromatin remodeling in anxiety and alcohol drinking behaviors using P and NP rats as an animal model of alcoholism. It was found that nuclear, but not cytosolic, histone deacetylases (HDAC) activity was higher in the amygdala of P rats compared to NP rats. We also found lower acetylation of histones (H3-K9 and H4-K8) and NPY expression and higher HDAC2 protein levels in the CeA and MeA, but not in the basolateral amygdala (BLA), of P rats compared with NP rats. Treatment with TSA inhibited HDAC activity and decreased HDAC2 protein levels and corrected the deficits in histone acetylation (H3-K9 & H4-K8) and NPY expression (mRNA and protein levels) in the CeA and MeA of P rats. Using chromatin immunoprecipitation assay with acetylated histone H3(K9&14), we found lower levels of acetylated H3 in the promoter of NPY in the amygdala of P rats as compared with NP rats, which is increased by treatment with TSA in P rats. In contrast, TSA treatment had no effect on the HDAC activity, levels of histone acetylation, or NPY expression in the amygdala of NP rats. Behaviorally, TSA treatment attenuated the anxiety-like behaviors and also decreased the alcohol intake in P rats, but has no effects on these behaviors in NP rats. Taken together, these results indicate that a deficit in chromatin remodeling associated reduction in NPY expression (i.e., epigenetic regulation) in the neurocircuitry of the amygdala may be involved in pathophysiology of alcoholism (Supported by NIH-NIAAA grants and VA Merit and Career Scientist Grants to SCP).

### S17

#### Functional Genetic Variants on NPY, Stress Response and Relapse of Substance Abuse

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Neuropeptide Y (NPY) plays an important role in regulating stress. Stress significantly contributes to increase risk of relapse in substance abuse population. We previously reported that a haplotype (allelic combination on the same chromosome) of NPY was associated with NPY mRNA level in human brain and blood samples. In this study, we investigated the relationship of functional NPY haplotype, plasma NPY level and stress response following a stress challenging paradigm in alcohol and cocaine dependence subjects. We further tested a correlation between plasma NPY and the time of relapse in this group. We analyzed 37 substance dependence (SD) and 28 healthy controls (HC) subjects who were exposed to stress, alcohol/drug cue and neutral relaxing cues using individualized imagery in a human laboratory. Diplotype for each subject was characterized. We compared high expression diplotype (HH) versus the combination of intermediate expression diplotype (HL) and lower expression diplotype (HLLL). We found that HH individuals showed significantly lower stress-induced NPY with greater heart rate and anxiety ratings, while the HLLL group showed the reverse pattern of NPY, anxiety and heart rate responses. This differential genetic modulation of NPY stress response was suppressed in the SD group. Lower NPY predicted subsequent higher number of days and greater amounts of post-treatment drug use. The finding that lower stress-related NPY is predictive of greater relapse severity provides support for therapeutic development of neuropeptide Y targets in the treatment of substance use disorders.

### S18

#### A novel, brain penetrant NPY-Y2R antagonist in animal models of alcohol-intake and anxiety

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The NPY-Y2 receptor has been a theoretically attractive target for treatment therapies for high alcohol-intake states and anxiety-disorders for quite some time. However, studies using different small molecule, non-peptidergic antagonists aimed at the NPY-Y2R have yielded mixed results. The non-blood brain barrier penetrating molecule BIIE0246 significantly suppresses operant responding for alcohol-solution in both naïve and post-dependent rats upon central administration, while the more recent, peripherally available JNJ-31020028 does not. With regards to anxiety-related behavior,

an anxiolytic-like effect of NPY-Y2R antagonist is more generally seen and reproduced. Here, we present data for a novel, small molecule NPY-Y2R antagonist in animal models of alcohol intake and anxiety-related behavior. A significant suppression of both operant and non-operant alcohol consumption was demonstrated, as well as a significant anxiolytic-like effect in the elevated plus-maze model as well as the open field test. In addition, treatment with the NPY-Y2R antagonist reversed the anxiogenic effect of an acute alcohol-administration as measured on the elevated plus-maze. These results present us with the opportunity to re-examine the usefulness of the NPY-Y2R as a therapeutic target for alcohol dependence disorders as well as anxiety-disorders. In addition to the novel data, a summary of the effects of NPY-Y2R antagonists in alcohol intake models as well as in models of anxiety-related behavior will be presented.

### Symposium V

#### MAINTAINING BALANCE: THE ROLE OF ENDOGENOUS CANNABINOID SIGNALING IN ALCOHOL-RELATED REWARD, AVERSION PROCESSING AND STRESS RESPONSIVITY

Chair: Loren H. Parsons

### S19

#### Acetaldehyde as a drug of abuse: Involvement of endocannabinoid- and dopamine neurotransmission

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Acetaldehyde (ACD), the first metabolite of ethanol, directly enhances dopamine neurotransmission (1) and has rewarding and motivational properties in paradigms tailored for studying addictive-like behaviours (2, 3). The endocannabinoid system affects distinct drug-related behaviours, since it may in turn fine-tune dopamine cell activity (4, 5). In light of this, the present study aimed at investigating the effects of a direct manipulation of the DAergic synapse, and the contribution of the endocannabinoid system on oral ACD self-administration in rats. ACD drinking-behaviour was evaluated in an operant paradigm consisting of acquisition and maintenance; extinction; deprivation and relapse; conflict. D2-receptor agonists, quinpirole (0.03 mg/Kg, i.p.) and ropinirole (0.03 mg/Kg, i.p.), and CB1-receptor antagonist, AM281 (1 mg/Kg, i.p.), were administered during different phases of the experiment. Our results show that oral ACD readily induced the acquisition and maintenance of an operant drinking-behaviour, even during reinstatement and conflict. Quinpirole decreased lever presses for ACD during extinction ( $p < 0.05$ ) and relapse ( $p < 0.01$ ;  $p < 0.001$ ). Ropinirole, administered during abstinence, reduced ACD intake during reinstatement ( $p < 0.001$ ). AM281 significantly decreased lever presses for ACD during extinction ( $p < 0.001$ ), relapse ( $p < 0.001$ ) and conflict ( $p < 0.001$ ). These data suggest that whereas the direct modulation of the dopaminergic synapse influences drug-seeking and relapse behaviour, the endocannabinoid system may also play a role in shock-paired compulsive ACD intake. These findings highlight the mandatory need for further investigation on the therapeutic potential played by the endocannabinoid system taking into account its crucial role in alcohol, and ACD neuropharmacology.

### S20

#### Role of 2-arachidonoylglycerol-mediated plasticity at inhibitory synapses onto midbrain dopamine neurons in increased consumption of and preference for ethanol in Sardinian alcohol-preferring rats

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Sardinian alcohol-preferring (sP) or non-preferring (sNP) rats are one of the few pairs of lines of rats selectively bred for their voluntary alcohol preference or aversion, respectively. The ventral tegmental area (VTA) dopamine (DA) neurons have long been implicated in many drug-related behaviors, including alcohol self-administration. Indeed, the VTA is a key component of brain reward circuitry. Endocannabinoids (eCBs), retrograde signaling molecules at many synapses in the brain, regulate reward seeking

by modulating DA signaling, and interact with alcohol to produce its reinforcing effects. Here we took advantage of significant differences in alcohol self-administration displayed by sP and sNP rats, and studied VTA DA cell synaptic properties *ex vivo*. sP rats showed a decreased probability of GABA release at two discrete sets of inhibitory synapses arising from rostral and caudal afferents onto VTA DA neurons. Because eCBs activating presynaptic cannabinoid-type 1 (CB1) receptors inhibit neurotransmitter release, we studied an endocannabinoid-mediated form of short-term synaptic plasticity, that is depolarization-induced suppression of inhibition (DSI). sP and sNP rats express different DSI at both rostral and caudal inhibitory synapses onto VTA DA neurons. Both DSI are mediated by 2-arachidonoylglycerol (2-AG), which activates CB1 receptors. However, the two discrete DSI do not depend upon differences in CB1 number and/or function, but rather to the rate 2-AG is degraded. Differences in molecular architecture of 2-AG signaling might, therefore, contribute to regulate responses to aversive intrinsic properties to alcohol, thus resulting in faster acquisition/initiation of alcohol drinking that may be associated to alcohol preference.

## S21

### Functional interplay between CRHR1 and endocannabinoid signaling in the regulation of stress and anxiety

J. Megan Gray, Maria Morena, Alex B. Kim, Dan Hermanson, Tiffany Lee, Haley Vecchiarelli, Ryan J. McLaughlin, Kowther Hassan, Jan Deussing, Sachin Patel and Matthew N. Hill

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Corticotropin releasing hormone (CRH) and endocannabinoid have opposing influences on how the brain responds to stress. The CRHR1 receptor acts throughout the brain and pituitary to increase endocrine and behavioral responses to stress, while the cannabinoid type 1 receptor (CB1) suppresses these outputs. Although these systems appear to have some overlapping distribution, few studies have investigated if these systems interact. Our studies reveal a novel mechanism of CRHR1 regulation of hydrolysis of the endocannabinoid anandamide (AEA) through an induction of fatty acid amide hydrolase (FAAH). Specifically, activation of CRHR1 in the amygdala enhances FAAH activity and suppresses AEA signaling in the amygdala. More so, CRHR1 activity, specifically on glutamatergic neurons in the basolateral nucleus of the amygdala (BLA), mediates stress-induced FAAH activity. Consistent with this, the majority of CRHR1 positive cells in the BLA co-express FAAH, and local inhibition of FAAH within the BLA is capable of attenuating CRH-induced anxiety and activation of the HPA axis. Consistent with this role of CRH-mediated regulation of endocannabinoid signaling, CRH overexpressing mice exhibit constitutive upregulation of FAAH activity and reductions in AEA content within the amygdala. Together, these data demonstrate that induction of FAAH activity by CRH contributes to the generation of anxiety in response to stress, and more so, these data may also support a mechanism by which enhanced amygdalar CRH signaling, which occurs during conditions of drug withdrawal, produces emotionally aversive states. Funding Sources: Canadian Institutes of Health Research, Alberta Innovates Health Solutions.

## S22

### Increased alcohol consumption in rats after subchronic antidepressant treatment

Francisco Alén<sup>1,2</sup>, Laura Orío<sup>2</sup>, Miguel Ángel Gorriti<sup>1</sup>, Raquel Gómez de Heras<sup>1</sup>, María Teresa Ramírez-López<sup>1</sup> MS, Miguel Ángel Pozo<sup>1,3</sup>, and Fernando Rodríguez de Fonseca<sup>1,2</sup>

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The use of antidepressants for alcoholism in humans has been a matter of controversy in recent years. Despite the existence of an important comorbidity for depression and alcoholism, some studies suggest that the use of certain antidepressants could worsen the prognosis of alcoholism. However there is a lack of studies in animal models exploring this phenomenon. We have monitored the effects of subchronic (15 days) SSRI-

SNRI or NRI-type antidepressant treatment on alcohol consumption and relapse. Rats trained to self-administer a 10% alcohol solution were withdrawn from alcohol access for 15 days. Along this period they received a subchronic treatment with comparable doses of Fluoxetine (SSRI) Venlafaxine (SNRI) or Atomoxetine (NRI) for 15 days, after which they resumed the alcohol self-administration sessions and changes in alcohol consumption were monitored. In parallel, the ability of these treatment schedules to induce locomotor sensitization to the psychostimulant amphetamine was assessed in another set of animals. Treatment with SSRI-type antidepressant Fluoxetine (10 mg/kg), SNRI-type antidepressant Venlafaxine (50 mg/kg) or NRI-type antidepressant Venlafaxine (10 mg/kg) for 15 days affected alcohol deprivation effect (ADE), -defined as an increased alcohol self-administration after a period of abstinence from this drug-, and subsequent alcohol consumption. Initially, Fluoxetine reduced the ADE in the first day of relapse, whereas venlafaxine did not affect it. Atomoxetine increased alcohol intake from the beginning. In the following days, animals previously treated with all antidepressants increased alcohol consumption, an effect that was found to last at least 5 weeks for SNRI and SSRI-type antidepressants and only a week for NRI-type. Treatment with the SSRI fluoxetine caused a locomotor sensitized response to a challenge dose of amphetamine (0.5 mg/kg ip), indicating the presence of a supersensitive dopaminergic transmission. Subchronic treatment with antidepressants along alcohol deprivation induce long-lasting increases in alcohol consumption in rats when the animals are re-exposed to alcohol self-administration. The effect of Fluoxetine on alcohol consumption could be related to a hypersensitivity of the motivational reward system, since this antidepressant sensitized the locomotor response after an amphetamine challenge. However noradrenaline uptake inhibitors were devoid of this effect. Our results have important implications for the clinic suggesting that a prolonged use of antidepressants should be revised in a context of alcoholism, especially if relapse is associated to a cessation of antidepressant therapy. Supported by ISCIII- Red de Trastornos Adictivos Grant RD012/0028.

## Symposium VI

HOW DOES STRESS INFLUENCE ALCOHOL DRINKING: MOLECULAR, NEUROIMAGING AND BEHAVIOURAL MEDIATORS  
Chair: Gunter Schumann

## S23

### Effect of psychosocial stress on brain activity and its consequence for psychopathology and alcohol drinking: a project from the IMAGEN study

Gunter Schumann

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In the IMAGEN project we aim to identify the neurobiological basis of individual variability in reinforcement behaviour and determine their predictive value for the development of addictions and other psychiatric disorders. Comprehensive behavioural and neuropsychological characterization, functional and structural neuroimaging and genome-wide association analyses of 2000 14-year-old adolescents at baseline are coupled with longitudinal follow-up and combined with functional genetics in animal and human models. We will present data on the effect of psychosocial stress on brain activity and its consequence for psychopathology, including alcohol-drinking behavior. Specifically, we have conducted a factor analysis of life events in adolescents. Using a Kernel-based approach we will analyse the association of these life event factors with brain activation during reinforcement-related behavior, including reward anticipation, impulsiveness (motor inhibition) and emotional perception (angry faces). To investigate a possible epigenetic mediation we will explore an association of brain activity patterns with methylation profiles. We will also assess the effect of environmental-induced changes in brain activity on psychopathology and alcohol drinking behavior.

## S24

**Genome-wide identification of methylation profiles associated with alcohol dependence, impulsiveness and brain activation.**

Sylvane Desrivieres, Barbara Ruggeri

Institute of Psychiatry, King's College, London for the IMAGEN Consortium.

In the IMAGEN project we aim to identify the neurobiological basis of individual variability in reinforcement behaviour and determine their predictive value for the development of addictions and other psychiatric disorders. Comprehensive behavioural and neuropsychological characterization, functional and structural neuroimaging and genome-wide association analyses of 2000 14-year-old adolescents at baseline are coupled with longitudinal follow-up and combined with functional genetics in animal and human models. We carried out a genome-wide analysis of DNA methylation profile of 18 MZ twin pairs discordant for alcohol dependence (AD), using peripheral blood DNA to identify CpG sites with significantly different methylation levels. Differentially methylated CpG sites were validated with an independent method in 36 MZ AD-discordant twin pairs. We further investigated the behavioural and physiological processes related to the most significant differential methylation, observed in the PPM1G gene locus. We assessed its association with alcohol related behavior and personality traits as well as brain activation in a functional neuroimaging epigenetics dataset of 578 adolescents from the IMAGEN study. Results of this study will be presented.

**S25**

**Stress and the development of excessive alcohol intake**

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Animal models can help to examine longitudinal effects of stress exposure on drug and alcohol intake. In non-human primates, early social isolation stress was associated with reduced serotonin turnover and an associated alteration in serotonin transporter availability *in vivo*, which was directly correlated with a low response to acute alcohol consumption and an increased long-term alcohol intake. In adolescents, similar alterations in serotonin transporter availability can result from genotype effects and are also associated with a low response to acute intoxication and a higher level of long-term alcohol intake. Associated mechanisms appear to include serotonergic-GABAergic interactions in the prefrontal cortex that affect sedation following alcohol consumption. Further human studies point to an additional role of dopaminergic neurotransmission, which in the amygdala and cingulate cortex of healthy controls was correlated with functional activation elicited by aversive stimuli. In detoxified alcohol-dependent patients, this dopaminergic modulation of aversive stimulus processing in the amygdala and cingulate cortex appears to be impaired, resulting in an increase in negative mood, which can increase the long-term relapse risk.

**S26**

**The role of glucocorticoid receptor function in stress-induced alcohol consumption**

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Numerous preclinical studies have focused on the identification of biological and environmental factors that modulate stress and alcohol interactions but the determinants of stress-induced alcohol consumption in rodents are not well understood. We therefore carried out a clustered meta-analysis on stress-induced alcohol consumption in 1520 rats. Stress-induced alcohol consumption in rodents is age-dependent, with adults being more sensitive than adolescents; has a high genetic load; and results from an interaction of stress and reward systems. It is hypothesized that corticosterone (CORT), acting on medium spiny neurons within the nucleus accumbens (NAC), is an important mediator of stress effects on alcohol intake. Indeed adrenalectomized (ADX) rats do not show stress-induced alcohol consumption. Since the ADX manipulation can be restored by CORT treatment a role of CORT and glucocorticoid receptor (GR) function is implicated in this behavior. To examine the contribution of GR function in

this behavior we subjected mice lacking specifically either GR in dopaminergic (GRDATCre) or dopaminoceptive (GRD1Cre) neurons to different stress procedures but could not find any genotype differences in stress-induced alcohol consumption when compared to wild-type littermates. These results imply that GR does not play a role in stress-induced alcohol consumption but rather non-genomic CORT effects within the NAC mediate stress-induced alcohol consumption.

**Symposium VII**

**A CASE FOR K: ROLE OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN STRESS AND ALCOHOL RESPONSES**

Chair: Candice Contet

**S27**

**Modulation of the behavioral effects and voluntary drinking of ethanol by the auxiliary subunits of the large conductance calcium-activated (BK) channel**

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Large conductance calcium-activated potassium (BK) channels play a key role in controlling action potential firing and neurotransmitter release. Ethanol is a potent activator of BK channel gating, but how this action may impact the behavioral effects and voluntary intake of ethanol still remains poorly understood. Association of auxiliary  $\beta$  subunits to the pore-forming  $\alpha$  subunit reduces ethanol-induced potentiation of BK currents *in vitro*. In the present study, we investigated whether BK  $\beta 1$  and  $\beta 4$  subunits influence ethanol intoxication, tolerance, dependence and drinking using knockout mice. We found that deletion of BK  $\beta 1$  or  $\beta 4$  did not impact the sensitivity to acute ethanol intoxication in ethanol-naïve mice. In contrast, BK  $\beta 1$  knockout mice developed less tolerance to ethanol-induced sedation and hypothermia following chronic intermittent ethanol exposure. In addition, physical withdrawal syndrome was exacerbated in both BK  $\beta 1$  and  $\beta 4$  knockout mice. We therefore speculate that BK channel auxiliary subunits may be recruited upon chronic ethanol intoxication to dampen ethanol-induced potentiation of BK currents. BK  $\beta 1$  and  $\beta 4$  did not modulate voluntary drinking in non-dependent mice, regardless of the pattern of access to ethanol. However, deletion of BK  $\beta 4$  attenuated, while deletion of BK  $\beta 1$  accelerated, the escalation of ethanol drinking during withdrawal from chronic intermittent ethanol inhalation, suggesting that BK  $\beta 1$  and  $\beta 4$  subunits exert opposite influences on the negative reinforcing properties of ethanol withdrawal. Modulating the expression, cellular distribution or interaction properties of BK channel auxiliary subunits may therefore represent a novel avenue for the treatment of alcoholism. *This work was funded by NIH grants AA020913 (CC), AA020893 (AJR) and AA013517 (GFK).*

**S28**

**SK channel adaptations promote maladaptive alcohol intake and relapse**

Hopf, F.W., Bowers, M.S., Seif, T. Simms, J., Chang, S.J., Chen, B.T., Martin, M., Cho, S.L., Tye, K., Mohamedi, M.M., Bartlett, S., A. Bonci Gallo Center, University of California at San Francisco, San Francisco, CA 94158, USA

Alcoholism imposes a tremendous social and economic burden. There are relatively few pharmacological treatments for alcoholism, with only moderate efficacy, and there is considerable interest in identifying additional therapeutic options. SK-type  $K^+$  channels are potent inhibitors of firing in many mesolimbic regions, and intermittent 2-bottle-choice and operant alcohol intake reduce SK channel function and enhance excitability in nucleus accumbens core (NAcore), determined using patch-clamp electrophysiology *in vitro*. We then reasoned that activating SK channels with a positive allosteric modulator (1-EBIO, chlorzoxazone) could reduce excessive alcohol intake as well as relapse after abstinence. This is especially important since chlorzoxazone is FDA-approved as a centrally-acting myorelaxant and thus represents an immediately accessible therapy for

alcoholism. In agreement, intra-NAcore infusion of 1-EBIO significantly reduces relapse, and systemic injection of chlorzoxazone significantly reduces excessive alcohol intake. These positive SK modulators have no behavioral effect in control conditions (operant sucrose intake, continuous 2-bottle choice intake) where NAcore SK function measured *in vitro* is normal. Also, passive repeated alcohol exposure reduces SK function in the VTA, which promotes NMDA receptor-dependent burst firing and is associated with increased cross-sensitization to the locomotor activating effects of cocaine. Thus, adaptations in SK channel function within mesolimbic regions can potentially promote alcohol intake, relapse, and sensitization, making the FDA-approved chlorzoxazone a novel and promising therapeutic target to treat alcohol use disorders. Future research can address how the potent SK regulation of synaptic plasticity could alter drinking, and possible genetic associations between alcoholism and SK channel splice variants linked to schizophrenia. Supported by RO1 AA015358 (F.W.H.), F32 AA015464 (M.S.B.), funds from the ABMRF (F.W.H.), and funds provided by the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco (S.B., A.B.).

### S29

**Repeated stress and emotion: The role of  $K_{Ca}$  channels in the amygdala**  
J. Amiel Rosenkranz, Wei Zhang, and Mallika Padival  
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Stress potentially precipitates episodes of depression and anxiety. The amygdala contributes to mood and emotion, and is sensitive to stress. Previous studies demonstrate amygdala hyperactivity in patients with a history of repeated or severe stress. In rodent models of stress, increased amygdala-dependent fear behavior has been observed in parallel with hypertrophy of neurons of the basolateral amygdala (BLA). The purpose of the current studies is to understand whether repeated stress increases BLA neuronal activity, and the mechanism for these effects. We found that repeated stress increases the activity of BLA neurons of adult rats. However, the mechanism for this effect depended upon BLA subnucleus. Repeated stress caused a decrease in the function of calcium-activated  $K^+$  ( $K_{Ca}$ ) channels of pyramidal-like neurons of the lateral nucleus (LA), while increasing excitatory synaptic drive of neurons in the basal nucleus (BA). These effects were not observed in rats that were resilient to the effects of repeated stress. Pharmacological targeting of  $K_{Ca}$  channels reversed the effects of repeated stress on LA-dependent fear conditioning, but not on BA-dependent behaviors. These studies indicate a significant role for  $K_{Ca}$  channels in stress-driven behavioral abnormalities, and may implicate  $K_{Ca}$  channels as potential targets to reverse the negative effects of stress, and perhaps facilitate resilience to affective disorders.

### S30

**Large conductance calcium- and voltage-activated potassium (BK) channels and the control of the neuroendocrine stress axis**  
Peter J Duncan, Mike Shipston  
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The anterior pituitary corticotroph is a central hub in the control of the hypothalamic-pituitary-adrenal (HPA) axis and the neuroendocrine response to stress. Corticotrophs are electrically excitable however how their electrical excitability is regulated and coordinated by hypothalamic neuropeptides and negative feedback from circulating glucocorticoids is largely unknown. Large conductance calcium- and voltage-activated potassium (BK) channels are expressed at multiple levels of the HPA axis and mice with a genetic deletion of BK channels show a stress hyporesponsiveness. BK channels play an important role in controlling the electrical activity of corticotrophs through the regulation of electrical bursting activity. Under basal conditions murine corticotrophs generate single action potentials that upon stimulation with the hypothalamic secretagogues corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) transition to 'pseudo plateau bursting' events that are hypothesized to promote ACTH secretion. Pharmacological inhibition, or genetic deletion of BK channels in corticotrophs, largely prevents CRH/AVP-induced bursting behaviour. Furthermore, early glucocorticoid negative feedback also prevents

CRH/AVP-induced bursting suggesting that the transition to bursting is a key regulatory mechanism that is controlled by BK channels. Understanding how corticotroph excitability is controlled, and the important role of BK channels in regulating electrical bursting behaviour, provides new insights into the physiology of HPA axis regulation and promises to define new approaches to therapeutic intervention in stress-related disorders. This work was supported by The Wellcome Trust. PJD was in receipt of an MRC PhD studentship.

### Symposium VIII

EXPLORING STRESS AS A MULTI-SYSTEM, MULTI-DIMENSIONAL RESPONSE: SEX SPECIFICITY AND TREATMENT IMPLICATIONS

Chair: Sarah Jo Nixon

Introduction: Howard Becker

### S31

**Acute alcohol and other environmental challenge effects on neuro-cardiac signaling: Gender differences and implications for addiction treatment**

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The ability to adapt physically and psychologically to the environment involves activating in response to challenge, and then returning to a state of rest. Bidirectional communication between the body and brain, referred to as neurocardiac signaling, is an important component of adaptation. While a neurocardiac 'stress response' is appropriate in the face of challenge, the continuation of a stress response when the challenge is diminished or has ended is maladaptive and may support inflexible behavior, such as that implicated in the transition from social to compulsive drinking. Heart rate variability (HRV) reflects the efficiency of bidirectional brain-heart communication in modulating arousal in response to stress and other challenges. This presentation will present a new metric that quantifies adaptive HRV spectral reorganization as it occurs when people are exposed to different types of challenges. We found that healthy women showed significantly more spectral reorganization towards lower spectral frequencies than men in response to both positive and negative emotional challenges, but not neutral cues, during alcohol and placebo beverage challenges. This suggests greater activation of an adaptive process involving cardiovascular resonance. We will also present results examining gender differences in stress resilience quantified by reduced vagal withdrawal following repeated presentations of the same negative challenge. These findings have implications for promoting behavioral flexibility towards alcohol and other drug use through behavioral interventions that improve automatic, adaptive neurocardiac processes in response to stress and other environmental challenges. Supported by NIH grants and contracts R01AA15248, P20DA17552, K02 AA000325, HHSN275301000003C, K24AA021778.

### S32

**Amygdala mechanisms underlying escalation of alcohol use**  
Heidi M.B. Lesscher

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Working definitions of "the stress response" are often confined to a restricted area of the HPA axis. However, it is well-known that processes subserved by the amygdala impact the interpretation of and response to "stressful" events and stimuli. Animal work conducted in recent years reinforces the pertinence of amygdala circuitry in the development of loss of control over substance use, one of the defining end stage characteristics of addiction. Mice and rats show rapid escalation of alcohol intake under restricted access conditions. Moreover, they become insensitive to adulteration of the alcohol with the bitter tastant quinine, reflecting inflexible and indifferent alcohol drinking. Our studies support a key role for the amygdala in the escalation to full-blown alcoholism. Local knockdown of protein kinase C epsilon in the central nucleus of the amygdala (CeA) prevented escalation of alcohol use. Subsequent microarray analysis, comparing amygdala gene expression at different stages during escalation of alcohol use, confirmed amygdala

involvement in this process and identified the adapter protein 14-3-3 zeta as a potential regulator of alcohol use. Indeed, knockdown of this protein (14-3-3 zeta) in the CeA markedly enhanced alcohol use. Moreover, mice with reduced 14-3-3 zeta levels showed persistent preference for alcohol, despite the adulteration with quinine, confirming the contribution of this protein to the development of alcoholism. These findings suggest the potential import of systematically exploring distinct, yet overlapping neurobiological systems underlying negative affect and stress. Ultimately, the identification of the mechanisms through which PKC epsilon and 14-3-3 zeta affect alcohol consumption may lead to new therapeutic strategies for the treatment of alcohol addiction. Furthermore, in light of human research reported in this symposium, these data reinforce the need for controlled studies of sex differences across species. Supported by ZonMw VENI (91679134) and Knowledge Utilisation (91501007) grants to HL.

### S33

#### **Targeting the noradrenergic system for stress-precipitated smoking relapse: An examination of gender differences**

Sherry McKee

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Stress drives smoking and precipitates relapse in those trying to quit. Emerging evidence identifies that affect regulation plays an especially critical role in the ability of women to initiate abstinence and avoid relapse. Targeting stress-reactivity for treatment development is a critical, yet underdeveloped area of research which may serve to increase rates of smoking cessation for women. Preclinical findings support the hypothesis that noradrenergic pathways are involved in stress-induced relapse, and that their manipulation may be of potential benefit in the prevention of stress-related drug relapse. Using our validated human laboratory model to examine stress-induced smoking, we evaluated whether guanfacine (an alpha2a agonist) preferentially counteracted stress effects on smoking in women compared to men. We then evaluated whether guanfacine significantly reduced smoking behavior during a brief 4-week treatment period. During the treatment phase, results demonstrated that guanfacine equally reduced smoking in women and men, but appeared to target gender-sensitive mechanisms. During the laboratory component, guanfacine preferentially reduced the effect of stress on smoking in women compared to men. In men compared to women, guanfacine preferentially reduced smoking-related reinforcement. We will also present results examining gender differences in mechanisms underlying stress-precipitated smoking lapse and smoking-related reinforcement (e.g., craving, mood, withdrawal, cardiovascular reactivity, hypothalamus-pituitary-adrenal axis reactivity, catecholamines, cognitive function, and ovarian hormones). Results such as these provide important evidence that targeting gender-sensitive mechanisms is a viable medication development strategy, and support the further testing of noradrenergic agents for tobacco dependence. Supported by NIH grants P50DA033945 (ORWH & NIDA), R21DA033597, RL1DA024857, UL1RR024139.

### S34

#### **Nicotine use in alcoholics: Normalizing stress and performance?**

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Although its rates in the general population have decreased in recent decades, cigarette smoking remains a highly comorbid condition among persons with substance use dependencies. Recent data reported by our laboratory indicate that ~ 90% of men and women seeking treatment for alcohol and other substances are current smokers. Our initial studies of this comorbidity compared the neurocognitive effects of nicotine administration in treatment seeking alcoholics relative to smokers from the general community. This work suggested that alcoholics benefit from the cognitive enhancing effects of nicotine to a greater extent than do community smokers. Furthermore, as predicted, this benefit was evident primarily in tasks with a high attentional demand. We then considered the possibility that nicotine's effect might be modulated through its action on the stress response, i.e., via normalization of the typically blunted response. This work indicated nicotine administration was associated with a "normalized" cortisol response during the Trier social

stressor. Importantly, this relationship was not dependent on sex. Follow-up analyses show that alcoholics receiving nicotine demonstrated a positive relationship between salivary cortisol and neurocognitive performance. However, there are striking sex differences. Alcoholic men who received nicotine demonstrated a consistent positive relationship between cortisol and performance across psychomotor, set-shifting, and visual-scanning/executive function tasks whereas those who received placebo showed no significant correlations. In contrast, alcoholic women showed no relationship between cortisol levels and performance, regardless of whether they received nicotine. Taken together these findings indicate that nicotine use in alcoholics may be supported by its direct effects on cognition and/or through its action on the stress response system and that sex may influence the relative import of each. These data reinforce the importance of considering sex as a critical factor in developing effective short-term treatment and reducing longer-term relapse risk. R01 DA-13667

### Symposium IX

#### **MEDIATION OF ACUTE AND CHRONIC ACTIONS OF ETHANOL ON NEURONS OF THE VENTRAL TEGMENTAL AREA: NEW ADVANCES TOWARD NOVEL THERAPEUTICS**

Chair: Mark Brodie

Discussant: Michela Marinelli

### S35

#### **Epigenetic regulation of GABA sensitivity of VTA neurons following chronic alcohol.**

Mark S. Brodie, Devinder S. Arora, Sudarat Nimitvilai, Lei Tang, Tara L. Teppen, Maureen A. McElvain, Amul J. Sakharkar, Chang You, Huaiibo Zhang, and Subhash C. Pandey  
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Dopaminergic (DA) ventral tegmental area (VTA) neurons are crucial for evaluation of reward salience and are activated by numerous drugs of abuse, including alcohol. Modulation of the activity of VTA neurons and adaptation of VTA neurons to the presence of chronic alcohol may induce critical neuroadaptations underlying the development of alcoholism. We have demonstrated that repeated ethanol administration to mice produces a decreased sensitivity of DA VTA neurons to inhibition of firing by exogenously administered GABA. We have begun to elucidate the role of HDAC-induced histone modifications in the regulation of ethanol mediated changes in GABA sensitivity of DA VTA neurons. Repeated alcohol administration, either in mice injected with ethanol or in rats given access to ethanol-containing diet, increases levels of expression of HDAC2 and decreases histone H3-K9 acetylation during withdrawal. Interestingly, acute treatment with ethanol one hour before sacrifice normalized the levels of HDAC2 and histone H3-K9 acetylation. Correlated with the changes in HDAC2 and histone H3-K9 acetylation during withdrawal was decreased sensitivity of DA VTA neurons to GABA (50 – 500  $\mu$ M); this hyposensitivity was reversed and the response to GABA normalized by in vitro treatment of slices with HDAC inhibitors, either suberoylanilide hydroxamic acid (SAHA) or trichostatin A (TSA) for two hours. As reduction of HDAC2 function by HDAC inhibitors normalized GABA sensitivity of DAergic neurons, it is possible that inhibition of HDACs can reverse ethanol-induced neuroadaptational changes in the abnormal neuronal physiology of reward circuitry induced by ethanol exposure. Supported by AA-09125 (MSB), AA-05846 (MSB) and AA-016690 (SCP) AA-019971 (NADIA project) (SCP), and by the Department of Veterans Affairs (SCP).

### S36

#### **Profound and selective decrease of dendritic spines in the nucleus accumbens of ethanol dependent rats**

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Neuronal refinement and stabilization are hypothesized to confer resilience to poor decision-making and addictive-like behaviors, such as excessive ethanol drinking and dependence. Accordingly, structural abnormalities are

likely to contribute to the appearance of alcohol withdrawal signs and symptoms, which occur from suddenly ceasing the use of alcohol after chronic ingestion, thus perpetuating the addictive cycle. Here we show that ethanol dependent rats display marked structural abnormalities in the mesolimbic (dopamine-containing) system such as a loss of dendritic spines in medium spiny neurons of the Nacc, accompanied by a reduction of TH-positive terminals and PSD-95 positive elements. Further analysis indicates that 'long thin', but not 'mushroom', spines are selectively affected. These changes are restricted to the withdrawal phase of ethanol dependence suggesting their relevance in the genesis of signs and/or symptoms affecting ethanol withdrawal, and thus the whole addicting cycle. Overall these results highlight the importance of spine function on the evolution of alcohol dependence and suggest that the selective loss of 'long thin' spines may affect learning dysfunctions and significantly contribute to further 'impoverish' the already deficient dopaminergic transmission whose hypofunctionality is a major factor for the emergence of the harmful consequences of alcohol abuse/dependence. Supported by Dipartimento Politiche Antidroga and PRIN.

### S37

#### **Differential Effects of Ethanol and Inhalants on Ventral Tegmental DA Neurons**

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Addiction is a complex brain disease characterized by compulsive drug taking and altered sensitivity of brain reward circuits. Midbrain dopamine (DA) neurons in the ventral tegmental area (VTA) are especially responsive to addictive drugs and display alterations in excitability and glutamatergic plasticity following even brief exposures to drugs of abuse. We examined the effects of ethanol and abused inhalants on VTA DA neurons to better understand how drugs with different mechanisms of action may affect reward-sensitive VTA DA neurons. A single in vivo exposure to toluene vapor enhanced the AMPA/NMDA ratio of VTA DA neurons and this effect persisted for up to three weeks. This effect was selective for mesoaccumbens VTA DA neurons as those projecting to the prefrontal cortex (PFC) were unaffected. However, bi-directional modulation of PFC output prior to vapor exposure either blocked or enhanced toluene's effect on VTA DA neuron plasticity revealing cortical control of midbrain reward circuitry. In slice electrophysiology recordings, both ethanol and toluene enhanced spontaneous firing of VTA DA neurons and this was blocked by the mixed ion channel antagonist quinine. The nicotinic antagonist mecamylamine also blocked toluene's effect on firing but had no effect on ethanol potentiation of firing. Nicotine enhanced VTA DA neuron firing and this action was attenuated by both toluene and ethanol. Toluene induced firing of VTA DA neurons was blunted by GABA receptor antagonists but enhanced by blockers of glutamatergic signaling. These agents had little effect on ethanol's ability to enhance DA neuron spiking. Finally, while ethanol blocked the desensitization of dopamine D2 mediated inhibition (termed dopamine reversal of inhibition, DIR), toluene had no effect DIR. Overall, these results reveal a complex interplay between different drugs of abuse and brain reward circuitry. Supported by AA009986 (JJW), DA013951 (JJW), DA030891 (JTB), AA09125 (MSB) and AA-05846 (MSB).

### S38

#### **Food and alcohol: common neuroadaptations in the VTA**

Hitoshi Morikawa, Linzy M. Hendrickson, Matthew B. Pomrenze, Takashi Okamoto, Brian E. Bernier, Simona Perra  
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Clinical evidence indicates a significant association between alcoholism and pathological overeating, suggesting that these two conditions may share overlapping neurobiological mechanisms. Food intake to meet caloric demands of the body is largely controlled by the actions of appetite-controlling peptides/hormones in the hypothalamus. However, feeding, like intake of ethanol and other drugs of abuse, is also driven by the hedonic value of food through activation of the mesolimbic dopaminergic (DA)

system originating in the ventral tegmental area (VTA). During long-term exposure to ethanol, the DA system exhibits reduced baseline activity and tolerance to ethanol-induced stimulation, which will drive compulsive intake of a large amount of ethanol to compensate for the DA deficit. At the same time, the DA response to ethanol-associated sensory stimuli intensifies, powerfully motivating ethanol seeking in response to those stimuli. Similar changes in the DA system have been observed in human subjects with overeating disorders/obesity and in animals after prolonged consumption of calorie-dense palatable foods, resulting in increased motivation to consume more and more palatable foods. Here, we show that repeated ethanol exposure and extended access to calorie-dense palatable foods ("cafeteria diet") lead to 1) a reduction in baseline DA neuron activity in the VTA and 2) enhanced synaptic plasticity of excitatory glutamatergic inputs onto DA neurons that would drive acquisition of the phasic DA response to sensory stimuli. These neuroadaptations in the VTA may be part of the neurobiological substrate underlying comorbidity between alcoholism and overeating disorders/obesity. Supported by NIH R01 AA015521.

### Symposium X

#### **UNDERSTANDING THE ROLE OF STRESS AND DISTRESS TOLERANCE ON ALCOHOL USE IN YOUTHS**

Chair: Paola Pedrelli

### S39

#### **Gender specific relationship between adolescent distress tolerance and cortisol response to stress**

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Negative reinforcement models emphasize that the motivational basis for substance use is the escape or avoidance of negative affective states. A proxy for negative reinforcement processes, distress tolerance, is defined as the ability to persist in goal directed activity when experiencing affective distress. Low distress tolerance is an established risk factor for adolescent and early adult frequency of alcohol use, alcohol related problems, and coping motives for alcohol use, yet the biological mechanisms underlying this construct remain unknown. The aim of the present investigation was to examine the gender specific relation between distress tolerance and salivary cortisol response to stress among adolescents. A total of 143 adolescents [ $M = 16.0$  ( $SD = 1.0$ ); 54.5% female; 54.5% African American] attended a 2-hour laboratory session during which salivary cortisol was collected at 20 min intervals between pre and post stress exposure, and measured as area under the curve (AUC) from pre-stress to 40 minutes post stress. Distress tolerance was measured with a computerized behavioral task. A hierarchical logistic regression analysis indicated that gender moderates the relation between AUC and distress tolerance such that among males, the odds of having low distress tolerance was negatively associated with AUC (Wald = 6.83;  $p < .01$ ; OR = 0.72; 95% CI = 0.56–0.92) and among females, the odds of having low distress tolerance was positively associated with AUC (Wald = 6.86;  $p < .01$ ; OR = 1.35; 95% CI = 1.08–1.69). These findings suggest that gender-specific differences in salivary cortisol response to stress are associated with a maladaptive behavioral response to stress.

### S40

#### **Distress tolerance, alcohol use and alcohol-related problems among female and male college students**

Paola Pedrelli, Susannah Parkin  
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Binge drinking in college students is common and is associated with numerous severe negative consequences. Processes at the bases of this hazardous behavior require further elucidation to effectively reduce this problem. Some studies suggest that distress tolerance (DT), defined as the ability to persist in goal-directed activities while experiencing affective distress, affect alcohol consumption and the occurrence of alcohol-related problems in college students. Others have not found the same results.

Inconsistent findings may be due to the fact that DT in this population has been primarily measured by self-report questionnaires and it has not been investigated separately in males and females. The current study examined the relationship between distress tolerance, alcohol use and alcohol-related problems in binge drinking (BD) and healthy control (HC) female and male college students. DT was assessed by administering a computer-based task, the Paced Auditory Serial Attention Task (PASAT), and quitting prior to the end of the task indicated low DT. This method has been shown a valid measure of DT. A total of 60 students (Mean age 19.8 + 1.1; 60% female; 62% BD; 72.2% Caucasian) were enrolled. Female students with low DT had higher number of BD episodes and of alcohol related problems than their peers ( $F(1,31)=5.6, p<0.05$ ;  $F(1,30)=4.7, p<0.05$ ). No effect was found among males. A computer-based measure of DT identified female students at risk for hazardous alcohol use and alcohol problems. Findings underline the importance of intervention programs targeting alcohol use and alcohol-related problems to address DT, in particular among female college. Funding: ABMRF/ The Foundation for Alcohol Research (PP).

#### S41

##### **Distress tolerance over time predicts increases in alcohol use across adolescence**

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Distress tolerance (DT) is the pursuit of goal-directed behavior in the face of affective distress. Although DT is an emerging construct of theoretical interest with multiple studies among adults indicating an inverse relationship with DT and problem behaviors such as substance use, little is known regarding its prospective relationship with the development of alcohol use in youth. Thus, we examined the course of DT and its potential time-varying relationship with the development of alcohol use behavior from early to later adolescence. We hypothesized lower DT would be associated with increased odds of alcohol use over time, after controlling for relevant covariates. This study employed data from a community sample of 277 5<sup>th</sup>-6<sup>th</sup> graders (M age = 11.0 years, 46.5% female; 51.3% White) at initial enrollment and assessed at 6 annual waves. Participants completed a modified YRBS to measure past year alcohol use and the Behavioral Indicator of Resiliency to Distress (BIRD), a computer-based behavioral measure of DT for youth. Generalized Estimating Equations (GEE) analyses included demographics, the effect of time, and the time-varying effect of DT as predictors of alcohol use in subsequent waves. Results: After controlling for covariates ( $p's<.05$ ), odds of alcohol use increased over the six years ( $B = .08, SE = .02, p=.001$ ). Moreover, lower average DT over time was associated with greater odds of alcohol use ( $B = -.001, SE = .002, p = .01$ ). Conclusions: Results suggest DT should be a target of early intervention for the development of adolescent alcohol use. Funding: R01DA18647 (CWL).

#### S42

##### **A randomized controlled trial of a behavioral economic intervention for substance abuse in a diverse college sample**

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Behavioral economic research indicates that low-levels of substance-free reinforcement is a risk factor for poor response to brief motivational interventions (BMIs). Previous research has demonstrated that one way of enhancing the efficacy of BMI's is the introduction of a supplemental session that directly targets substance-free reinforcement and delayed reward discounting. The current study intended to extend the aforementioned study by adapting the typical motivational interviewing and substance-free activity sessions (SFAS) to address the risk factors of an ethnically diverse sample and by focusing on both alcohol and drug misuse. In addition to encouraging engagement in constructive alternatives to substance-use and reducing delayed reward discounting, the SFAS addressed stress-related variables. Because college students who experience higher levels of stress are more inclined to increase their alcohol consumption, the SFAS focused on introducing substance free alternatives to using alcohol and drugs when coping with stress. Participants were 97 heavy drinking college students,

who completed a baseline assessment and an individual alcohol-focused BMI. Participants were then randomized to either the SFAS session or an education control session. Mixed model analyses revealed that participants in both conditions showed similar reductions in alcohol consumption at follow-up, however, participants in the BMI+SFAS condition showed larger effect size reductions compared to the control group. Additionally, those in the SFAS condition endorsed fewer days of marijuana use at follow-up. These results suggest that traditional alcohol and drug BMI's can be enhanced by the addition of substance free activity session, which focuses on alternatives to substance use.

#### Symposium XI

##### **APPLYING THE NEW GENOMICS TO ALCOHOL DEPENDENCE**

Chair and Introduction: R. Adron Harris

#### S43

##### **Biological networks underlying lifetime consumption of alcohol within human prefrontal cortex**

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Alcohol Dependence (AD) is a complex psychiatric disorder influenced by environmental and biological factors. Variation in gene expression is hypothesized to contribute to substance abuse. Using RNA Sequencing (RNA-Seq) we determined the coordinate expression of genes, alternative spliced transcripts, and exons from the prefrontal cortex (PFC), a brain region implicated in both stress and substance abuse, for alcoholics and matched control subjects. Applying network methodologies our analysis determined the average overall connectivity among biological features is significantly reduced in alcoholic PFC, which may reflect a general loss of homeostasis due to AD. The pairwise relationships for gene expression within AD PFC defined distinct gene modules that were largely distinct from controls in terms of co-variation. Comparing the upper- and lower-quartile for gene-sets correlated to lifetime consumption of alcohol revealed higher connectivity within modules associated with alcohol drinking, suggesting neuronal plasticity related to alcohol consumption. Modules significantly associated with lifetime consumption of alcohol were over-represented for ion-channels and additional genes affecting synaptic plasticity and long-term potentiation. These results indicate a biological network of genes, known to interact with alcohol in experimental models, may underlie lifetime alcohol consumption in dependent individuals. Extending the network analysis for transcriptional isoforms further refined our results to identify AD modules of specific splice variants. Clarifying specific isoforms, and coordinately regulated sets of isoforms, will assist in the design of pharmacotherapies that target networks impacting the neurobiology of alcohol dependence and related phenotypes. *This work was supported by NIAAA grants R01AA012404, U01AA020926, RC2AA019382, and T32AA007471.*

#### S44

##### **microRNA contribution to the development of alcohol dependence**

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Alcohol Dependence (AD) is a complex, chronic disease, which both human and animals can develop. AD progresses due to frequent alcohol exposures and withdrawals. It is thought that in AD alcohol causes permanent changes in complex gene expression networks in the brain. However, the mechanisms of these changes are largely unknown. microRNAs are master regulators of gene expression networks, and our and others work showed that alcohol affects microRNAs. To better understand the role of microRNAs in AD we used a multiplex approach focused on a specific microRNA called miR-9 – an important regulator of neuronal function, also involved in the development of molecular tolerance to alcohol. Using COGA (the Collaborative Studies on Genetics of Alcoholism) human DNA samples we determined that there are specific SNPs in miR-9 regulatory regions highly associated with AD. Interestingly, some of these SNPs are potential binding

sites of transcription factors as well as epigenetic regulation of miR-9 expression. Moreover, by exposing mouse striatal cultures to alcohol for various periods of time we observed that levels of miR-9 depend highly on the interplay between alcohol exposure and withdrawal. These results can enhance our insight into the molecular differences between binge drinking and chronic consumption associated with AD. Finally, we determined that levels of miR-9 in the postmortem prefrontal cortex of people who suffered from AD are decreased. Together, microRNAs are intermediaries of alcohol actions in the brain and can be considered as attractive, novel therapeutic targets of AD. *This work was supported by NIAAA grants AA01748 and AA017920 to AZP.*

#### S45

##### **Synaptomics: Using genomic expression profiling to identify chronic ethanol-induced alterations in the synaptic transcriptome**

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Chronic ethanol exposure leads to behavioral alterations including sensitization, tolerance, and dependence. The molecular mechanisms underlying these behavioral responses are largely unknown but are thought to involve changes in gene expression that produce altered neuronal function and synaptic plasticity. One mechanism contributing to synaptic plasticity is thought to entail the trafficking of mRNA/miRNA molecules to the dendritic spines where local protein synthesis is affected. We have used whole genome microarray studies and RNA-seq analysis to profile synaptic transcriptome changes in mRNA and miRNA abundance during the initiation phase of ethanol sensitization. Our studies have identified a remarkably diverse portion of the transcriptome that is enriched in the synaptoneurosome fraction of forebrain tissue in DBA/2J mice. A significant subgroup of ethanol-responsive genes are contained in this synaptic transcriptome, suggesting that changes in dendritic transcripts contribute to synaptic plasticity underlying behaviors seen with chronic ethanol exposures as with ethanol sensitization. Supported by NIAAA grants P20AA017828 and U01AA016667.

#### S46

##### **Reverse engineering transcriptional networks to identify critical neuroadaptations during protracted withdrawal from alcohol dependence**

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Compulsive use of alcohol has been hypothesized to be driven by multiple sources of reinforcement that change with the individual's transition from social drinking to abuse and substance dependence on alcohol. The overarching hypothesis behind the present project is that understanding the dysregulations of the gene regulatory network that underlie the neuroadaptive changes associated with excessive alcohol drinking will allow the identification of new and more effective therapeutic targets for alcohol abuse. Specifically, we are using an integrated systems biology approach to identify the master regulator genes (MRs) that regulate the expression of gene signatures associated with excessive drinking in the chronic ethanol-induced dependence (CEID) model. The present systems biology strategy is based on the dissection of context-specific regulatory networks rather than on the identification of genes that are statistically associated with the phenotype. With this strategy, the emphasis comes from the high-throughput data, rather than from what is previously known in the literature or by the investigator. This strategy identifies a small number of genes connected within pathways dysregulated in the disease and that are likely to be causal. This facilitates the analysis of the molecular context determining the phenotype by providing specific testable hypotheses for experimental validation. Nr3c1, the gene

coding for the glucocorticoid receptor (GR), was identified as one of the highest ranking MRs showing differential activation in rats with a history of dependence, consistent with a key functional role for GR in the CEID paradigm. Supported by NIAAA R01 AA021667.

#### Symposium XII

##### YOUNG INVESTIGATOR AWARD

Chairs: Antonio Noronha and Fulton Crews

#### S47

##### **Excessive alcohol drinking in the context of sensitized stress signaling**

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The progression from recreational alcohol drinking to dependence affects numerous neurobiological systems and can be conceptualized as a transition from positive to negative reinforcement mechanisms. Stress-associated neuroadaptations that predict or reflect excessive drinking would likely provide novel molecular candidates for therapeutic intervention. In collaboration with Dr. Nicholas W. Gilpin (LSUHSC-New Orleans), neuronal activation patterns and synchronicity were assessed in a traumatic context by measuring brain regional ERK phosphorylation in an animal model of PTSD. Animals that persistently avoided a traumatic stress-paired environment over several weeks exhibited an escalation in alcohol drinking. Re-exposure to the stress-paired context produced bidirectional changes in prefrontal ERK phosphorylation between avoider and non-avoider groups, as well as altered synchronous activity between prefrontal and amygdala subregions that may reflect a differential phenotype associated with post-traumatic escalation of drinking. In search of neural signatures of stress sensitization in the alcohol-dependent state, Drs. George F. Koob and Leandro F. Vendruscolo (The Scripps Research Institute) and I recently found that dependent rats display significant increases in glucocorticoid receptor (GR) phosphorylation at Ser211/232 in the central amygdala (CeA) compared to non-dependent rats. This neuroadaptation is consistent with a central stress system sensitization in alcohol dependence since phosphorylation at Ser211/232 is a biomarker of transcriptionally active GR. In concert with this neuroadaptation, injection of the GR antagonist mifepristone directly into the CeA reduces alcohol self-administration in dependent rats. These studies provide strong evidence for a potentiation of stress-related neural circuitry that may promote a transition to excessive drinking. This work was generously supported by the following grants from NIAAA: AA020839, AA018400, AA008459, and by the Pearson Center for Alcoholism and Addiction Research.

#### S48

##### **Chronic alcohol exposure disrupts cognitive function and D2/D4 receptor modulation of prefrontal cortical function**

Heather Trantham-Davidson, Sam Centanni, L. Judson Chandler  
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The medial prefrontal cortex (mPFC) plays a critical role in decision-making and cognitive control of behavior, and these functions are highly influenced by dopaminergic (DA) activity in the mPFC. Previous studies in humans have reported disruption of cognitive processes following repeated episodes of excessive alcohol consumption and withdrawal, suggesting a possible link to altered mPFC function and DA activity in the mPFC. Recurrent network activity between excitatory pyramidal neurons is shaped by recruitment of inhibitory GABAergic neurons, specifically those that are characterized as fast-spiking interneurons (FSIN's). Dopaminergic inputs to mPFC exert powerful effects on network activity via D1/D2 receptor modulation of the balance between excitatory and inhibitory tone. Behavioral assessment of cognitive function revealed delay-dependent impairment of working memory performance in CIE rats compared to controls. These deficits were also associated with a reduction in cognitively modulated neuronal synchrony during the phase of the task, which required the rats to use memory to guide decision-making. Electrophysiological assessment of neuronal activity in acute brain slices revealed that CIE did not alter D1 receptor modulation of pyramidal neurons or FSIN's. However, in slices from CIE animals, the



effects of both D2 and D4 stimulation were lost in both cell types and this persisted at 1 month following ethanol exposure. While the cellular mechanisms that underlie the loss of D2/D4 modulation of cortical activity following CIE are unclear and are currently under investigation, our preliminary analysis of spike characteristics and synaptic responses suggests that CIE primarily disrupts D2 signaling in pyramidal neurons and preferentially affects D4 signaling in FSIN's. It is reasonable to suggest that this loss will significantly impact DA modulation of PFC activity and may contribute to the cognitive dysfunction associated with CIE exposure. This is consistent with observations in detoxified alcoholics of altered D2 receptor signaling and impaired cognitive function that persists well into abstinence. We suggest that loss of D2/D4 receptor modulation following CIE exposure may shift the cortical network into a D1-receptor dominated state that may contribute to cognitive deficits observed in human alcoholics. Preliminary experiments suggest that mGluR1 stimulation of FSIN's could be used as a novel therapeutic strategy that will restore the excitatory-inhibitory balance required for network synchrony that supports cognitive function.

#### S49

##### **Ethanol produces CRF dependent regulation of glutamatergic transmission in the Central Amygdala**

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In the central amygdala (CeA), ethanol (EtOH) enhances GABAergic transmission via activation of local corticotropin releasing factor (CRF) receptors, potentially by increasing CRF release. However, CRF receptor 1 deletion from glutamatergic synapses has been shown to produce more robust affective phenotypes than CRF receptor 1 deletion from GABAergic synapses. We recently showed that CRF enhances glutamatergic transmission in the bed nucleus of the stria terminalis and in the CeA. Given that EtOH may induce CRF release in the CeA and the role of CRF-glutamate interactions in the regulation of affective phenotypes typically seen in EtOH withdrawal, we sought to determine if EtOH acutely alters glutamatergic transmission in the CeA via a CRF dependent mechanism. Utilizing whole-cell patch-clamp electrophysiology recordings of CeA neurons from adult male C57BL/6J mice, we found that bath application of EtOH (5-100mM) significantly enhances spontaneous excitatory postsynaptic current (sEPSC) frequency in a concentration-dependent manner (ANOVA:  $F(4,21)=3.596, p<0.05$ ;  $EC_{50}=18.31mM$ ). 100mM EtOH significantly increased sEPSC frequency from baseline levels ( $132.0 \pm 8.4\%$ ,  $n=8, p=0.007$ ), an effect completely abolished by pretreating CeA slices with CRF receptor 1 and 2 antagonists, NBI27914 and Astressin2B ( $98.3 \pm 11.05\%$ ,  $n=10, p=0.88$ ), suggesting EtOH requires intact CRF signaling to increase presynaptic glutamate release in the CeA. The CeA contains CRF producing neurons and extrinsic CRF sources, however it is not yet known which source of CRF is required for EtOH potentiation of CeA glutamate release. To that end, experiments are currently in progress to determine EtOH-sensitive CRF sources required for modulation of CeA neurotransmission

#### S50

##### **Phosphodiesterase 10A inhibition reduces alcohol self-administration even in stress history and alcohol-dependent rats**

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Alcohol use disorders are chronically relapsing conditions with particularly high rates of recidivism and treatment resistance among those with comorbid diagnoses of stress-related disorders. Recently we identified phosphodiesterase 10A (PDE10A) as a putative mediator of stress history-related elevations in relapse-like alcohol self-administration: *Pde10a* mRNA levels were elevated in the basolateral amygdala of stress history rats. In the prelimbic prefrontal cortex, *Pde10a* expression was elevated and correlated with relapse-like alcohol self-administration in otherwise low-drinking rats that showed heightened alcohol intake upon renewed access. Thus, we hypothesized that inhibition of PDE10A would reduce alcohol self-

administration, particularly among rats with a history of stress or alcohol dependence. Systemic administration of the selective PDE10A inhibitor TP-10 significantly reduced relapse-like alcohol self-administration, even in stress history rats that showed elevated relapse-like self-administration. TP-10 also decreased alcohol self-administration in alcohol-preferring Scr:SP rats, as well as in alcohol-dependent and nondependent rats, under both fixed and progressive ratio requirements. TP-10 also reduced saccharin self-administration, suggesting a general role for PDE10A in supporting the motivation to self-administer alcohol or non-alcohol reinforcers. Site-specific infusion of TP-10 into the dorsolateral striatum, but not nucleus accumbens, significantly reduced alcohol self-administration. Analysis of the microstructure of alcohol self-administration demonstrated significant reductions in number, size and duration of bouts of self-administration, even at the minimally effective dose which did not alter the rate of alcohol-reinforced lever pressing. Thus, acute pharmacological inhibition of PDE10A potentially reduced self-administration of reinforcing substances, including alcohol, and did so in vulnerable populations with high self-administration, such as alcohol-dependent rats, alcohol-preferring rats and high-relapsing stress history rats.

#### Symposium XIII

##### **CORICOSTRIATAL CIRCUITRY AND HABITUAL ETHANOL-SEEKING BEHAVIOR**

Chair: L. Judson Chandler  
Discussant: Ruben Gonzalez

#### S51

##### **Firing patterns of dorsal striatal neurons to alcohol-associated cues**

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The transition from goal-directed to habitual behavioral control may involve a shift of the prominent striatal circuit, with the dorsomedial striatum (DMS) important for goal-directed behavior and the dorsolateral striatum (DLS) required for habit formation. Using extracellular electrophysiology, we investigated DMS and DLS neuronal activity in rats trained to self-administer alcohol on habit-promoting or habit-resistant reinforcement schedules. In both goal-directed and habit-directed rats, the DMS neuronal population showed increased firing at the presentation of start-of-session cues, as well as at the time of reinforcement and reinforcement-associated cues, while the most prominent phasic activity in the DLS surrounded the action of the lever response. However, the degree of overlap between DMS and DLS firing patterns was greater in rats trained on the habit-promoting schedule. In a second study, we trained rats to self-administer either sucrose or a sweetened ethanol solution on habit-promoting or habit-resistant reinforcement schedules. After 5 days of extinction, we measured neural activity in the DMS and DLS during presentation of the reward-conditioned cues and an aliquot of reward. Across groups, the DMS neuronal population showed increased firing at the presentation of the cue, while DLS neurons showed excitations to the cue only in habit-trained rats. Moreover, this DLS excitation was enhanced in rats trained to drink alcohol on the habit-promoting schedule. These data suggest that both habit formation and alcohol reward promote DLS activation in an animal model of cue-induced relapse. Funded by NIH R01AA018008, ABMRF/The Foundation for Alcohol Research, and the UNC Bowles Center for Alcohol Studies.

#### S52

##### **Innate and acquired differences in the nucleus accumbens in the acquisition and expression of habitual alcohol seeking**

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While alcohol consumption for most individuals is controlled and goal-directed, some individuals go on to develop inflexible stimulus-mediated habitual drinking. Identification of neurobiological differences that create a predisposition to habitual ethanol seeking is expected to inform the

development of novel pharmacotherapeutic strategies for the treatment of alcoholism. The regulation of habitual behavior likely requires normal synaptic plasticity in key neural substrates including the nucleus accumbens (NAc). Our data highlight innate and acquired differences in NAc neurobiology that can promote habitual behavior. A growing body of literature has shown roles for cell adhesion molecules in addiction. Here we show that individual differences in expression of the proplastic, sialylated form of neural cell adhesion molecule (PSA-NCAM) in the NAc were related to innate differences in Pavlovian approach behavior and the propensity to develop habitual ethanol seeking. Given our finding that chronic intermittent exposure to ethanol (CIE) can drive habitual behavior, and further evidence that CIE down-regulates mGluR2 expression in the NAc (Meinhardt, et al., 2013), we used pharmacological manipulations to demonstrate that increased mGluR2 signaling in the NAc shell can restore flexible behavior after the development of habitual ethanol seeking. Interestingly, the addition of PSA to NCAM has also been shown to alter the cellular response to glutamate. Ongoing work is investigating the relationship between innate and dependence-induced alterations in accumbens plasticity, and in particular, how changes in glutamate signaling and PSA-NCAM expression causally alter habitual ethanol-seeking.

### S53

#### **Endocannabinoid signaling in orbitofrontal cortex modulates habit formation**

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Stress and addiction disorders, including alcohol-use disorder involve dysfunctional executive control, characterized by an impaired ability to break habits and shift to goal-directed control. However, the neural bases underlying how the brain switches action strategy is unknown. We found that the orbital frontal cortex (OFC) was necessary to break habits and shift towards goal-directed control. Optogenetic activation of OFC projection neurons increased goal-directed actions, while chemogenetic inhibition biased habitual control, suggesting plasticity of these circuits is necessary for the shift to automatic control. Previous findings show endocannabinoid-type 1 receptor (CB1) involvement in habit formation, and CB1 receptors are present on excitatory cortical projection neuron terminals. We hypothesized that endocannabinoid signaling could be shaping the plasticity of OFC circuits during action learning. We used a novel variant of an instrumental task where we train the same mouse to perform the same action for the same reward in a goal-directed versus habitual manner to examine mechanisms underlying action shifting. We initially found impaired habit formation in mice following deletion of CB1 receptors in forebrain projection neurons. Next we found CB1 receptor deletion specifically within OFC or in OFC projection neurons impaired habit formation, but left goal-directed actions intact. Further, chemogenetic inhibition of OFC projections to dorsal striatum blocked goal-directed actions. Our findings suggest the shift from goal-directed to automatic actions is controlled by CB1 receptor modulation of OFC excitatory output to dorsal striatum, with CB1 receptor deletion perturbing the ability of these circuits to shift and thereby altering habitual control over actions.

### S54

#### **Changes in the behavioral and neural control in the transition from goal-directed to habitual alcohol-seeking**

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It has long been hypothesized that alcohol seeking becomes habitual over time. We have investigated the time course of this transition for alcohol self-administration, and the neural systems controlling it. Rats were trained to make a lever-press response to earn alcohol. We then used outcome devaluation to test the sensitivity of the alcohol-seeking response to changes in the value of alcohol across the course of extended self-administration. We find that following limited training, rats decrease performance of the instrumental response when alcohol is devalued. However, following extended training, this sensitivity is lost and rats continue to perform the

alcohol-seeking response despite the decreased value of alcohol. We investigated the role of subregions of the dorsal striatum in controlling performance at these two time points. After limited training, inactivation of the dorsomedial striatum (DMS) decreased responding suggesting this area supports goal-directed performance. Inactivation of the dorsolateral striatum (DLS) at this time point was without effect. Following extended training, when behavior no longer showed sensitivity to devaluation, inactivation of the DMS was without effect whereas inactivation of the DLS renewed sensitivity to devaluation indicating that the structure is essential for the expression of a habitual response. Similarly, infusion of a D2 antagonist into the dorsolateral striatum rescues sensitivity to devaluation. Thus dopamine in the dorsolateral striatum plays a critical role in alcohol self-administration after extensive training. Together, these results indicate that behavioral flexibility decreases across extended alcohol self-administration and demonstrate an increased reliance on the dorsolateral striatum as responding becomes habitual. Supported by R01 AA018025 (PHJ) and the ABMRF (LHC).

### Symposium XIV

#### **TRANSLATIONAL STUDIES ON GENETIC AND GENOMIC ANALYSES OF STRESS, ALCOHOL DEPENDENCE, AND DRINKING**

Chair: Howard C. Becker

Introduction: Robert Williams

Discussant: Wolfgang H. Sommer

### S55

#### **Chronic Intermittent Ethanol Exposure Influences Voluntary Ethanol Intake in BXD Strains of Mice**

MF Lopez<sup>1</sup>; LA Ralston<sup>1</sup>; RW Williams<sup>2</sup>; MF Miles<sup>3</sup>; HC Becker<sup>1</sup>

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The recombinant inbred BXD strains of mice were generated by crossing and inbreeding ethanol-preferring C57BL/6J (B6) and ethanol-avoiding DBA/2J (D2) inbred strains. On a series of studies, a panel of 43 BXD strains has been tested for voluntary ethanol intake using a model of dependence and relapse drinking. Mice were tested for baseline ethanol intake using a 2-bottle (15% v/v ethanol vs. water) limited access procedure (2-hr/day) for 6 weeks (Baseline). Then, mice received 4 weekly cycles of chronic intermittent ethanol (CIE) vapor exposure or control air exposure (16 hr/day x 4 days) alternated by 5-day drinking test cycles. The original study, evaluated 121 mice (males and females) with n=1/line/sex/group. C57BL/6J male mice served as positive controls. Results indicated: a- differences in voluntary ethanol intake across BXD strains during baseline; b- CIE exposure induced either decrease or increase in ethanol intake, or no change in BXD strains; c- the change in ethanol intake was not related to the level of ethanol intoxication during CIE cycles; d- CIE exposure induced the expected escalation in ethanol drinking in C57BL/6J mice while intake in control mice remained stable. Ongoing follow up replicate studies are centered in increasing the number of mice/line/group/sex on BXD lines selected based on their ethanol intake after CIE exposure in the original study. These studies aim to further explore the genetic influence on the propensity to escalate (or avoid) ethanol drinking as a function CIE exposure. Supported by NIAAA grants AA020929, AA014095, and AA010761.

### S56

#### **Identification and validation of cross-species conserved gene networks associated with progressive ethanol consumption**

Angela M. Batman

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Chronic ethanol exposure leads to behavioral alterations including sensitization, tolerance and dependence. The molecular mechanisms underlying these behavioral responses are largely unknown but are thought to involve changes in gene expression that produce altered neuronal function and synaptic plasticity. Repeated exposure to cycles of ethanol vapor, followed by withdrawal and oral consumption, leads to a progressive increase in ethanol consumption and is thought to be an important model for

mechanisms occurring during the development of alcoholism in humans. We have used whole genome expression profiling with microarrays and RNA-seq, together with scale-free network analysis approaches to identify gene networks associated with progressive ethanol consumption in C57Bl/6 and BXD inbred strains of mice. This work has identified novel, brain-region specific networks which correlate with ethanol consumption. Co-analysis with expression data from a primate model of chronic ethanol consumption identified conserved cross-species networks correlating with consumption. Network topology and bioinformatics analysis was used to select hub genes in candidate networks for validation using viral vector and pharmacological studies with rodent 2-bottle choice or operant models. This approach has identified novel candidate mechanisms for future study as possible sites of intervention in alcoholism. Supported by NIAAA grants P20AA017828 and U01AA016667.

#### S57

##### **Epigenetic signatures associated with chronic alcohol use**

Betsy Ferguson<sup>1,2</sup>, Rita Cervera Juanes<sup>1</sup>, and Lucia Carbone<sup>1,2,3</sup>

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Epigenetic DNA modifications occur in response to 'environmental' perturbations, such as those elicited by diet, exposures to toxins, or stress. A growing number of studies comparing DNA methylation patterns in target genes between alcoholic and healthy populations have shown that long-term alcohol use can induce epigenetic changes. We are leveraging the monkey model of alcohol self-administration (Grant et al., 2008) and next-generation sequencing to investigate epigenetic changes associated with chronic alcohol use on a genomic scale. Blood-based DNA samples are collected at the onset of the study, while animals are alcohol naive, and then again after 12 months of chronic alcohol consumption. Genomic CpG methylation levels are measured and compared within subject, using whole genome bisulfite sequencing. On a global scale, we identified a slight decrease in CpG methylation rates following chronic alcohol consumption. At the same time, we could detect both hyper- and hypo-methylation within individual gene bodies and promoters. CpG methylation rates in these differentially methylated regions were validated in an expanded set of individuals using targeted bisulfite sequencing. In addition, effects on RNA expression levels of the same genes in the same individuals were evaluated using qRT-PCR. Notably, some of these genes for which differential methylation levels were identified following 12 months of alcohol consumption belong to the serotonergic and dopaminergic signaling pathways. We will therefore focus our presentation on these genes. Research supported by: NIH grants AA020928 and OD0110923

#### S58

##### **Sequencing the transcriptome in ethanol consuming rhesus macaques**

Robert Hitzemann, Priscila Darakjian, Shannon McWeeney, Nikki Walter, Betsy Ferguson, Christa Helms and Kathy Grant  
Oregon Health & Science University & Veterans Affairs Research Service, Portland, OR 97239, USA

Beginning with Lewohl et al. (2000) we now have ample evidence that alcoholism is associated with marked changes in the brain transcriptome. However, despite the careful selection of alcoholic and control samples, it is not possible to cleanly dissect the effects of chronic alcohol abuse from environmental effects and alcohol x environment interactions. A non-human primate (NHP) model of alcoholism addresses these issues. Details of the NHP model are described elsewhere (Grant et al. 2008). The sample here is 32 rhesus macaques, encompassing four different cohorts (ranging in size from 5 to 11). Following a 3-month ethanol induction period, the subjects were given unrestricted access to ethanol for 22 hours/day for 12 months. The 12-month average ethanol intakes ranged from 0.3 to 4.1 g/kg/day; the median consumption was 2.4g/kg/day. For area 32 and the CeA, ribosome depleted and strand specific libraries were prepared for single-end, 100 bp sequencing. Data (to date only for area 32) were analyzed using both gene-level summarization and at the level of individual exons. No association was detected between consumption and any of the glutamate or GABA subunits. A significant negative association was observed with *Snord115* which is

involved in regulating the alternative splicing of *Htr2c*, *Crhr1*, *Dpm2*, *Taf1*, *Ralgps1* and *Pbmr1*. *Crhr1* is not expressed in area 32 and the expression of *Htr2c* is too low to accurately assess alternative splicing. For *Htr2c* and *Crhr1*, the data from the CeA should be informative. Supported by AA 13484, AA 10760, AA 13510 and AA 19431.

#### Symposium XV

##### **STRESS AND ALCOHOL: A LIFESPAN PERSPECTIVE**

Chairs: C. Fernando Valenzuela

Discussant: Lindsey Grandison

#### S59

##### **Transgenerational epigenetic effects of fetal alcohol exposure on neuroendocrine-immune axis**

Dipak.K. Sarkar, Dmitry Govorko, Rola A. Bekdash, Changqing Zhang  
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The idea that exposure to adverse environmental conditions and lifestyle choices during pregnancy can result in fetal programming that underlies disease susceptibility in adulthood is now widely accepted. We evaluate whether alcohol exposure in utero produces epigenetic modification of genes critically involved in controlling neuroendocrine-immune axis functions. We conducted epigenetic, behavioral and physiological studies to determine transgenerational epigenetic effects on the neuroendocrine immune axis. Our data revealed that fetal alcohol exposure (FAE) increased DNA methylation of proopiomelanocortin (POMC) gene and decreased mRNA expressions of POMC and IFN-g genes. These methylation and gene expression defects in FAE offspring persisted in the F2 and F3 offspring. Determination of hormonal responses to stress revealed that adult FAE offspring had elevated stress hormones (corticosterone, ACTH) responses. FAE animals also showed higher anxiety like behaviors. Upon a carcinogen NMU treatment, FAE female rat offspring displayed an increase in mammary tumor incidence, growth and malignancy rate. Determination of immune status revealed that FAE rats had reduced innate immune functions. These changes in innate immune functions were correlated with a reduced activity of POMC neurons and the IFN-g level in FAE offspring. Supplementation of POMC neurons via transplantation decreased the stress response and anxiety like behaviors, increased NK cell functions and macrophage activities and decreased the incidence of mammary cancer in female FAE rats. These data suggest that alcohol exposure in utero produces epigenetic modification of POMC gene that increases body stress response and the susceptibility to tumorigenesis in endocrine tissues and these abnormalities transmit for many generations. (Supported by NIH Grant AA016695 and R37 AA08757).

#### S60

##### **Ethanol exposure during the brain growth spurt impairs modulation of GABA<sub>A</sub> receptor-dependent transmission by dopamine in the rat basolateral amygdala**

C. Fernando Valenzuela, Marvin Diaz.

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Fetal alcohol spectrum disorder is often associated with mood alterations, including high responsiveness to stress and anxiety. It is thought that these alterations are, in part, a consequence of basolateral amygdala (BLA) dysfunction. Within the BLA, local GABAergic interneurons provide inhibitory input to glutamatergic pyramidal neurons and are regulated by dopamine (DA) inputs from the ventral tegmental area. DA levels within the BLA are increased by novel and stressful stimuli. DA increases local GABAergic interneuron firing via DA D1 receptor (D1R) activation, preventing over-excitation of the BLA, and modulating behavioral responses to stress. We hypothesized that 3<sup>rd</sup> trimester-equivalent ethanol impaired D1R-dependent modulation of GABA<sub>A</sub> receptor-dependent transmission in the BLA. Dams and pups were exposed to vaporized ethanol or room air from postnatal day (P) 2-12 for 4 hours per day. Exposure to ethanol resulted in BECs of ~50 mM in the pups. Electrophysiological experiments were performed in offspring at P40-50 using the acute slice preparation and whole-cell patch-clamp techniques. We found that exposure to ethanol did not disrupt basal action potential-dependent spontaneous inhibitory

postsynaptic currents (sIPSCs) in pyramidal neurons. These events are mediated by activation of GABA<sub>A</sub> receptors by GABA released from local interneurons. In agreement with previous reports, we found that application of a high concentration of DA increased both the frequency and amplitude of sIPSCs in BLA pyramidal neurons from air-exposed rats. As expected, these effects were blocked by a D1R antagonist. Importantly, these effects of DA were significantly reduced in slices from ethanol-exposed rats. These data suggest that chronic 3<sup>rd</sup> trimester-equivalent ethanol exposure impairs D1R-mediated regulation of GABAergic transmission within the BLA and this functional deficit may, in part, underlie the anxiety-like behaviors that characterize fetal alcohol spectrum disorder. Supported by AA-014973 and minority supplement AA-014973-S1.

## S61

### Early-life stress, the HPA axis and addiction neurocircuitry

Delia Bellelli<sup>1</sup>, Ben G. Gun<sup>1</sup>, Linda Cunningham<sup>1</sup>, Edward P. Maguire<sup>1</sup>, Scott Mitchell<sup>1</sup>, Jerome Swinny<sup>2</sup> and Jeremy Lambert<sup>1</sup>.

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Adverse early life experiences, for example in the form of poor maternal care, negatively affect normal brain development and program an abnormal stress response by encoding long-lasting molecular changes in the brain. Such perturbations predispose to a higher incidence of psychiatric disturbances and an increased susceptibility to addiction in adulthood. Genetic linkage analysis has suggested a region of chromosome 4 containing a cluster of GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) genes including those that encode for  $\alpha 2$ ,  $\alpha 4$ ,  $\beta 1$  and  $\gamma 1$  subunits to increase the propensity for drug abuse (Edenberg *et al.*, 2004). Furthermore, a genetic association of GABRA2 gene haplotypes with drug taking was evident in individuals who had experienced childhood trauma (Enoch *et al.*, 2010). The nucleus accumbens is an established substrate for mediating drug reward and is increasingly recognised as an important locus for influencing stress-induced behaviours. The majority of accumbal neurons are GABA-ergic medium spiny neurons, which we demonstrated to express synaptic  $\alpha 2$ -GABA<sub>A</sub>Rs (Dixon *et al.*, 2010). Given the strong association of childhood trauma, drug abuse and the GABRA2 haplotype we have developed a mouse model of early life stress (ELS) and utilized a combination of immunohistochemistry, electrophysiology and behavior to explore how negative early-life events may influence neurotransmitter function both in the hypothalamic stress neurocircuitry and in the nucleus accumbens to consequently influence the behavioural effects of addictive drugs. Our findings reveal specific alterations in neurotransmitter function in both brain regions and in the behavioral response to addictive drugs, which offer insights relevant to human studies. Acknowledgments: Supported by Wellbeing of Women UK (Grant # RG1265), Medical Research UK (G1000008) and Tenovus Scotland.

## S62

### Role of the Noradrenergic Systems in Stress-Induced Reinstatement of Alcohol Seeking in Rats

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Relapse to alcohol use after abstinence can be provoked by stress. We have used an animal model of relapse, known as the reinstatement procedure, to study the neurobiological basis of stress-induced relapse to alcohol. One focus of our research over the past several years has been on the role of the noradrenergic systems in stress-induced relapse to alcohol. In these experiments, rats were trained to self-administer alcohol in operant chambers and reinstatement of alcohol seeking was assessed following extinction of the self-administration behavior. Administration of the alpha-2-adrenoceptor antagonist, yohimbine, which causes stress-like responses in humans and experimental animals, reliably enhances alcohol intake and reinstates alcohol seeking in rat models of relapse. Consistent with this, administration of alpha-2 receptor agonists such as lofexidine or clonidine attenuate reinstatement of alcohol seeking induced by footshock stress or yohimbine. We also evaluated the ability of the alpha-1 adrenoceptor antagonist prazosin

(0.5-2 mg/kg), and the alpha-2 adrenoceptor agonist guanfacine (0.125-0.5 mg/kg) on yohimbine-induced reinstatement of alcohol seeking and on yohimbine-induced increases in alcohol consumption. Prazosin attenuated yohimbine-induced reinstatement of alcohol seeking at all doses tested whereas significant effects of guanfacine were observed only with the highest dose employed. Although neither of these compounds affected basal alcohol self-administration, both drugs attenuated yohimbine-induced increases in alcohol self-administration. Our current work examines the central mechanisms underlying the modulatory effects of prazosin on stress-induced relapse to alcohol. Together, these data indicate that the noradrenergic systems play a critical role in stress-induced relapse to alcohol. Drugs targeting these systems might therefore be of potential use in the treatment of alcohol dependence. Both prazosin and guanfacine have been approved for treatment of, respectively, PTSD and ADHD. The observation that alcohol abuse is frequently co morbid with these disorders further suggests that these two compounds might be of potential use in the treatment of alcohol dependence. Supported in part by NIAAA: AA13108.

## Symposium XVI

### CLINICAL TREATMENTS ROUNDTABLE

Chair: Barbara J. Mason

Discussant: Kenneth R. Warren

The Clinical Roundtable will use examples of translational approaches, proof-of-concept studies in patients, Phase III trials for regulatory approval and extension to a new indication for an approved compound as strategies for medication development for alcohol dependence. The results presented will serve as the basis for discussions about methodological innovations in medication development for alcoholism, validation of pre-clinical targets, and implications for treatment of alcohol dependence.

## S63

### A selective NOP receptor agonist MT-7716 with efficacy in animal models of alcoholism

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We describe a novel nociception orphanin FQ peptide (NOP) receptor agonist, (R)-2-{3-[1-(Acenaphthen-1-yl)piperidin-4-yl]-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl}-N-methylacetamide hydrochloride hydrate (MT-7716), and its *in vivo* activity in preclinical alcoholism models. In the two bottle choice test (choice between 10% alcohol and water), MT-7716 (0.3, 1 and 3 mg/kg, bid) given orally for 14 days dose-dependently decreased voluntary alcohol intake in genetically selected alcohol preferring Marchigian Sardinian (msP) rats. The effect became gradually stronger following repeated administrations, and was still significant at one week after discontinuation of the drug. Oral naltrexone (30 mg/kg, bid) for 14 days also reduced ethanol intake, however the effect was limited to the treatment period. MT-7716 also blocked reinstatement of cue- and yohimbine-induced alcohol seeking behavior in msP rats, suggesting that MT-7716 is effective to treat relapse caused by both ethanol-associated environmental stimuli and stress. To investigate the effect of MT-7716 on alcohol withdrawal symptoms, Wistar rats were withdrawn from alcohol after the treatment with alcohol containing liquid diet for 7 days. MT-7716 significantly attenuated somatic alcohol withdrawal symptoms. Altogether these findings indicate that MT-7716 is promising candidate for alcohol abuse treatment compatible with chronic administration.

S64

**Effects of CRF1 antagonists on overactive anxiety and stress circuit in humans disorders: effects on social anxiety and potential relevance for alcoholism**

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Corticotropin Releasing Factor (CRF) regulates the hypothalamus-pituitary axis and arousal-emotional response associated to stress via activation of CRF1, a G-protein coupled receptors abundantly expressed in pituitary, hypothalamus and other limbic structures, including amygdala. Most of the current understanding of the possible role of CRF in human psychiatric disorders is based on animal studies, showing a robust role in alcohol dependence as well as in chronic anxiety and depression. It is believed that increased endogenous CRF would lead to an excessive engagement of the limbic circuits involved in the control of negative emotion and anxiety. The present study was designed to assess the effects on these limbic circuits of two novel CRF1 antagonists, GW876008 and GSK561679. The study was run in subjects with Social Anxiety Disorder (SAD). The fMRI protocols included an emotional face processing task (EFP) and the Public Exposition of Recorded Performance (PERP) task, followed of a 6-minute resting state fMRI. Thirty-six subjects with a primary diagnosis of SAD and a Liebowitz Social Anxiety Scale (LSAS) score > 50 entered a randomized, double-blind, double-dummy, placebo-controlled, single dose, three-way crossover incomplete block design trial aimed to study the two novel CRF1 antagonists vs. one comparator, alprazolam, and placebo. Results showed that, in EFP, both alprazolam and GSK561679 produced significant attenuation in bilateral amygdala, while GW876008 showed no effect. Similar attenuation was observed at the PERP task only with GSK561679, including a prefrontal deactivation, a feature observed also in resting state fMRI. These results indicate that GSK561679 is predicted a more clinically effective CRF1 antagonist than GW876008, showing normalizing effects on the hyperactive stress and anxiety circuit of SAD subjects. It is tempting to suggest that similar effects would be seen in treatment of alcoholics, either during withdrawal or in preventing relapse induced by stress or cue-associated negative emotions.

S65

**A Glucocorticoid Antagonist Shows Therapeutic Potential for Alcohol Dependence in a POC Human Laboratory Study**

Barbara J. Mason

Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA 92037, USA

A focus for medication development in alcoholism is the post acute withdrawal, protracted abstinence phase. This phase may include stress and alcohol cue-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis and impaired glucocorticoid receptor feedback and activity, which can activate the mesocorticolimbic dopamine system. We hypothesize that the glucocorticoid antagonist, mifepristone, will show POC efficacy in non treatment-seeking outpatient alcoholics by significantly decreasing the number of drinks consumed relative to placebo. Subjects are medically healthy, male or female paid volunteers, 18-65 years of age, meeting DSM-IV criteria for current alcohol dependence and not seeking treatment. Subjects are randomly assigned to double-blind dosing for 1 week with mifepristone 600mg/d or placebo. Human laboratory cue reactivity procedures are conducted on the last day of dosing and salivary cortisol and VAS craving ratings collected. Subjects return after 1-week and 1-month to assess long term drug effects on drinking, cortisol, mood and sleep. Preliminary analyses found mifepristone was associated with a significantly greater reduction in drinking than placebo (p=0.01). Reduction in drinking was predicted by higher mifepristone plasma level, greater change in salivary cortisol levels from baseline to follow-up, and greater suppression of stress and alcohol cue-induced craving in the human laboratory model. A positive signal in this non treatment-seeking, alcohol-dependent sample lends support to the potential utility of mifepristone in the treatment of alcohol dependence. A neurobiological explanation for the results may be related to normalization of the HPA axis/ brain extrahypothalamic stress systems and a subsequent normalization of the mesocorticolimbic dopamine system. Supported by NIAAA grant number R01AA012602.

S66

**A Double-Blind, Placebo Controlled Trial Assessing the Efficacy and Safety of Varenicline Tartrate for Alcohol Dependence**

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Converging lines of data suggest that nicotinic acetylene receptors play a significant role in the rewarding effects of both nicotine and alcohol dependence, indicating a promising molecular target for the treatment of both disorders. Alcohol and tobacco use often occur in tandem with interactions occurring at the pharmacologic, genetic and neurochemical levels. Preclinical studies of varenicline have shown decreases in alcohol consumption in rodent models and human laboratory studies have demonstrated reductions in alcohol consumption and craving in heavy drinking smokers. The present study is the first multisite study designed to assess the efficacy and safety of varenicline, an  $\alpha 4\beta 2$  nicotinic acetylene partial agonist, for the treatment of alcohol dependence. Two hundred smokers and non-smokers meeting criteria for alcohol dependence were recruited across 5 clinical sites. Patients received double blind varenicline (2 mg) or matched placebo and a computerized behavioral intervention. The varenicline group had significantly fewer days of heavy drinking and reduced craving for alcohol. The average treatment effect on alcohol use was similar for smokers and non-smokers. Varenicline was well tolerated by both smokers and non-smokers with few unexpected adverse events. Challenges to further development and approval of varenicline for alcohol dependence will also be discussed.

S67

**An extension of the current treatment paradigm for alcohol dependence: Results from nalmefene phase III trials**

Karl Mann, Antoni Gual, Wim van den Brink

Dept. of Addictive Behavior & Addiction Medicine, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany

Treatment for alcohol depends has been focused on total abstinence using psychotherapeutic and pharmacological interventions. However, many alcohol-dependent individuals are not able or inclined to achieve total abstinence, resulting in a medical condition that is under-diagnosed and under-treated. In Europe, only a small fraction (8.3%) of the people diagnosed with alcohol dependence receives treatment. Nalmefene is an opioid system modulator with antagonist activity at the  $\mu$  and  $\delta$  opioid receptors and partial agonist activity at the  $\kappa$  opioid receptor. Three multi-centre, double-blind, placebo-controlled Phase III studies, designed to assess the efficacy and safety of nalmefene in reducing alcohol consumption, enrolled close to 2000 patients with alcohol dependence. Nalmefene as-needed has been shown to reduce the total amount of alcohol consumption and number of heavy drinking days and to improve liver functions and clinical status in two 6-month studies (identically designed), and one 1-year study in patients with alcohol dependence. The benefit of nalmefene was further studied in the pooled subgroup of patients from the 6-month studies. The patients in this subgroup were consuming alcohol at a high drinking risk level at the initial assessment (screening) and at the start of treatment (baseline). In this subgroup, identified as most likely to benefit, the net treatment effect over placebo in terms of reduction of alcohol consumption was more pronounced.

## Symposium XVII

### STRESS, MEMORY AND ALCOHOL ABUSE DISORDER

Chairs: Segev Barak, Dorit Ron

#### S68

##### **Mammalian target of rapamycin complex 1 (mTORC1), protein translation and alcohol drinking – recent findings**

Dorit Ron

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The serine/threonine kinase, mammalian target of rapamycin in complex 1 (mTORC1) has been implicated in synaptic plasticity, learning and memory. We previously reported that excessive consumption of alcohol activates the mTORC1 pathway in the nucleus accumbens (NAc) of rodents and that inhibition of mTORC1 decreases alcohol-induced locomotor sensitization, place preference, alcohol-seeking and consumption (Neasta et al., 2010). mTORC1 plays an essential role in dendritic protein translation. We therefore hypothesized that the consequence of alcohol-dependent mTORC1 activation is the mRNA to protein translation of synaptic proteins that in turn contribute to plasticity mechanisms underlying alcohol-dependent behaviors. To test this hypothesis, rats with a history of excessive alcohol consumption were sacrificed after the last 30-minute binge drinking session or after 24 hours of withdrawal, the NAc was dissected, and the levels of mRNAs undergoing translation were assessed by RT-PCR in polysomal RNA fractions. We found that both binge drinking and alcohol withdrawal increased the mRNA levels of several synaptic proteins including collapsin response mediator protein-2 (CRMP-2). Alcohol-mediated *CRMP2* mRNA levels were increased in the polysomal but not in the total RNA fraction, suggesting a translational but not transcriptional change. We further showed that the increase in *CRMP2* translation by alcohol is mTORC1-dependent and that it corresponds with an elevation in the levels of the protein in the synaptosomal fraction of the NAc. CRMP-2 participates in mechanisms underlying neurite outgrowth and dendritic assembly by binding tubulin to promote assembly of microtubules. We found that excessive alcohol drinking enhanced the binding of CRMP-2 with tubulin and that microtubule content was higher in the NAc of rats consuming alcohol as compared with rats drinking water only. Finally, systemic administration of lacosamide, which blocks CRMP-2 binding to tubulin and prevents microtubule polymerization, decreased rat binge drinking of alcohol. Together, our results suggest that alcohol-mediated, mTORC1-dependent translation of CRMP-2 in the NAc is a key step in structural plasticity that underlies alcohol-drinking behaviors. Neasta J, Ben Hamida S, Yowell Q, Carnicella S and Ron D. A role for mTOR complex 1 signaling in neuroadaptations underlying alcohol-related disorders. *Proc Natl Acad Sci USA* 107:20093-20098, 2010. This work was supported by NIAAA P50 AA012870 and the State of California.

#### S69

##### **The way stress remaps memory systems in our brain**

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Classically, stress is considered to suppress memory-formation related plasticity in brain areas such as the hippocampus. Indeed, the exposure to stress was found to suppress the formation of long-term potentiation (LTP) in the CA1 area of the hippocampus. Similarly, the basolateral amygdala, which is activated by exposure to stress, was found to suppress LTP in CA1, suggesting that stress and amygdala activation have the same impact on memory processes. However, and in contrast to their impact on CA1, it was found that both stress and amygdala activation enhance LTP formation in the dentate gyrus (DG). This dual effect of stress on memory suggests that it does not either suppresses or enhances memory but rather that stress alters the map of activation that is recruited to form memory. The result should be that the quality or characteristics of the memory formed under stress are different from that formed under less stressful conditions. Furthermore, because stress was found to induce plasticity in the amygdala it is conceivable that the way the amygdala modulates memory processes in the hippocampus would also be modified. In support of that we found that altering the pattern of amygdala activation modified its impact on LTP but

only in the DG and not CA1. This differential effect indicates that following exposure to stress the DG assumes a more pivotal role than the CA1 in defining the outcome with respect to memory formation.

#### S70

##### **Erasure of alcohol-associated memories by mTORC1 inhibition prevents relapse**

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Relapse to alcohol abuse is often caused by craving due to retrieval of alcohol-associated memories. Memory reconsolidation is a process in which memories are temporarily destabilized upon their retrieval (reactivation), and then undergo re-stabilization in order to persist. During this process, the reactivated memory becomes labile to manipulations, enabling interference with unwanted memories. Activation of the kinase mammalian target of rapamycin complex 1 (mTORC1) is required for synaptic proteins translation, and is implicated in synaptic plasticity, learning and memory. Here, we tested whether reconsolidation of alcohol-related memories upon their retrieval activates the mTORC1 pathway, and whether mTORC1 inhibition disrupts the memories, therefore preventing relapse. Reactivation of alcohol-related memories increased the phosphorylation of the mTORC1 substrates 4E-BP and S6 kinase, and the S6K substrate S6, in the prefrontal cortex and amygdala, indicating activation of the mTORC1 pathway. To test whether mTORC1 inhibition during memory reconsolidation interferes with subsequent alcohol seeking, we administered the mTORC1 inhibitor rapamycin (20mg/kg, i.p., or 50ug/side, intra-amygdala) immediately after memory reactivation, and observed reduced alcohol seeking and intake 24 hrs and even 2 weeks later. Importantly, alcohol seeking was not affected by rapamycin when the memory reactivation session was omitted, or when rapamycin was administered 5 hrs after memory reactivation, suggesting that memory retrieval initiates a time-limited 'reconsolidation window', during which memories are labile. Our findings suggest that reactivation of alcohol-associated memories activates the mTORC1 pathway in specific brain regions, and that inhibition of this pathway can be used to disrupt unwanted memories and reduce relapse. *Supported by NIH/NIAAA Grant P50 AA017072 (DR, PHJ) and funds from the State of California through the UCSF (DR, PHJ).*

#### S71

##### **microRNA-206 in rat medial prefrontal cortex regulates BDNF expression and alcohol drinking**

Heilig, M., Jenica D. Tapocik, Estelle Barbier, Meghan Flanigan, Matthew Solomon, Ali Pincus, Andrew Pilling, Hui Sun, Jesse R. Schank, Courtney King, Laboratory of Clinical and Translational Studies, NIAAA, MD USA; Chemical Biology Branch, NIDA, MD USA

Escalation of voluntary alcohol consumption is a hallmark of alcoholism, but its neural substrates remain unknown. In rats, escalation occurs following prolonged exposure to cycles of alcohol intoxication, and is associated with persistent, wide-ranging changes in gene expression within the medial prefrontal cortex (mPFC). Here, we examined whether induction of microRNA (miR) 206 in mPFC contributes to escalated alcohol consumption. Following up on a microarray screen, qPCR confirmed that a history of dependence results in persistent (over 3-weeks) up-regulation of miR-206 expression in the mPFC, but not in the ventral tegmental area (VTA), amygdala (AMG), or nucleus accumbens (NAc). Viral-mediated overexpression of miR-206 in the mPFC of non-dependent rats reproduced the escalation of alcohol self administration seen following a history of dependence and significantly inhibited brain derived neurotrophic factor (BDNF) expression. Bioinformatic analysis identified 3 conserved target sites for miR-206 in the 3'UTR of the rat BDNF transcript. Accordingly, BDNF was downregulated in post-dependent rats on microarray analysis, and this was confirmed by qPCR. In vitro, BDNF expression was repressed by miR-206 but not miR-9 in a 3'UTR reporter assay, confirming BDNF as a functional target of miR-206. Mutation analysis showed that repression was dependent on the presence of all three miR-206 target sites in the BDNF



3'UTR. Inhibition of miR-206 expression in differentiated rat cortical primary neurons significantly increased secreted levels of BDNF. In conclusion, recruitment of miR-206 in the mPFC contributes to escalated alcohol consumption following a history of dependence, with BDNF as a possible mediator of its action.

#### Symposium XVII

#### BRAIN REWARD AND STRESS SYSTEMS IN EXCESSIVE ALCOHOL DRINKING

Chair: Nicholas W. Gilpin

Discussant: Adolf Pfefferbaum

#### S72

#### Lateral hypothalamus is critical for context-induced relapse to alcohol seeking after punishment

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Alcoholics typically abstain because of negative consequences associated with excessive drinking and relapse is often triggered by exposure to contexts previously associated with alcohol use. We recently developed a rat model that captures some characteristics of this human condition: exposure to the alcohol self-administration environment (context A) after punishment-induced suppression of alcohol seeking in a different environment (context B) provoked relapse to alcohol seeking in alcohol-preferring P-rats. The mechanisms of context-induced relapse after punishment are unknown. Here, we studied the role of lateral hypothalamus (LH) and its forebrain projections in this relapse. We first assessed the effect of reversible inactivation of LH by GABA<sub>A</sub>+GABA<sub>B</sub> receptor agonists (muscimol+baclofen) on context-induced relapse to alcohol seeking. We then assessed LH-projection-specific activation during context-induced relapse by measuring in LH projections areas double-labeling of the retrograde tracer cholera toxin subunit B (CTb, injected into LH) with the neuronal activity marker Fos. Context-induced relapse after suppression of alcohol seeking by punishment was blocked by muscimol+baclofen injections into LH. In contrast, muscimol+baclofen injections dorsal to LH were ineffective; additionally, LH inactivation had no effect on alcohol self-administration, demonstrating anatomical and behavioral specificity. Double-labeling analysis of Fos+CTb demonstrated that context-induced relapse was associated with selective activation of accumbens shell and potentially ventral BNST but not medial prefrontal cortex, lateral septum or dorsal BNST neurons projecting to LH. Results demonstrate a critical role of LH in context-induced relapse to alcohol seeking after punishment and suggest a role of accumbens shell and ventral BNST projections to LH in this relapse.

#### S73

#### The role of midbrain dopamine neurons in controlling variable alcohol drinking behaviors

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Individuals within a population display variability in alcohol drinking behaviors: some people consume alcohol in a controlled manner while others are susceptible to engage in excessive and pathological alcohol consumption. To understand these differences, we established an alcohol drinking model that parses out low and high alcohol drinking mice within the genetically identical mouse strain C57BL/6J. This mouse model provides us with a unique opportunity to investigate neurophysiological mechanisms without confound of diverse genetic backgrounds. To examine the possible role of ventral tegmental area (VTA) dopamine (DA) neurons in mediating the low and high alcohol drinking behaviors, we performed *in vivo* electrophysiological recordings. Surprisingly, we found that high alcohol drinking mice maintain *in vivo* VTA DA firing properties similar to ethanol-

naïve mice, yet the VTA DA neurons of low alcohol drinking mice display higher firing rates and bursting. Optogenetically mimicking this increase in DA neuron activity in previously high alcohol drinking mice reduced their alcohol drinking. We further identified interesting adaptive changes in two ion currents known to modulate DA neuron firing:  $I_h$  and  $K^+$  currents. Low alcohol drinking mice have reduced  $K^+$  currents, while high alcohol drinkers maintained their “normal” firing by a significantly reduced  $I_h$  current, a phenomenon that is absent in low alcohol drinkers and ethanol-naïve mice. These data demonstrate that VTA DA neurons play a key role in controlling individual alcohol drinking behaviors, and suggest that  $I_h$  tolerance in the brain’s reward circuit may drive susceptible individuals to drink more to obtain initial alcohol effects.

#### S74

#### Individual differences in stress-induced behavioral dysregulation mediated by corticotropin-releasing factor (CRF) in central amygdala (CeA)

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Only some humans exposed to traumatic stress develop a traumatic stress disorder (e.g., PTSD), and these individuals exhibit co-morbid long-term increases in alcohol drinking and pain processing. Our lab has developed a predator odor stress model in which animals that exhibit high traumatic stress reactivity also exhibit persistent increases in alcohol drinking and hyperalgesia. The overarching hypothesis of these studies was that stress produces hyperfunctional corticotropin-releasing factor (CRF) signaling in CeA that mediates stress-induced behavioral dysregulation in some but not all stressed animals. We utilized a predator odor stress model to examine stress effects on *ex vivo* CRF-positive cell counts and CRF protein levels in CeA, and systemic and intra-CeA pharmacology to determine the role of CRF1Rs in mediating stress-induced behavioral changes. Stressed rats were divided into two groups based on avoidance of predator odor-paired context: Avoiders and Non-Avoiders. Avoiders exhibit increased CRF protein expression in CeA 3 weeks following predator odor stress. Predator odor stress increases alcohol drinking, and this effect is reversed by systemic CRF1R antagonism. Predator odor stress produces thermal hyperalgesia in Avoiders, this effect is mimicked by intra-CeA CRF infusion, and both of these effects are CRF1R-dependent. Systemic antagonism of CRF1Rs reduces stress-induced thermal hyperalgesia in Avoiders, and this effect is dependent on an intact and functional CeA. Collectively, our results suggest that individual differences in stress-induced behavioral dysregulation is mediated by neuroadaptations in CRF-CRF1R systems in CeA of rats that exhibit high stress reactivity. This work was supported by NIH grant AA018400.

#### S75

#### Mechanisms of stress-enhanced fear learning in a rat model of post-traumatic stress disorder

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Post-traumatic stress disorder (PTSD) patients exhibit high rates of alcohol abuse. In rats stress with 15-shocks enhances conditioning to 1-shock in a novel context. This stress-enhanced fear learning (SEFL) mimics several PTSD features, including resistance to exposure therapy and amnesic treatment. We previously showed that SEFL produces long-term increases in voluntary alcohol intake without affecting sucrose/quinine consumption. Here we assessed the involvement of basolateral amygdala (BLA) in SEFL and examined the role of corticosterone (CORT) in SEFL-induced biochemical changes within the BLA. First, selective bilateral inactivation of the BLA with muscimol before but not after stress eliminated SEFL. Next, the CORT synthesis inhibitor metyrapone administered pre- but not post-stressor decreased the CORT response to the stressor and blocked SEFL, but

CORT levels were not significantly changed during the 1-shock-context test 6 days later. Pre-stress CORT injections rescued the freezing response from metyrapone; however, CORT without stress did not produce SEFL. Western blots of BLA tissue revealed increases in the glutamate  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor 1 (GluA1) subunit in SEFL versus control rats; the increases were prevented by metyrapone pretreatment. Similarly, the  $\gamma$ -aminobutyric acid (A) receptor (GABA<sub>A</sub>R)  $\alpha$ 3 subunit was increased in SEFL rats and prevented by metyrapone pretreatment. These data indicate CORT, as well as the BLA, are necessary for SEFL initiation, which leads to increased expression of BLA GluA1 and GABA<sub>A</sub>R  $\alpha$ 3 subunits. Further molecular studies should help define the BLA mechanisms behind the high alcohol intake of SEFL rats. Support: NIH grants MH088184 (MSF), AA021037 (EMM), AA016100, AA022408 (IS).



## POSTERS

## P76

**Role of IL-1 Receptor Antagonist in Ethanol-Induced Regulation of the GABAergic Transmission in the Central Amygdala**

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IL-1 receptor antagonist, encoded by the IL-1rn gene, is an endogenous antagonist of IL-1 receptor. Behavioral studies showed a reduction in ethanol drinking in IL-1rn knockout (KO) mice compared to wild type (WT) mice. In this study, we examined the effects of ethanol at the GABAergic synapses in central nucleus of the amygdala (CeA) of KO and WT mice. We found that the baseline frequency of spontaneous IPSCs (sIPSCs), but not miniature IPSCs (mIPSCs), was significantly increased in KO mice compared to WT mice, indicating increased presynaptic action-potential dependent GABA release in the CeA of KO mice. No difference in the baseline amplitude, rise and decay time of both sIPSCs and mIPSCs were observed between KO and WT mice. Acute ethanol (44 mM) increased the frequency of sIPSCs and mIPSCs in WT mice, but it did not induce potentiation of either mIPSC nor sIPSC frequency in the KO mice. Ethanol increased the rise and decay of CeA sIPSCs in both KO and WT mice, suggesting also postsynaptic effects. The pre-treatment of CeA slices with IL-1 receptor antagonist (Kineret; 100 ng/ml) reversed the increased sIPSCs frequency in KO mice without altering the frequency in the WT mice. Importantly, Kineret restored ethanol-induced potentiation of the sIPSC frequency in the KO mice. These results suggest that the IL-1 receptor signaling is involved in a regulation of the baseline GABAergic transmission in the CeA and plays a critical role in the EtOH effects at these synapses. Supported by NIH/NIAAA INIA West Consortium U01-AA013498 and AA013517.

## P77

**How do fenofibrate and tesaglitazar (agonists of peroxisome proliferator-activated receptors) reduce ethanol intake and preference in C57BL/6 mice?**

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We proposed that a normally functioning immune system limits alcohol drinking (Harris and Blednov, 2012), whereas overactivation of neuroimmune signaling may promote excessive alcohol consumption. The ligands of peroxisome proliferator-activated receptors (PPARs) inhibit the activation of inflammatory gene expression and proinflammatory transcription factor signaling pathways. Recently, we demonstrated that fenofibrate (FENO, agonist of PPAR $\alpha$ ) and tesaglitazar (TESA, dual agonist of PPAR $\alpha$ , $\gamma$ ) significantly reduced ethanol intake and preference in C57BL/6 mice (Blednov et al., RSA 2013). To understand the behavioral nature of this effect, we studied conditioned taste aversion (CTA) and acute ethanol withdrawal (AWT), which normally negatively correlate with ethanol consumption, in C57BL/6 mice after 8 days of pre-treatment with FENO (150 mg/kg daily) or TESA (1.5 mg/kg daily). FENO significantly increased the development of CTA to a low, normally inactive dose of ethanol (2.0 g/kg), whereas TESA significantly reduced saccharin consumption independently when paired with administration of ethanol. Both drugs significantly increased the severity of AWT and reduced ethanol's protective properties on handling-induced convulsions. Such protection is normally positively correlated with the sedative properties of ethanol and negatively correlated with the severity of AWT (Blednov et al., 2012). Indeed, pre-treatment with both FENO and TESA significantly reduced the duration of ethanol-induced loss of righting reflex. Overall, these results demonstrate that activation of PPAR ( $\alpha$  or  $\alpha$ ,  $\gamma$ ) increases the negative properties of ethanol, thereby reducing alcohol intake in mice. Supported by NIH/NIAAA INIA Consortium (AA U01 13520 - INIA Project; AA06399).

## P78

**Activation of PPAR $\gamma$  receptors with natural agents reduces alcohol drinking and stress-induced relapse in Marchigian-Sardinian alcohol-preferring (msP) rats**

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*Andrographis paniculata* (*A. paniculata*, fam. Acanthaceae) is a herbaceous plant commonly used in Siddha, Ayurvedic and tribal medicines for multiple clinical applications. Andrographolide, the main active ingredient of the plant, is thought to exert its pharmacological properties through a PPAR $\gamma$ -mediated mechanism. As recently shown in our laboratory, PPAR $\gamma$  activation by pioglitazone potently and reliably reduced alcohol drinking, stress-induced relapse and withdrawal. Here we examined whether alcohol intake and seeking were also attenuated by treatment with the natural agents *A. paniculata* and andrographolide in genetically selected alcohol preferring msP rats. The two-bottle free choice paradigm was used to assess voluntary alcohol drinking while yohimbine-stress was used to evoke alcohol seeking in animals subjected to a self-administration/extinction paradigm. Subchronic treatment with *A. paniculata* (0, 15, 150 and 450 mg/kg) significantly reduced voluntary alcohol intake. The extract (0, 150 and 450 mg/kg) also abolished the reinstatement of previously extinguished alcohol-paired responses when elicited by the pharmacological stressor yohimbine (1.25 mg/kg, i.p.) but failed to alter cue-induced reinstatement of alcohol seeking. Reduced alcohol consumption was also observed with andrographolide (0, 5, 10 mg/kg). Notably, the effect of andrographolide (10 mg/kg) was blocked by intracerebroventricular pretreatment with the selective PPAR $\gamma$  antagonist GW9662 (5  $\mu$ g/rat), thus suggesting a central PPAR $\gamma$  receptor-mediated mechanism of action. In conclusion, here we demonstrate the efficacy of natural PPAR $\gamma$  agonists in decreasing both alcohol intake and stress-induced relapse-like behaviors. These results further support the evidence that PPAR $\gamma$  may serve as a potential target for treatment of alcoholism.

## P79

**Acute Ethanol Administration Increases Cerebrocortical And Hippocampal Corticosterone Levels But Does Not Alter Allopregnanolone Levels In C57BL/6J And DBA/2J Mice**

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The ethanol-induced increase in brain allopregnanolone levels in rats contributes to sensitivity to ethanol's behavioral effects. However, ethanol's effects on allopregnanolone may differ across species. We investigated the effects of acute ethanol administration on allopregnanolone, progesterone and corticosterone levels in cerebral cortex and hippocampus of C57BL/6J and DBA/2J mice, two inbred strains with different alcohol sensitivity. Male C57BL/6J and DBA/2J mice received ethanol (1, 2, 3 or 4 g/kg, i.p.) or saline and were euthanized 1 hour later. For the time-course study, ethanol (2 g/kg) was administered 15, 30, 60 and 120 minutes before euthanasia. Steroids were measured by radioimmunoassay. Acute ethanol administration did not alter cerebrocortical and hippocampal levels of allopregnanolone and progesterone in these strains at any of the doses and time points examined. Acute ethanol dose-dependently increased cerebrocortical corticosterone levels by 319%, 347% and 459% in C57BL/6J mice at 2, 3 and 4 g/kg, and by 371%, 507%, 533% and 692% in DBA/2J mice at 1, 2, 3 and 4 g/kg, respectively. Similar changes were observed in the hippocampus. Ethanol's effects on cerebrocortical corticosterone levels were also time-dependent in both strains. Morphine administration (5 mg/kg) increased cerebrocortical allopregnanolone levels in C57BL/6J (+77%) and DBA/2J mice (+81%), suggesting that the impairment in brain neurosteroidogenesis may be specific to ethanol. These results underline important species differences on ethanol-induced brain neurosteroidogenesis. Acute ethanol increases corticosterone levels but does not alter allopregnanolone and progesterone concentrations in cerebral cortex and hippocampus of C57BL/6J and DBA/2J mice.

**P80****Further Characterization of Binge-like Drinking in Sardinian Alcohol-Preferring Rats Exposed to an Unpredictable Schedule of Limited Access to Multiple Alcohol Concentrations**

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This paper represents an update - with respect to the previous Volterra meeting - on the set up, characterization, and validation of a novel and unique experimental procedure of alcohol drinking capable of promoting exceptionally high intakes of alcohol in Sardinian alcohol-preferring (sP) rats (one of the few rat lines selectively bred worldwide for excessive alcohol consumption). sP rats were exposed to the 4-bottle “alcohol (10%, 20%, and 30%, v/v) vs water” choice regimen during one of the 12 hours of the dark phase of the daily light/dark cycle; the time of alcohol exposure was changed daily under a *semi*-random order and was unpredictable to rats. Alcohol intake was found to be highly positively correlated ( $r=0.984$ ,  $P<0.0001$ ) with the time of alcohol exposure and ranged from an average of approximately 0.7 g/kg (drinking session occurring during the 1<sup>st</sup> hour of the dark phase) to an average of approximately 2.2 g/kg (drinking session occurring during the 12<sup>th</sup> hour of the dark phase). Alcohol drinking during the 12<sup>th</sup> hour of the dark phase resulted in (a) blood alcohol levels averaging approximately 100 mg% and (b) severe signs of alcohol intoxication (e.g., markedly impaired performance at a Rota-Rod task). These results demonstrate that unpredictable, limited access to multiple alcohol concentrations may result in exceptionally high intakes of alcohol in sP rats. A progressively increasing emotional “distress” associated to the rats’ expectation of alcohol might be the neurobiological basis of this behavior. Supported by NIAAA-funded “Integrative Neuroscience Initiative on Alcoholism” (INIA-Stress) Consortium.

**P81****The economics of protecting children against early environmental stress: The costs and benefits of preventing alcohol abuse**

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The societal burden of alcoholism is staggering—with the US cost of alcohol abuse alone totaling over \$223 Billion annually (Sacks et al., 2013). A substantial body of research has documented how early environmental stress contributes to the development of alcohol abuse (Enoch, 2011; Hawkins, 1992). Building on this work, researchers have developed strategies that can successfully reduce the effects of such stressors and prevent future abuse (Kumpfer & Rose, 2003). Despite these promising findings, little work has examined the economics of protecting children against early risk factors linked to alcohol abuse (e.g., family conflict). To better understand the costs and benefits of prevention, we evaluated four evidence-based preventive strategies for reducing alcoholism within a NIAAA funded randomized-controlled-trial (N=12,346; Spoth et al., 2004). In 6<sup>th</sup> grade ( $M=11$  years old), participants in the intervention group received a family-based program that targeted risk factors linked to future alcohol abuse. Participants were then followed through the end of high school. Family program participants were 5% less likely to begin using alcohol before graduation. This program cost between \$278-\$348 USD per family and translates into a cost of between \$5,560-6,960 USD to prevent a child from ever drinking alcohol before the end of high school costs (*unpublished*). This work illustrates how an investment in reducing environmental stress in childhood can effectively prevent alcohol abuse. Other alcohol-related outcomes, subgroup analyses, and policy implication will be discussed.

**P82****Kappa opioid receptors modulate basolateral amygdala projections to the bed nucleus of the stria terminalis**

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The BNST plays a key role in regulation of stress and alcohol related behavior. The relationship between the dynorphin-KOR system and BNST output and related behaviors is unknown. We used a combination of electrophysiological and optogenetic techniques to probe the ability of the dynorphin-KOR system to modulate circuit function. In addition, we assessed the impact of chronic intermittent ethanol (CIE) exposure on this system. These findings point towards KOR signaling in the BNST as an important neuronal locus for alcohol induced and stress induced anxiety like behavior. Using patch clamp electrophysiology, we found that KOR activation inhibited glutamate inputs from the BLA, but not the PFC. We then assessed KOR effects on miniature synaptic transmission, and demonstrated a presynaptic locus of action. Additionally, we found that deletion of KOR receptors from BLA neurons prevent KOR mediated inhibition of glutamate transmission in the BNST. Next, KORs were found to signal via a p38 map kinase-dependent, calcium-dependent mechanism. We assessed how chronic intermittent alcohol exposure altered this system. One week of CIE exposure differentially alters KOR modulation of glutamatergic and GABAergic signaling in the BNST. Ongoing studies are probing the nature of these changes and their relationship to KOR-dependent changes in anxiety-like behavior seen following alcohol exposure. These results suggest that KORs inhibit glutamate transmission in the BNST. In addition, KORs are activated in part by local dynorphin release, and this KOR activation modulates pathway-specific inputs into the BNST. This circuit functions to modulate anxiety related and alcohol related behaviors *in vivo*.

**P83****Genomic signature of PPAR agonists in mouse amygdala: Role in alcohol consumption**

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PPARs are nuclear hormone receptors that act as ligand-activated transcription factors. PPAR agonists are used to treat hyperlipidemia and diabetes, and are neuroprotective, hepatoprotective and decrease nicotine and alcohol self-administration. We used an unbiased genomic screen to characterize the gene expression profile induced in the mouse amygdala, a key brain region for alcohol dependence, by two PPAR agonists that are effective (fenofibrate and tesaglitazar) and one that is non-effective (bezafibrate) at reducing alcohol consumption. Differential expression analysis found 549 genes commonly regulated by the two effective treatments. We used the non-effective treatment to filter out non-related genes, leaving 424 potentially important targets. We studied the transcriptome using weighted gene co-expression network analysis and identified the groups of genes (modules) that were treatment-responsive. Neuropeptides that have established roles in both stress response and alcohol-drinking behavior (e.g. Penk1, NPY, Tac1 and AVP) are coordinately expressed in the amygdala and are responsive to only the effective treatments. We further assessed the biological pathways, ontologies and cell-types overrepresented in the differentially expressed genes and treatment responsive modules. Results indicate that PPAR agonists have a strong neuronal signature in the amygdala and that fenofibrate and tesaglitazar (the effective treatments) target a subset of GABAergic interneurons in the amygdala that bezafibrate does not. These results demonstrate that changes in alcohol drinking in mice after receiving PPAR agonists are accompanied by changes in gene expression in the amygdala, a critical step in bringing these drugs into therapeutic use for alcohol dependence. Supported by NIH/NIAAA.

**P84****Cell type-specific tonic GABA currents and ethanol sensitivity of central amygdala neurons in naïve and ethanol-dependent rats**

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We have previously shown a cell-type specific tonic GABA<sub>A</sub> receptor signaling and differential ethanol sensitivity in the central amygdala (CeA) of a CRF receptor-1 reporter mouse model. However, the presence of tonic

GABA<sub>A</sub> receptor signaling in the rat CeA and the changes in that tonic signaling with chronic ethanol exposure remain understudied. In the present study we utilized whole cell voltage- and current-clamp recordings in CeA neurons from naïve male Sprague Dawley rats and rats exposed to 5 weeks of chronic intermittent ethanol vapor (CIE). We found that in naïve rats, low threshold bursting (LTB) and a subset of regular spiking (RS) CeA neurons have an ongoing tonic conductance that is mediated by the  $\alpha 1$  GABA<sub>A</sub> receptor subunit and is insensitive to acute ethanol. Late spiking (LS) and a separate population of RS neurons do not display an ongoing tonic conductance but have the potential for tonic signaling that is mediated by the  $\delta$  GABA<sub>A</sub> receptor subunit and can be activated by increased ambient GABA concentration or acute ethanol. In rats exposed to CIE, we found that the tonic conductance displayed by LTB neurons was lost and that LS neurons displayed a significant ongoing tonic conductance. Collectively, these data demonstrate cell type-specific tonic signaling in the rat CeA and provide new insight into how acute and chronic ethanol differentially alters specific aspects of CeA circuitry. This work was supported by NIAAA grants F32AA020430, AA06420, and AA015566.

#### P85

##### **Deconstructing the role of amygdalar neurotensin neurons in motivated behaviors**

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The extended amygdala is well known to modulate fear, anxiety and behaviors related to alcohol dependence. It is not well understood, however, how discrete genetically defined populations of neurons within this region contribute to the expression of these behaviors. Neurotensin, a 13 amino acid residue neuropeptide, has been implicated in mediating reinforcing behavior, and the neurotensin system is implicated in alcohol dependence in both rodent and human studies. Despite the potentially critical role of this peptide, little is known about the neurons in the extended amygdala that express this peptide nor how alcohol alters their function. We evaluated the function and connectivity of neurotensin neurons in the extended amygdala using cre-dependent optogenetic viral targeting strategy in transgenic mice expressing cre driven by the neurotensin promoter. Consistent with the neurotensin mRNA expression in the Allen Mouse Brain Atlas, we found that a discrete population of neurons in the extended amygdala expressed channel rhodopsin coupled to eYFP. Using a real-time place preference paradigm, we observed that mice prefer to spend time on the side of a chamber where these neurotensin neurons are being stimulated. Ongoing experiments are probing the cellular phenotype of these neurons, investigating their projections and observing how alcohol modulates their physiology.

#### P86

##### **The Neuropeptide S differently modulates alcohol seeking in msP and Wistar rats**

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Anxiety, stress, environmental factors and individual predisposition to abuse contribute to the maintenance of alcohol intake and relapse to alcohol seeking in alcoholic patients. We previously demonstrated that the Neuropeptide S (NPS), a neuropeptide characterized by a unique pro-arousal/anxiolytic pharmacology, exacerbates cue-induced reinstatement of alcohol seeking leaving unaltered alcohol self-administration in Wistar rats. The intriguing pharmacological properties of NPS stimulated us to extend its investigation in genetically selected alcohol preferring Marchigian Sardinian (msP) rats, an animal model in which excessive alcohol drinking is associated with high anxiety and sensitivity to stress. In this study we examined the effect of intracerebroventricular (ICV) injection of NPS (0.1-2.0 nmol/ICV) on alcohol self-administration, cue-induced reinstatement of

alcohol seeking and NPS induced priming under extinction condition in msP and Wistar rats. Consistent with our previous work in Wistar rats NPS did not affect alcohol self-administration but exacerbated cue-induced reinstatement of alcohol seeking. Moreover, under extinction condition a priming injection of NPS enhanced responding at the alcohol-paired lever. The effect of NPS in msP rats was completely different; ICV peptide injection decreased alcohol self-administration but left unaltered cue-induced reinstatement. These findings suggested two major conclusions: First msP rats might be characterized by a different neuronal or functional organization of the NPS system that may possibly contribute to their excessive drinking and/or anxious-like phenotype. Second, we speculate that in msP rats, alcohol drinking reduction following NPS injection results from the anxiolytic activity of the peptide, which in these animals may attenuate the need to self-medicate with alcohol.

#### P87

##### **Role of protein phosphatase 2A in the development of ethanol relapse**

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Relapse is one of the most important problems of alcoholism. We previously found that the increase of histone deacetylase 5 (HDAC5) in ethanol relapse. Several studies have shown that protein phosphatase 2A (PP2A) may regulate the HDAC5 nuclear shuttling. In the present study, we investigated the expression of PP2A in the mouse ethanol relapse model. Using the escalating ethanol dosage schedule, the mice were fed the ethanol diet as follows: 1st day: 1 w/v%; 2nd and 3rd day: 3 w/v% and from the 4th to 10th day: 4 w/v% ethanol diet, respectively. The control mice were given the same volume of ethanol-free liquid diet with sucrose substituted in isocaloric quantities for ethanol. The mice chronically treated with ethanol revealed severe withdrawal signs. Ten days after withdrawal, we performed a conditioned place preference to evaluate ethanol relapse. At the dose of 0.5 g/kg of ethanol, which produced neither preference nor aversion in control group, the alcohol group showed significant rewarding effects. PP2A was increased in limbic forebrain (containing nucleus accumbens) at ethanol relapse state. Moreover, intracerebroventricular treatment of the PP2A inhibitor cantharidin acid during ethanol withdrawal significantly suppressed the ethanol-induced rewarding effect. Our findings suggest that the increase in PP2A caused the dephosphorylation of HDAC5, resulting in the increase of HDAC5 nuclear transport in ethanol relapse. This research was supported by The Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Young Scientists (B) No. 25860394.

#### P88

##### **Differences in stress axis response to methamphetamine may play a role in genetic risk for methamphetamine intake**

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Lines of mice selectively bred for extreme differences in methamphetamine (MA) intake also exhibit extreme differences in sensitivity to rewarding, reinforcing and aversive effects of MA. MA High Drinking (MAHDR) mice are sensitive to rewarding and reinforcing, but not aversive, effects of MA, whereas MA Low Drinking (MALDR) mice show the opposite sensitivities. High sensitivity to aversive drug effects likely plays a protective role in continued drug use. Similar to other drugs of abuse, including alcohol, MA activates the stress axis. We hypothesized that level of activation plays a role in the aversive effect of MA and that MALDR mice would show greater MA-induced activation, compared to MAHDR mice. Mice were injected (IP) with saline, 1, 2, or 4 mg/kg MA and blood was taken from separate groups at 5, 10, 15, 30, 60 and 120 min for CORT and 5, 10, 15 and 30 min for ACTH. Hormone levels were assayed by radioimmunoassay. The dependent variable was change in hormone level in MA-injected mice from that of saline-injected mice. In MALDR mice, these doses of MA increased CORT to a similar degree, with peak levels occurring at 60 min post-injection. No significant MA-induced changes in CORT were found in MAHDR mice. ACTH levels were also elevated in MALDR mice after MA injection, with

peak levels occurring at 10 to 15 min post-injection; these MA doses had similar effects. There was no significant elevation of ACTH levels in MAHDR mice. Level of stress axis activation by MA may play a role in risk for MA use. Supported by the Department of Veterans Affairs, and NIH NIDA grants T32 DA07262 and P50 DA018165.

#### P89

##### **The Serotonin System in the Nucleus Accumbens Shell Regulates EtOH-Seeking: Involvement of 5HT7 Receptors and Response to Conditioned Cues**

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Conditioned drug cues contribute significantly to drug craving and increase the likelihood that an individual will relapse. Recent data from our laboratory show that odor cues that signal access to or the absence of alcohol differentially alter cFos expression and DA levels within the nucleus accumbens shell (AcbSh), a neurological substrate for drug reward. There is evidence that 5-HT7 receptors in the AcbSh are involved in the regulation of self-control/behavioral inhibition as microinjections of a 5-HT7 receptor agonist increased and microinjections of an antagonist decreased behavioral self-control. The first series of experiments examined the effects of 5-HT7 receptor agents on context-induced EtOH-seeking (spontaneous recovery). The 5-HT7 receptor antagonist SB269970 was microinjected (0, 0.1, 1, and 10  $\mu$ M/side) into the AcbSh or the nucleus accumbens core (AcbC) prior to EtOH-seeking testing. In additional subjects, the 5-HT7 receptor agonist LP-12 was microinjected (0, 25, and 100  $\mu$ M/side) into the AcbSh prior to EtOH-seeking testing. To determine the effects of conditioned cues on serotonin levels within the AcbSh was conducted using excitatory, inhibitory, and neutral odor cues. For 10 consecutive daily sessions, alcohol-preferring (P) rats self-administer 15% EtOH or water in the presence of an olfactory cue (CS+). On days 71-77, P rats were given daily operant sessions with EtOH and water unavailable in the presence of a 2nd odor cue (CS-). From days 58-77, all rats were exposed to a 3rd odor in a non-drug paired environment for 1 hr (exposure occurred at least 2 hours separated from operant testing). Rats were implanted with guide cannulae aimed at the AcbSh on day 84. Microdialysis was performed on day 91 in a non drug-paired environment (animals were habituated to this environment). Standard microdialysis procedures were used; samples were collected every 8 min, and rats were exposed to the CS+, CS-, or CS0 for a total of 24 minutes. Microinjection of the 5-HT7 receptor antagonist SB269970 into the AcbSh, but not AcbC, enhanced EtOH-seeking in the P rat. In contrast, microinjection of the 5-HT7 receptor agonist LP-12 into the AcbSh blocked the expression of EtOH-seeking. Exposure to the CS+ produced a rapid, pronounced reduction in 5HT levels in the AcbSh for the 24 min period of odor exposure (55-64% reduction for all 3 samples taken during odor exposure). The data indicate that altering the activity of the 5-HT7 receptor within the AcbSh has a bi-directional effect on EtOH-seeking in P rats. The data also indicate that the neurochemical effects of presenting a CS+ (reduction in 5HT levels in the AcbSh) are complementary to the pharmacological data. Thus, the inhibition of 5-HT release within the AcbSh may represent an underlying neurological mechanism contributing to both EtOH-seeking and conditioned cue enhancement of EtOH-seeking.

#### P90

##### **Inhibition of IKK $\beta$ reduces ethanol consumption in C57BL/6J mice**

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We hypothesized that inhibiting the TLR4/NF- $\kappa$ B (toll-like receptor 4/nuclear factor kappa B) pathway will decrease voluntary alcohol consumption based on our previous findings that over-activation of neuroimmune signaling promotes excessive alcohol consumption. We tested this by selectively regulating inhibitory kappa B kinase beta (IKK $\beta$ ), a key regulator of the nuclear factor kappa B cascade. First, we studied the effect of a systemically administered small molecule inhibitor of IKK $\beta$ , 2-[(aminocarbonyl) amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide

(TPCA-1), on alcohol intake using 24 hour two-bottle choice (2BC) and 2BC with limited access to alcohol (2BC-DID) tests in C57BL/6J mice. In both tests, the inhibitor reduced alcohol intake and preference within 6 hours post-administration. Secondly, we knocked down IKK $\beta$  in either the nucleus accumbens (NAc) or the central amygdala (CeA) of mice using lentiviral-mediated Cre-Lox recombination. Knockdown of IKK $\beta$  post viral transduction was observed to be 40% after 3 weeks and 90% after 8 weeks. Four weeks post viral injection, local deletion of IKK $\beta$  in either the NAc or the CeA reduced alcohol intake and preference in the 2BC test by greater than 40% at higher ethanol concentrations (i.e., 12, 14, 16%). Taken together, these results demonstrate that blockage/knockdown of IKK $\beta$  decreases voluntary alcohol consumption, indicating that IKK $\beta$  is a potential therapeutic target for regulating alcohol intake. Research supported by NIH/NIAAA and the INIA Consortium (AA020683; AA13520; AA06399).

#### P91

##### **L- and P/Q-type calcium channels mediate ethanol enhancement of GABA release in rat central nucleus of the amygdala**

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GABA transmission in the central nucleus of the amygdala (CeA) is a crucial mediator of the effects of acute and chronic alcohol consumption, and we have previously found that ethanol (44 mM) increases both action potential-dependent and -independent GABA release. GABA release is controlled by calcium influx through voltage-gated calcium channels (VGCCs; L-, P/Q- and N-type calcium channels expressed on cell bodies and presynaptic terminals). To investigate the role of VGCCs in ethanol-induced GABA release, we performed whole-cell voltage clamp electrophysiology in the CeA of Sprague Dawley rats to record spontaneous and miniature inhibitory postsynaptic currents (s/mIPSCs). Nifedipine (10  $\mu$ M), an L-type calcium channel blocker, abolished ethanol enhancement of action potential-dependent GABA release, as measured by sIPSC frequency. As L-type calcium channel activity can trigger calcium-induced calcium release (CICR), we used 2-APB (42  $\mu$ M) to block IP<sub>3</sub> receptors and once again, ethanol's effect on action potential-dependent GABA release was lost. We also studied ethanol induction of action potential-independent GABA release and found that it was abolished by  $\omega$ -agatoxin TK (500 nM), a P/Q-type calcium channel blocker, as measured by mIPSC frequency. Thus, we have demonstrated that ethanol enhancement of GABA release from isolated presynaptic terminals and across the synaptic network of the CeA are mediated by P/Q-type calcium channel activity and L-type calcium channel activity/IP<sub>3</sub> receptor-driven CICR, respectively. Collectively, these data suggest that ethanol employs multiple calcium channels in its effects on GABA release, and changes in calcium channel structure or function may represent a novel mechanism by which chronic ethanol alters CeA activity. Supported by NIH/NIAAA AA015566, AA017447, AA006420, AA0016895.

#### P92

##### **Cellular and molecular mechanisms of ethanol action at the nanoscopic level**

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Ethanol affects the signaling of several important neurotransmitter and neuromodulator systems in the CNS. It has been recently proposed that ethanol alters the dynamic lateral organization of proteins and lipids in the plasma membrane, thereby affecting surface receptor-mediated cellular signaling. Our aim is to establish whether pharmacologically relevant levels of ethanol can affect the lateral organization of plasma membrane and cytoskeletal proteins, and investigate the relevance of such perturbations for

mu-opioid receptor (MOP) function. Quantitative methods with single-molecule sensitivity, Fluorescence Correlation Spectroscopy (FCS) and super-resolution pair-correlation Photoactivated Localization Microscopy (pcPALM) were used to nondestructively study ethanol effects on the lateral organization of opioid receptors in the plasma membrane. We observed that short exposure to pharmacologically relevant levels of ethanol (20 min exposure to 20 and 40 mM ethanol) alter the lateral organization and dynamics of MOP, cause a reorganization of GPI-linked proteins and induce actin polymerization. Pretreatment with the MOP antagonist naltrexone (200 nM for 3 hours) is protective against ethanol action and significantly reduces the extent to which ethanol remodels the lateral organization of lipid raft-associated proteins in the plasma membrane. Methods with single-molecule sensitivity reveal details of ethanol action at the nanoscale level with a high spatial resolution (15-25 nm) and sub-millisecond temporal resolution, giving new mechanistic insight on the cellular and molecular mechanisms behind.

### P93

#### **Enhanced sensitivity to amphetamine and cross-sensitization with acute stress following prenatal alcohol exposure: A marker for increased vulnerability to substance use disorders?**

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Prenatal alcohol exposure (PAE) increases the prevalence of substance use disorders (SUD). The interaction of the hypothalamic-pituitary-adrenal (HPA) and dopamine systems is implicated in vulnerability to SUD. PAE alters both HPA and dopamine systems, resulting in increased HPA and reduced tonic dopamine activity, but the effects of PAE on the interaction between stress and dopamine systems are not fully understood. In the present study, adult Sprague-Dawley male and female offspring from PAE, pair-fed (PF), and *ad libitum*-fed control (C) groups were exposed to repeated amphetamine (AMPH) or saline injections and assessed for behavioral sensitization (augmented typical and atypical behaviors) and cross-sensitization with acute restraint stress (increased HPA activity), both known to be positively correlated with vulnerability to SUD. Amphetamine sensitization occurred earlier and at a lower threshold in PAE compared to control males and females, suggesting enhanced sensitivity of underlying dopamine systems to drug-induced changes. Cross-sensitization between amphetamine and stress was evident in PAE but not C or PF animals. Further, compared to controls, saline-pretreated PAE males displayed *increased* dopamine receptor (D<sub>1</sub> and D<sub>2</sub>) expression, while AMPH-pretreated PAE males displayed *decreased* D<sub>1</sub> and D<sub>2</sub> expression in the IL subregion of the mPFC. The novel finding that PAE alters dopamine-stress interactions in a manner consistent with increased vulnerability to SUD provides insight into possible mechanisms underlying the increased prevalence of mental health problems and SUD among individuals with FASD, as well as the efficacy of possible pharmacological treatments targeting dopaminergic function. Funded by grants from the Canadian Foundation for Fetal Alcohol Research (CFFAR) to JW and LAMG, and NIH/NIAAA R37 AA007789 to JW. LAMG is also supported by grants from CIHR, NSERC and Alzheimer's Society for Canada. KAU was funded by IMPART (CIHR STIHR Training Program).

### P94

#### **Role of the orphan GPCR, GPR88 in ethanol-associated behaviors**

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The striatum including the nucleus accumbens regulates reinforcing actions of ethanol and ethanol-seeking behaviors. The orphan G protein-coupled receptor, GPR88, is highly expressed in the striatum and GPR88 mRNA is modified in response to anti-depressant and neuropharmacological treatments, but its contribution to neural and behavioral effects of ethanol is unclear. Using a gene expression study, we previously showed that GPR88 is regulated in the extended amygdala following 4 weeks of abstinence to

several drugs of abuse, including ethanol. The present study was conducted to investigate the role of GPR88 on ethanol-related behaviors. We first demonstrate that GPR88 mRNA is enhanced in the striatum 1 hour after acute ethanol exposure. Behaviorally, GPR88 knockout mice showed an augmented level of voluntary ethanol consumption and preference compared to wild-type mice. Importantly, no alterations in water, saccharine, or quinine intake were observed. In addition, our results document that mice lacking the GPR88 gene show a decreased ethanol-induced place preference suggesting a decreased rewarding value of ethanol. This first set of data position GPR88 as a modulator of ethanol-drinking behaviors. This work was supported by National Institute on Alcohol Abuse and Alcoholism, grant #16658.

### P95

#### **Traumatic brain injury increases alcohol drinking in rats**

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Traumatic brain injury (TBI), increasingly frequent in military personnel, is also a well-recognized sports-medical problem. Higher incidence of post-TBI alcohol abuse is reported in military servicemen with prior history of high alcohol use. The underlying neurobehavioral mechanisms are not known. TBI induces neuroinflammation, neurobehavioral deficits, and stress and anxiety-like behavior. Prefrontal cortex neuroinflammation has been reported to increase operant alcohol self-administration in rodents. Objective: Test the prediction that post-TBI neuroinflammation is associated with increased anxiety-like behavior and alcohol self-administration. Male Wistar rats were trained to self-administer alcohol vs. water (fixed-ratio 1 schedules, two-lever contingency, 30 min sessions, for four weeks) prior to counterbalanced assignment to TBI (lateral fluid percussion; 2 ATM; 25 ms; N=11) or surgical sham (N=13) groups. Pre- and post-TBI neurobehavioral assessments and post-TBI elevated plus maze (EPM) and drinking were determined. Brain TLR4/HMGB1 expression and astrocyte/microglia activation were determined two weeks post-TBI. Alcohol self-administration was increased in TBI and sham animals, particularly in animals with high pre-TBI baseline drinking, at post-TBI day 15. Neurobehavioral scores were worse in TBI animals (5x, 2x, and 1x more than shams at 24 hours, 72 hours, and 7 days post-TBI, respectively). Anxiety scores (EPM), astrocyte activation, and TLR4 expression at the site of injury were accentuated in TBI animals vs. sham. These findings suggest a possible mechanistic link between neuroinflammation and anxiety behavior with post-TBI increased alcohol drinking. **Supported by:** NIAAA-007577

### P96

#### **Activation of PPAR $\gamma$ receptors prevents alcohol-induced neurodegeneration and impaired cognitive flexibility in rats**

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Excessive alcohol exposure leads to region-specific neurodegeneration and subsequent cognitive deficits. Mechanisms involved in excitotoxicity, pro-inflammation and generation of reactive oxygen species are pivotal elements in alcohol-induced neurotoxicity. Recent findings have demonstrated that PPAR $\gamma$  receptor activation shows anti-inflammatory and anti-oxidant properties. Here we examine whether treatment with the PPAR $\gamma$  agonist pioglitazone is beneficial in modulating neurodegeneration and cognitive damage produced by an alcohol insult. Wistar rats were subjected to a 4-day binge intoxication procedure in which alcohol (20% vol/vol) was administered orally every 8 hours to reach doses of 11-15 g/kg/day. Control rats received equal volumes of the vehicle (water with 6% sucrose and 14.7% milk powder). Across the 4-day period, both groups received pioglitazone (0, 30, 60 mg/kg, os) twice daily at 12-h intervals. Degenerative cells were detected by fluoro-jade B (FJ-B) immunostaining. In order to evaluate cognitive functions, additional experiments were designed to test the effects of pioglitazone on cognitive flexibility as assessed in an operant reversal learning task and the Morris water maze. Binge-like alcohol exposure produced widespread neuronal damage particularly in the dentate gyrus of

the hippocampus and the adjacent entorhinal cortex. Pioglitazone dose-dependently reduced FJ-B positive cells in both these structures and the 60 mg/kg dose rescued alcohol-impaired reversal learning ability in both the operant and the Morris water maze tasks while reference memory was not altered. In conclusion, these data suggest a neuroprotective role of PPAR $\gamma$  receptor agonists, which are also effective in preventing functional deficits that accompany alcohol intoxication.

#### P97

##### **Alcohol dependence modulates anti-anxiety neuropeptides in extended amygdala**

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The extended amygdala, comprised of the central amygdala (CeA), lateral bed nucleus of the stria terminalis (BNST), and nucleus accumbens (NAc) shell, mediates emotion, reward and expresses high quantities of pro- and anti-anxiety neuropeptides. Two such anti-anxiety neuropeptides, neuropeptide Y (NPY) and nociceptin modulate inhibitory neurotransmission in CeA and BNST; NPY also promotes dopamine release in NAc. Because NPY and nociceptin reduce anxiety-like behavior and excessive alcohol drinking, this study was designed to examine NPY and nociceptin expression in the extended amygdala during the development of alcohol dependence. We hypothesized that transition to alcohol dependence would recruit anti-anxiety neuropeptide systems, and that withdrawal would be defined by NPY and nociceptin deficits. To induce alcohol dependence, male Wistar rats were exposed to chronic intermittent (14hrs on/10hrs off daily) ethanol (CIE) vapor (BALs ~175-250 mg/dl) or ambient air (controls) for 1 or 28 days, and sacrificed during either intoxication or withdrawal. Immunoreactivity was quantified by immunohistochemical densitometry analysis. Analyses revealed that 28 days CIE increased NPY and nociceptin immunoreactivity in BNST. This effect was reversed ten hours into withdrawal, suggesting that once "normal" levels of NPY and nociceptin are actually deficits during withdrawal, indicating an allostatic shift in neuropeptide content. In contrast, 28 days CIE reduced NPY levels in NAc, and this effect extended into withdrawal. These results show chronic alcohol-induced neuroadaptations in NPY and nociceptin expression in extended amygdala may have implications for high anxiety and reduced brain reward function as they relate to excessive alcohol drinking in the dependent organism. This work was supported by NIH grants AA016436 and AA018400.

#### P98

##### **Clinical and experimental evidence of a link between adrenergic genes, early life stress and alcohol-related phenotypes**

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Alcohol use disorder (AUD) is a complex phenotype, and both genetic and environmental factors are contributing to early age onset and disease vulnerability. Environmental stressors are highly influential, especially during critical periods, such as childhood and adolescence. The adrenergic system is involved in the stress response, and both studies of experimental animals and humans have shown evidence for a link between the adrenergic system and stress response and alcohol-related effects and traits. We analysed candidate tag-polymorphisms in the  $\alpha$ -2A adrenergic receptor (*ADRA2A*) and noradrenaline transporter (*SLC6A2*) genes in a sample of adolescents from a clinic for substance misuse and their families, and found associations of these polymorphisms with AUD, and gene-by-environment interaction effects of these markers with childhood physical abuse in relation to AUD. Furthermore, we investigated the expression of the *adra2a* gene in hypothalamus of rats in an experimental model with controlled environmental conditions to study the consequences of early-life stress, and

of ethanol binge drinking. The results indicate a combined effect of stress and ethanol on *adra2a* gene expression in hypothalamus. The present results strengthen a role of adrenergic genes in alcohol-related phenotypes, also in view of the known effects of selective  $\alpha$ 2-adrenoceptor agonists on alcohol consumption and NE transporter inhibitors in mood disorders. Further studies on the molecular mechanisms involving the regulation of these genes and how genetic variation in these genes affects adrenergic drugs' response are warranted.

#### P99

##### **The association of mental, physical and neighborhood stress with adolescent alcohol use**

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Alcohol use is common among adolescents in the United States, with approximately 27% of 10<sup>th</sup> graders and 40% of 12<sup>th</sup> graders reporting drinking in the past month. Drinking during adolescence may be related to different types of stress resulting from mental health concerns (e.g., anxiety and depression), physical problems (e.g., headaches, trouble sleeping), as well as stressful events in the neighborhood environment (e.g., adolescents' perceptions of abandoned buildings, crime, drug selling, and graffiti in their neighborhood). Few studies have simultaneously examined all three stressors in a U.S. sample of adolescents. We used logistic regression to examine cross-sectional associations controlling for age and gender, in a diverse sample of 2,290 U.S. adolescents with a mean age of 16 (44% Hispanic; 25% White; 20% Asian; 6% mixed; 5% other). We assessed the association between mental health, stress, physical problems, and neighborhood disorder stressors and drinking behavior (past year and past month). Results indicated that youth were more likely to drink if they reported trouble sleeping, low energy or feeling tired, worse mental health, and greater neighborhood disorder. These associations were statistically significant for both any past month and any past year drinking, as well as any heavy drinking (five or more drinks) in the past month or past year. Thus, even having one drink during this developmental period was associated with greater stress. Findings emphasize the importance of helping youth cope with stress across a variety of contexts in order to potentially prevent both initiation of alcohol use and heavier drinking.

#### P100

##### **Influence of a corticotropin-releasing factor receptor 1 gene polymorphism on anxiety-like behaviours of marchigian sardinian alcohol-preferring rats**

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Marchigian Sardinian alcohol preferring (msP) rats exhibit high stress sensitivity along with anxious-like phenotype. Genetic analysis showed that over-expression of the CRF system of msP rats is linked to two single nucleotide polymorphisms occurring in the promoter region of the CRF1 receptor (CRF1-R). We examined whether these point mutations are associated to heightened anxiety and increased stress sensitivity. The msP rats were re-derived to obtain two distinct lines carrying the point mutations (AA) and wild type (GG), respectively. The phenotype of these two rat lines were assessed in comparison with those of unselected Wistar rats on preclinical models of anxiety. Both msP lines demonstrated higher anxiety-like behaviour in the elevated plus maze and fear conditioning paradigms compared to Wistars. Surprisingly, the AA rats showed a decreased burying in the defensive burying compared to GG and Wistar, likely due to stress hyper-sensitivity. Restrained Wistars performed in a similar manner as AA. The selective CRF1-R antagonist, antalarmin dose-dependently reduced the burying in Wistar rats. In the GG rats however, antalarmin at 10 mg/kg significantly increased burying while at 20 mg/kg, it decreased the burying behaviour. Similar results were obtained for AA although the differences were not statistically significant. These data indicate that the polymorphisms at CRF1-R are associated to increased sensitivity to stress and inhibition of active behavioural responses to stress. Our results also suggest that there is a

general innate dysregulation of the brain stress system common to msP rats that are not directly related to the polymorphisms. This study was supported by NIH/NIAAA AA017447 (MR and RC).

**P101**

**Mathematical modeling of ethanol effects on the HPA axis dynamics**

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Alcoholism and stress are mutually related and difficult to disentangle. Numerous studies show that **a**) ethanol impinges on the hypothalamic-pituitary-adrenal (HPA) axis dynamics, altering individual response to stress, and **b**) that individuals under stress are more prone to drinking. Molecular mechanisms underlying the relationship between stress and alcohol consumption are not well understood and difficult to characterize experimentally due to the intricate nature of the problem. We use here mathematical modeling and numerical simulations to emulate ethanol effects on the HPA axis dynamics and study mechanisms through which these interactions are integrated to yield an altered response to stress. We present a low-dimensional mathematical model in which the complex regulation of HPA axis activity arises from the intrinsic nonlinearity of underlying biochemical interactions and the entanglement of investigated species via feedback mechanisms, rather than from any stochastic or noisy input from the surroundings. Modeling shows that the underlying non-linearity enables the HPA axis to quickly adjust its dynamics in response to perturbations with ethanol, and promptly restores its balance thereafter. In addition, modeling shows that chronic exposure to ethanol changes the dynamic regulation of HPA axis activity, leading to reduced and eventually loss of adaptive potential. Mathematical modeling and numerical simulations may critically contribute to the elucidation of dynamic mechanisms that regulate the function of the HPA axis at the organism level. These integrative methods enable us to mimic *in silico* the effects of acute and chronic exposure to ethanol, and investigate the impact of ethanol on the HPA axis dynamics and the response to stress.

**P102**

**Heavy prenatal alcohol exposure differentially impacts the relationship between pituitary volume and behavior in male and female adolescents**

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Rodent studies demonstrate that prenatal alcohol exposure produces hypothalamic-pituitary-adrenal (HPA) axis dysregulation with sex-dependent effects on behavior. Pituitary volume may be a relatively stable and state-independent index of HPA axis function in humans. To determine if prenatal alcohol exposure produces measurable changes in pituitary volume that relate with problem behavior in human adolescents, we manually traced the pituitary in T1-weighted structural magnetic resonance images (MRI). Pituitary volumes were calculated for male and female adolescents with and without prenatal alcohol exposure and were correlated with primary caregiver ratings of behavior on the Child Behavior Checklist (CBCL). Control female adolescents presented with significantly greater pituitary volume compared to males, however this sex-effect was absent in adolescents with histories of prenatal alcohol exposure. In prenatal alcohol exposed males, scores on the CBCL aggressive behavior scale were negatively associated with pituitary volume. The lack of a sex difference in pituitary volumes between the alcohol-exposed groups suggests such exposure may interfere with adolescent typical sexual dimorphism of the pituitary. In prenatal alcohol exposed males pituitary volume may reflect cortisol levels, which have previously been reported to be negatively associated with aggressive behavior in clinical populations. These findings suggest the endocrine system is impacted by prenatal alcohol exposure, which may have implications for stress reactivity in this population. This

work was supported by NIAAA grants R01 AA010417, R01 AA019605, U24 AA014811, U01 AA014834, and T32 AA013525.

**P103**

**Alcohol-Responsive Synaptic MicroRNAs Coordinately Regulate MRNAs Following Chronic Alcohol Consumption**

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Local translation of mRNAs in synaptic compartments of the cell plays a major role in synaptic structure and function. Chronic alcohol use causes persistent changes in synaptic mRNA expression that might be regulated by microRNAs in the synapse. To determine the microRNAs that may regulate synaptic alcohol-induced mRNA adaptations, we profiled the transcriptome of synaptoneurosomes from the amygdala of mice undergoing a chronic voluntary alcohol consumption paradigm. We found 67 mature mouse microRNAs and 1,531 mRNAs differentially-expressed between the alcohol-drinking mice and the controls. 38 premature microRNAs were found differentially-expressed, 11 of which correspond to 12 alcohol-responsive mature microRNAs. To predict mRNA-microRNA interactions we used two approaches: (1) mRNA-microRNA co-expression using weighted gene co-expression network analysis (WGCNA) and (2) mRNA-microRNA target prediction using mirSVR scores in miRanda. WGCNA highlighted many co-expressed mRNAs and microRNAs that have high correlations with alcohol consumption. The biological pathways associated with the co-expressed mRNAs include long-term potentiation and depression, glutamate signaling, neuroimmune processes, RNA-processing and translation. Prediction analysis revealed that the 67 differentially-expressed microRNAs were predicted to target 1,039 of the 1,531 differentially-expressed mRNAs, and the 1,531 differentially-expressed mRNAs were predicted to be targeted by 15 of the 67 differentially-expressed microRNAs. These 15 microRNAs showed 15-50% fold-change after alcohol treatment and were each predicted to target 60-400 mRNAs responsive to alcohol. We constructed a list of the most probable alcohol-responsive microRNA-mRNA interactions in the synapse and propose specific microRNAs which may be effective in manipulating local synaptic translation as potential treatments for alcoholism. Work supported by NIH Grants AA022557, AA13520, AA012404, RC2AA019382, AA020683

**P104**

**Marchigian Sardinian alcohol-preferring rats exhibit deficient anandamide regulation of stress-induced increases in excitatory amino acid transmission in the central amygdala**

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Marchigian Sardinian alcohol-preferring (msP) rats exhibit enhanced preference for alcohol and increased susceptibility to affective responses associated with stress and anxiety. Endogenous cannabinoid (eCB) signaling is known to play an important homeostatic role in the regulation of affective state. The current studies examined the neurochemical correlates of enhanced stress and anxiety in the central amygdala (CeA) of msP versus wildtype rats. Briefly, msP and wildtype Wistar rats (n=5-7 per group) were examined for anxiety-like behavior on the elevated plus maze following a 30-min restraint procedure. To characterize amygdalar neurochemistry, we implanted a separate cohort of animals (n=8 per group) with cannulas into the CeA region. Rats were then prepared with microdialysis probes (1 mm active membrane), and dialysate levels were retrieved in 15-min collection fractions before, during and after 30-min restraint. Similar procedures were used in a separate cohort of rats (n=8-10 per group) to examine eCB overflow. The results revealed that msP rats exhibited enhanced anxiety-like behavior than wildtypes regardless of restraint procedures. msPs also exhibited enhanced basal and stress-induced changes in the excitatory amino acid transmitters glutamate and aspartate in the CeA. In contrast, we observed that basal and stress-induced changes in anandamide (AEA) were lower in msP than wildtypes. We suggest that the enhanced stress and anxiety phenotype in

msPs is, in part, due to enhanced stress-induced increases in excitatory transmission in the CeA. Moreover, these neurochemical differences likely reflect underactive AEA regulation of excitatory circuits that serve to constrain stress-induced changes and increase susceptibility to alcohol-seeking. Supported by RO1 AA020404 and AA017447.

#### P105

##### **Chronic ethanol exposure results in time- and brain region-dependent changes in gene coexpression networks**

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Repeated ethanol exposure and withdrawal in mice has been shown to increase voluntary drinking and represents an animal model of physical dependence. We examined time- and brain region- dependent changes in gene coexpression networks using chronic intermittent ethanol (CIE) exposure in mice. We measured gene expression in prefrontal cortex (PFC), nucleus accumbens (NACB), and amygdala (AMYG) from C57BL/6J mice following four weekly cycles (4 days 16 hr ethanol vapor/8 hr room air + 7 days off) of CIE vapor exposure. Animals were sacrificed at 0, 8, and 120 hr following the last ethanol exposure. Each brain region exhibited a large number of differentially expressed (DE) genes (2000-3000;  $p < .05$ ) at the 0 and 8 hr time points, but fewer changes were detected at the 120 hr time point (400-600). Within each brain region, there was little overlap of DE genes across time (~20%). All brain regions were significantly enriched with DE immune-related genes at the 8 hr time point. Weighted gene correlation network analysis (WGCNA) was used to investigate the modular structure of the data at a gene network level. Many detected modules were highly enriched in DE genes at the 0 and 8 hr time points with virtually no enrichment at 120 hrs. Differentially enriched modules (for ethanol-responsive and cell-specific genes) were discerned in each brain region and time point. These results support the hypothesis that chronic alcohol exposure causes global 'rewiring' of co-expression systems involving glial and immune signaling as well as neuronal genes. This work was supported by NIAAA grants AA016648, AA012404, U01 AA014095, U01 AA020929, and P50 AA010761.

#### P106

##### **Effect of comorbid Alcohol use disorders on plasma pro-inflammatory chemokines in cocaine addicts seeking outpatient treatment**

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Cocaine addiction is commonly associated with high prevalence of mental disorders and other substance use disorders, mainly alcohol use disorders (AUD). Evidence indicates that the immune system might be involved in the pathogenesis of cocaine addiction and the associated psychiatric disorders. This study assessed the plasma pro-inflammatory chemokines, the cocaine symptom severity and the psychiatric comorbidity in abstinent cocaine users with cocaine use disorders (CUD). Outpatient cocaine users with lifetime CUD and age/gender/body mass-matched controls with no history of drug abuse were recruited. They were psychiatrically assessed with a diagnostic interview (PRISM). Chemokines in plasma were determined using a multiplex suspension protein array for CX3CL1/fractalkine, CXCL12/SDF-1 and CCL2/MCP-1. CUD-subjects displayed decreased levels of CXCL12/SDF-1 and CCL2/MCP-1 compared with controls. Also, a significant and positive correlation between cocaine symptom severity and chemokines (CX3CL1/fractalkine and CXCL12/SDF-1) were observed in these outpatients. This correlation allowed us to distinguish 2 degrees of cocaine symptom severity with different comorbid psychiatric profile. Regarding comorbid disorders, AUD (alcohol abuse and dependence) were diagnosed in approximately 65% of all CUD-subjects. Chemokine concentrations were compared between CUD-subjects with comorbid AUD and with no AUD, and we detected no differences. However when the severe CUD-subgroup was analyzed, we observed a significant increase of

chemokines (CX3CL1/fractalkine and SDF1) in CUD-subjects with AUD. Therefore, CUD-subjects with high severity of cocaine addiction displayed low plasma levels of chemokines. However, the diagnosis of comorbid AUD in severe cocaine users was associated with an increased level of chemokines in comparison with those with no AUD. Severe cocaine addicts could consume other drugs of abuse (i.e. alcohol) as a compensatory response to the effects on mediators such as chemokines. Further studies will be necessary to elucidate the effect of AUD in circulating chemokines in subjects with no CUD, as well as which is the role of chemokines in addiction. Supported by Instituto de Salud Carlos III, Red de Trastornos Adictivos UE-FEDER 2006 (RD06/0001/0000 and RD06/0001/1009) and 2012 (RD12/0028/0001 and RD12/0028/0009); Consejería de Salud, Junta de Andalucía (PI-0228/2013).

#### P107

##### **Effects of early life trauma on alcohol- and stress-related phenotypes in alcoholics with and without posttraumatic stress disorder (PTSD)**

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There is a high degree of comorbidity of alcohol dependence and PTSD. Exposure to childhood trauma may predispose individuals to develop PTSD and has been associated with increased severity of both alcohol- and PTSD-related phenotypes. We examined the effects of childhood trauma in treatment-seeking alcoholics both with and without PTSD. Data were obtained from 403 alcohol-dependent inpatients (128 females, 275 males); of these, 123 (30.5%) were also diagnosed with PTSD. Exposure to childhood abuse (emotional, physical, or sexual abuse) was assessed using the Childhood Trauma Questionnaire (CTQ). A subset of patients ( $n=101$ ) underwent the Trier Social Stress Test (TSST), and were also assessed for PTSD symptom severity. Of the full patient sample, 257 subjects (63.8%) reported at least one form of childhood abuse. Subjects reporting abuse began drinking to intoxication at an earlier age ( $F=16.1$ ,  $p < 0.0001$ ), and had higher levels of alcohol dependence severity ( $F=6.5$ ,  $p=0.01$ ), anxiety ( $F=5.3$ ,  $p=0.02$ ), neuroticism ( $F=23.8$ ,  $p < 0.0001$ ), and aggression ( $F=10.6$ ,  $p=0.001$ ). Subjects with PTSD ( $n=123$ ) also had higher levels of anxiety ( $F=7.1$ ,  $p=0.008$ ), neuroticism ( $F=5.2$ ,  $p=0.02$ ), and aggression ( $F=9.3$ ,  $p=0.002$ ), and these effects were additive with those observed for childhood abuse. In the TSST sample, subjects with both childhood abuse and PTSD ( $n=46$ , 45.5%) exhibited blunted ACTH and cortisol responses to the TSST, as well as higher arousal symptoms across the duration of their inpatient treatment. These findings confirm that childhood and PTSD, both individually and in combination, result in a more severely affected phenotype among alcohol dependent individuals.

#### P108

##### **Ethanol produces CRF dependent regulation of glutamatergic transmission in the Central Amygdala**

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In the central amygdala (CeA), ethanol (EtOH) enhances GABAergic transmission via activation of local corticotropin releasing factor (CRF) receptors, potentially by increasing CRF release. However, CRF receptor 1 deletion from glutamatergic synapses has been shown to produce more robust affective phenotypes than CRF receptor 1 deletion from GABAergic synapses. We recently showed that CRF enhances glutamatergic transmission in the bed nucleus of the stria terminalis and in the CeA. Given that EtOH may induce CRF release in the CeA and the role of CRF-glutamate interactions in the regulation of affective phenotypes typically seen in EtOH withdrawal, we sought to determine if EtOH acutely alters glutamatergic transmission in the CeA via a CRF dependent mechanism. Utilizing whole-cell patch-clamp electrophysiology recordings of CeA neurons from adult male C57BL/6J mice, we found that bath application of EtOH (5-100mM) significantly enhances spontaneous excitatory postsynaptic current (sEPSC) frequency in a concentration-dependent



manner (ANOVA:  $F_{(4,21)}=3.596$ ,  $p<0.05$ ;  $EC_{50}=18.31mM$ ). 100mM EtOH significantly increased sEPSC frequency from baseline levels ( $132.0 \pm 8.4\%$ ,  $n=8$ ,  $p=0.007$ ), an effect completely abolished by pretreating CeA slices with CRF receptor 1 and 2 antagonists, NBI27914 and Astressin2B ( $98.3 \pm 11.05\%$ ,  $n=10$ ,  $p=0.88$ ), suggesting EtOH requires intact CRF signaling to increase presynaptic glutamate release in the CeA. The CeA contains CRF producing neurons and extrinsic CRF sources, however it is not yet known which source of CRF is required for EtOH potentiation of CeA glutamate release. To that end, experiments are currently in progress to determine EtOH-sensitive CRF sources required for modulation of CeA neurotransmission.

#### P109

##### **Adverse childhood experiences predict heavier drinking and greater alcohol intake during intravenous (IV) alcohol self-administration in non-dependent drinkers**

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The objective of this study was to examine the influence of Adverse Childhood Experiences (ACE) on drinking history and IV alcohol self-administration, using the Computer-Assisted Self-infusion of Ethanol (CASE) method, in non-dependent drinkers. Participants ( $N=217$ ) were assessed for ACE using the Childhood Trauma Questionnaire (CTQ) yielding a score for overall trauma severity (CTQTotal), total number of traumatic events experienced (CTQNumCat), and severity scores for five subtypes of trauma. Recent drinking history was assessed using the Timeline Follow Back (TLFB). Self-administration measures for the CASE subset ( $N=86$ ) included peak (PEAK) and average (AVG) BrAC and total ethanol (EtOH). Subjective responses included the Drug Effects Questionnaire (DEQ) and the Alcohol Urge Questionnaire (AUQ). Personality measures included the NEO Five Factor Personality Assessment, Alcohol Effects Questionnaire (AEFQ), and the Sensitivity to Punishment and Reward Questionnaire (SPSRQ). There was a 38% prevalence of at least one form of childhood trauma in the sample. Individuals that reported childhood abuse had heavier drinking histories. Within the CASE subset, TLFB, CTQTotal, CTQNumCat, and history of physical or emotional neglect were positively associated with self-administration measures. DEQ subjective measures of "liking" and "wanting" alcohol and AUQ total score during self-administration were associated with CTQTotal, CTQNumCat, and emotional neglect. Sensitivity to reward, NEO measures, and facets of the AEFQ were associated with emotional and physical neglect, emotional abuse, and CTQTotal. These data support growing evidence of the impact of ACE on alcohol intake behavior and related measures, including subjective effects, expectancies, and reward sensitivity in non-dependent drinkers.

#### P110

##### **Comorbidity of posttraumatic stress disorder (PTSD) and alcoholism: a rat model of PTSD leads to escalated context-induced alcohol consumption**

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High rates of comorbidity exist between alcoholism and PTSD. However, it is unclear how exposure to traumatic experience increases the risk for alcoholism in adulthood. We developed a novel rat model of PTSD with subsequent alcohol-drinking assessments. The model is designed to emphasize specific PTSD stressor criteria ('threatened injury or death') by exposing them to live predator stress and determine if they develop high rates of alcohol drinking. Rats were exposed to a snake (live predator exposure; LPE) for 10 minutes at post-natal day (PND)31, 46 and 61. Corticosterone levels were taken before, immediately following, and 90 minutes after the last stress exposure. At PND75 anxiety-like behavioral responses were assessed in the elevated plus-maze. Rats were tested for two-bottle choice drinking at PND76, and were tested again at PND100 after exposure to the predator-associated context. At PND103, rats were exposed to a fear

conditioning and expression test. LPE resulted in blunted corticosterone levels compared to control animals immediately following the third stress test, consistent with an aberrant stress response. LPE rats also spent more time in the closed arms of the elevated plus maze indicative of a classic anxiety-like phenotype. During fear conditioning, LPE animals displayed more freezing during contextual and cue expression tests than control animals. No baseline drinking differences were observed, however, context exposure significantly increased drinking in LPE animals. Overall, exposure to a live predator induces PTSD-like symptoms and significantly escalates context-induced consumption, offering a novel comorbidity model to study the link between PTSD and alcoholism. # = author contribution.

#### P111

##### **Attenuated hypothalamo-pituitary adrenal axis re-activity predicts persistent avoidance of predator odor stress-paired context in rats**

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Post-traumatic stress disorder (PTSD) develops in a subset of individuals exposed to traumatic stress and is highly co-morbid with Alcohol Use Disorder (AUD). Our lab has established a rat model that allows for a priori detection of individuals susceptible to stress-induced escalation of alcohol drinking. Utilizing an aversive conditioning procedure, animals are divided into groups based on high or low avoidance of predator odor-paired stimuli, a diagnostic criterion of PTSD. Using this model, we have shown that high stress-reactive (HSR) rats exhibit persistent avoidance and consume significantly more alcohol than low stress-reactive rats (LSR) and unstressed controls. Both PTSD and AUD are defined by dysregulation of hypothalamo-pituitary axis (HPA) function. The purpose of this study was to examine HPA activity in HSR and LSR rats following a traumatic stress. We hypothesize that attenuated HPA responses drives stress-induced increases in alcohol consumption in HSR rats. Chronically-catheterized male Wistar rats (300g) underwent a place conditioning procedure to assess avoidance of an odor-paired chamber. Fifty-percent of rats exhibited persistent avoidance (HSR). LSR rats, but not HSR rats, exhibited significant increases in plasma adrenocorticotropic hormone (ACTH) and corticosterone post-odor exposure ( $226 \pm 34$  vs.  $116 \pm 8$  vs. pg/ml, respectively) following odor exposure. Interestingly, low ACTH levels were predictive of high avoidance ( $R^2=0.506$ ). In a separate group of rats, stress did not alter corticotropin-releasing factor (CRF) protein levels in the paraventricular nucleus (PVN) 20 days post-stress, as measured by radioimmunoassay (RIA). Future studies will examine the contribution of HPA axis dysfunction to stress-induced escalation of alcohol drinking in high-stress reactive subpopulations of rats. This work was supported by NIH grants: AA018400 (NWG) and T32AA007577 (GJB).

#### P112

##### **Novel NMDA receptor-based proteomic analysis reveals chronic ethanol regulation of mGluR LTD in Hippocampus**

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NMDA receptors (NMDAR) are a major target of both acute and chronic ethanol, yet much remains to be understood regarding the impact of chronic ethanol exposure on glutamate synapses. To address this gap, we employed a novel discovery-based proteomic approach to identify GluN2B associated proteins in synaptic and extrasynaptic locations of hippocampus following chronic ethanol. In the synaptic fraction of hippocampal tissue, we found that chronic ethanol treatment increased GluN2B associated Arc (immediate early gene) and Homer 1 (scaffold protein), while decreasing synaptic GluA2 (AMPA subunit) following chronic ethanol. This biochemical signature predicts enhanced and/or engaged long-term depression (LTD) mechanisms in the hippocampus. Consistent with this idea; we found that group I mGluR dependent DHPG-induced LTD could not be elicited in area CA1 of hippocampal slices prepared from CIE treated mice. These data suggest an unanticipated interconnection between ethanol regulation of NMDA receptors and mGluR-dependent LTD in the hippocampus.

**P113**

**Uncovering Coordinated MicroRNA/Protein Changes Induced by a Chronic Intermittent Ethanol Paradigm in Mouse Brain**

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Alcohol abuse causes dramatic neuroadaptations in the brain. Previous microarray/proteomics studies in human alcoholics and animal models have identified candidate microRNAs (miRNAs), genes, and proteins, but no single approach can fully account for the impact of these individual changes on brain function. For the first time, we combine miRNA and proteomic profiling with a systems approach to data analysis that integrates differential expression, coexpression networks, miRNA target predictions, protein-protein interactions, and gene annotations to unveil key neurobiological rearrangements associated with the transition to alcohol dependence. We analyzed cerebral cortices (CTX) and midbrains (MB) from C57BL/6J mice subjected to a chronic intermittent ethanol (CIE), two-bottle choice paradigm. miRNA and protein levels were significantly altered in CIE-exposed dependent mice compared with their non-dependent controls. Multiple protein isoforms showed region-specific differential regulation as a result of post-translational modifications. More importantly, our integrative analysis identified modules of coexpressed miRNAs that were highly correlated with CIE effects and predicted target genes encoding differentially expressed proteins. Coexpressed CIE-relevant proteins, in turn, were often negatively correlated with specific miRNA modules. We found that modules most associated with the effects of CIE treatment coordinate molecular imbalances in endocytic- and energy-related pathways. The coordinated dysregulations identified above may play a key role in the escalation of ethanol consumption associated with dependence. Our unique integrative systems approach to addiction will advance knowledge of brain remodeling mechanisms in response to drug abuse, uncover organizational principles of CTX and MB proteomes, and define potential new molecular targets for treating alcohol addiction. Supported by NIAAA grants AA016648, AA0107838, AA019382, AA020683, and Integrative Neuroscience Initiative on Alcoholism (INIA).

**P114**

**Dysregulation of stress-induced synaptic plasticity in the paraventricular nucleus of the hypothalamus in alcohol dependence**

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Alcohol use disorders are associated with a persistent dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and corticotropin-releasing hormone (CRH) signaling that leads to inappropriate stress responses, thereby increasing relapse susceptibility in abstinent alcoholics and increasing alcohol consumption in alcohol dependent rats. However, the cellular and molecular mechanisms responsible for the persistent dysregulation of the HPA axis in alcohol dependence are still unknown. We recently described an intricate cellular mechanism by which stress induces synaptic plasticity in the paraventricular nucleus of the hypothalamus (PVN) that contributes to homeostatic HPA axis responses to stressors in rats. Acute stress induces a CRH-dependent depression of NMDAR function in parvocellular neurosecretory cells (PNCs) in the PVN, which allows for the unmasking of short-term synaptic potentiation (STP) of glutamatergic transmission following high-frequency stimulation (HFS, 100Hz for 1sec, repeated 4 times). Here, we demonstrate that chronic intermittent ethanol (CIE, 30 doses, 5-6 g/kg, gavage) treatment of rats followed by >40 days of withdrawal produces potentiation of NMDAR function associated with increased GluN2B-containing NMDAR expression in PNCs. We also show that that STP can be induced in PNCs of CIE rats without acute stress. By contrast, STP is impaired in PNCs from acutely stressed CIE rats, but not their vehicle treated controls. This long-lasting alteration of NMDAR function and stress-induced synaptic plasticity may contribute to the development of a dysregulated stress system and to stress-induced increases in alcohol consumption of CIE rats. Supported by NIAAA grants AA016100 and AA022408.

**P115**

**Effects of allyphenylene on chronic alcohol intoxication model**

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Alcohol withdrawal refers to a cluster of symptoms that may occur from suddenly ceasing the use of alcohol after chronic or prolonged ingestion. These symptoms make alcohol abstinence difficult and increase the risk of relapse in recovering alcoholics. Our study was aimed at identifying novel candidate compounds, which might attenuate alcohol withdrawal signs in the rat. In previous studies, we demonstrated that allyphenylene, endowed with effective adrenergic  $\alpha_{2C}$ -agonism/ $\alpha_{2A}$ -antagonism and serotonergic 5-HT<sub>1A</sub>-agonism, significantly reduced morphine withdrawal syndrome and associated depression. Here, we evaluated the effects of allyphenylene on alcohol withdrawal signs in Wistar rats. For this purpose, animals were subjected to a 4-day chronic alcohol intoxication (by intragastric administration), and at 24 hours following cessation of alcohol exposure, they were treated intraperitoneally (ip) with allyphenylene (0.05 mg/kg and 0.5 mg/kg). Somatic withdrawal signs were scored after ip treatment. In a subsequent experiment, to evaluate the effects of allyphenylene on alcohol withdrawal-induced anxiety, another group of rats was subjected to ethanol intoxication and after 1 week was tested for anxiety behavior in the elevated plus maze (EPM) and in the defensive *burying* tests. The blood alcohol levels were assessed on third and fourth day of chronic alcohol intoxication. Results showed that allyphenylene did not reduce the expression of somatic withdrawal signs but attenuated anxiety-like behaviors in defensive *burying* tests associated with chronic alcohol intoxication. Allyphenylene did not affect anxiety scores in EPM. These findings suggest that modulation of the aforementioned receptor systems activity attenuates the expression of the affective but not the somatic signs during alcohol withdrawal.

**P116**

**Withdrawn.**

**P117**

**The role of the rostromedial tegmental nucleus in ethanol withdrawal**

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Alcohol withdrawal is associated with a hypodopaminergic state and increased negative affect, both of which are thought to play a significant role in the propensity for relapse. The rostromedial tegmental nucleus (RMTg) consists of GABAergic neurons that project to, and synapse on, dopamine (DA) neurons of the VTA. Activation of the RMTg results in inhibition of VTA DA neurons and produces conditioned place aversion. The present study examined the role of the RMTg in ethanol-induced withdrawal by measuring cFos expression in the RMTg and its associated neurocircuitry. Adult male Long-Evans rats were rendered ethanol dependent using chronic intermittent exposure to ethanol vapor, assigned to four experimental groups corresponding to 0, 6, 12, and 24 hr of withdrawal, and sacrificed at the corresponding time point after their final ethanol exposure. Significant withdrawal-induced changes in RMTg cFos expression were observed with very low levels at 0 hr that rose and peaked at 12 hr ( $p < 0.01$ ). The increase in RMTg cFos was closely paralleled by a 10-fold increase in cFos in the lateral habenula, which sends a dense glutamatergic projection to the RMTg. In contrast, the central amygdala, which projects indirectly to the RMTg, showed only a 2-fold increase in cFos expression. The time course of RMTg cFos expression closely resembles that of withdrawal-induced DA decline suggesting that the RMTg may play a role in the withdrawal-induced hypodopaminergic state. In addition, these data indicate the presence of strong selectivity of activation of RMTg-associated neurocircuitry across acute withdrawal.

**P118**

**Elevated ethanol intake following intermittent ethanol withdrawal requires endogenous GABA-A receptor signaling within the central nucleus of the amygdala**

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GABAergic transmission in the central nucleus of the amygdala (CeA) is implicated in regulating alcohol intake, and chronic ethanol exposure alters GABA signaling. In these studies, we investigated the effects of GABA receptor manipulations within the CeA for ethanol intake in mice following intermittent ethanol withdrawal. Adult male C57BL/6 mice were implanted with dual cannulae aimed at the CeA and allowed access to two-bottle choice to 15% (v/v) ethanol versus water for 2 hr occurring 30 min prior to lights out for 14 days to establish a stable baseline. Consumption matched groups were then exposed to either 3 bouts of intermittent ethanol vapor exposure (3 cycles of 16 hr of ethanol vapor + 8 hr of air; multiple-withdrawal (MW)) or air (control), each followed by a 7 day period of limited access consumption. Once significant differences in alcohol intake by group were accomplished, drug and vehicle infusions into the CeA began. Intra-CeA administration of the GABA-A antagonist bicuculline (0, 1.0, and 10.0 ng/side) and the GABA-A agonist muscimol (0, 10, 30 ng/side) both selectively reduced alcohol intake only in MW animals. This multiple withdrawal specific effect appeared to be selective for manipulation of GABA-A receptor signaling as intra-CeA administration of the GABA-B antagonist saclofen (0, 1.0, and 5.0 mg/side) and the GABA-B agonist baclofen (0, 5.0, 10.0, and 50.0 ng/side) decreased drinking across groups. Together these results suggest that the GABA-A receptor is differentially involved in mediating ethanol intake in withdrawn versus control mice. Supported by AA016981 and VA Research.

**P119**

**New strategies to study endogenous opioid system regulation in alcoholism: evaluation of ethanol mechanisms by molecular imaging, genetic and epigenetic analysis**

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Alcohol induces changes in the central nervous system by altering, directly or indirectly various neuromodulatory and neurotransmitter systems. A reasonable body of evidences indicates a linkage of the endogenous opioid system (EOS) to the development and/or maintenance of alcoholism. We here study mechanisms of adaptive transformations evoked by alcohol in the EOS at (1) cellular and (2) genetic levels. (1) Advanced fluorescence imaging by Confocal Laser Scanning Microscopy and Fluorescence Correlation Spectroscopy are used to study ethanol effects on MOP, KOP and NOP in live PC12 cells. We observed that ethanol (20 mM) differentially alters opioid receptor mobility and surface density in the plasma membrane, whereas pre-exposure to naltrexone partially counteract these effects. (2) Genome-wide Single-Nucleotide Polymorphisms (SNPs) in genes encoding for the EOS components have shown associations with alcoholism, however evidence for how they exert an effect on target genes are still not clear. We here report preliminary findings on the connection between genetic variants and DNA methylation at opioid gene promoters that provide new insight in the nature of genetic and epigenetic interactions in the manifestation of individual risk of becoming an alcohol abuser. The focus on alcohol effects on the dynamical aspect of adaptive transformations, as well as the gene x environment effects, might be of relevance to a better understanding of the role of the EOS in alcoholism and be of help in search for new treatments.

**P120**

**Translational Neuroimaging in Alcoholism: Identification of Altered Brain Connectivity in postdependent rats**

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Discrepancies between disease mechanisms in human and animal models hinder medication development for alcohol use disorders (AUD). New translational tools such as neuroimaging-based methods may improve the predictive validity of animal studies. TRANSALC aims to advance the development of pharmacotherapy for AUD by identifying brain responses via magnetic resonance imaging (MRI) that are comparable between patients and animal models of the disorder. Such brain signatures are hypothesized to occur in common "AUD-networks" and may be helpful in translating medication effects observed in experimental animals for potential AUD treatments. We are using resting state functional magnetic resonance imaging (rsfMRI) a quantitative description of functional connectivity in 3-D brain networks in postdependent rats. Analysis of rsfMRI data revealed altered PFC and amygdala connectivity within an *a priori* defined 'addiction-network' during abstinence in post-dependent rats. While alcohol drinking in postdependent rats did not result in noticeable effects on functional connectivity, 2 months basal alcohol consumption in control rats strongly affected connectivity measures of the habenula region. We will discuss these initial resting-state connectivity data from post-dependent rats in light of previous functional studies in this model and compare these findings to available rsfMRI data from human alcoholics.

**P121**

**MT-7716, a potent NOP agonist, preferentially reduces ethanol seeking and reinforcement in post-dependent rats**

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Dysregulation of the nociceptin system has been implicated in alcohol abuse and alcoholism and growing evidence suggests that targeting this system may be beneficial for treating alcoholism. To further explore the treatment target potential of the nociceptin system, the novel non-peptide, small-molecule opioid receptor-like 1 (ORL-1) agonist MT-7716 was examined for its effects on ethanol self-administration and stress induced reinstatement of alcohol seeking in non-dependent and post-dependent rats. Male Wistar rats were trained to self-administer ethanol and then made ethanol dependent via repeated intragastric ethanol intubation. The effects of MT-7716 (0.3 and 1 mg/kg; PO) on alcohol self-administration were determined two weeks following dependence induction, when baseline self-administration was restored. Effects of MT-7716 on stress-induced reinstatement were tested in separate cohorts of rats, one and three weeks post-withdrawal. MT-7716 reduced alcohol self-administration and stress-induced reinstatement of alcohol seeking in post-dependent rats, but was ineffective in non-dependent animals. Moreover, the prevention of stress-induced reinstatement by MT-7716 was more pronounced at 3 weeks post-dependence. The results further confirm treatment target potential for the NOP receptor and identify non-peptide NOP agonists as promising potential treatment drugs for alcohol abuse and relapse prevention. The findings also support dysregulation of the nociceptin system as a factor in alcohol seeking and reinforcement. This study was supported by NIH/NIAAA AA014351 (to FW and RC)

**P122**

**The mean and the individual: A person-centered approach to neuropsychological recovery in men and women with substance use disorders**

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Cognitive deficits are common in persons with substance use disorders (SUDs) and may interfere with recovery. Although cognition tends to improve with abstinence, there are substantial individual differences in the nature and extent of impairment. We propose that there are also substantial individual differences in the time-course and extent of cognitive recovery, which differentially affect how individuals in recovery from SUDs are able to benefit from treatment, and manage stressors that may lead to relapse. Few studies have cognitively assessed this population at more than two points in time, hampering knowledge of intra-individual trajectories of cognitive change. This study integrated a variable-centered and a person-centered approach to the assessment of cognitive recovery to address the question of individual stability and change in executive function, verbal ability, memory, and complex information processing speed. We assessed 197 individuals at treatment entry, and then on three additional occasions in the following year. Confirmatory factor analysis of latent cognitive abilities at these four time-points was used to minimize measurement error and the extent to which recovery was conflated with practice effects on specific tests. Empirical growth plots of individual-level changes were explored within the context of the statistical significance of group-level changes in the latent constructs between baseline and subsequent assessments. Clear differences in individual trajectories of latent cognitive abilities over time were evident, and not all individuals demonstrated the same trajectory of change. Implications for individualized treatment planning and targeted cognitive interventions that help individuals optimally manage interoceptive and exteroceptive stress are discussed.

**P123**

**The specificity of an alcohol habit: neuronal encoding in dorsal striatum during a rat model of habitual drinking and contingency degradation**

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Habitual behavior is defined as being insensitive to change in reward value and is a key component of alcoholism. Alcohol exposure may facilitate formation of habitual reward-seeking in rodents. We previously used extracellular electrophysiology to find that pre-press excitatory responses predominated in the sensorimotor, dorsolateral striatum (DLS) and post-reinforcement responses predominated in the associative, dorsomedial striatum (DMS). However, cells in the DMS and DLS of rats with more habit-like alcohol seeking exhibited phasic excitations following a reinforced press. Whether this effect was specific to alcohol and how it could be reversed was unknown. We therefore performed electrophysiological recordings in rats trained to self-administer 0.2% saccharin (Sac) or 0.2% saccharin with 15% ethanol (Sac/E) during self-administration, degradation of press-reinforcement contingencies, and after bilateral DLS infusion of the non-specific dopamine receptor antagonist  $\alpha$ -flupenthixol. More DLS excitations were observed in rats self-administering ethanol (Sac: 6/28 cells, Sac/E: 22/44 cells). Surprisingly, DMS neurons also demonstrated increased pre-reinforced press responses in Sac/E rats (Sac: 4/54 cells, Sac/E: 16/76 cells). DMS pre-press responses were reduced by contingency degradation in Sac/E rats, which showed blunted alcohol seeking after this exposure. In both Sac and Sac/E rats, DLS  $\alpha$ -flupenthixol infusion decreased the proportion of DMS cells with post-reinforced press responses. Thus, overlap between the DMS and DLS represented by similar neuronal response patterns to reinforcement may contribute to habitual behavior, and this may require dopamine transmission in the DLS. Funded by NIH (R01AA018008, T32NS007431, T32AA007573), and the UNC Bowles Center for Alcohol Studies.

**P124**

**Prevention of alcohol drinking using optogenetic and pharmacogenetic inhibition of CRF neurons in the central nucleus of the amygdala**

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Alcohol drinking in dependent but not in non-dependent rats has been shown to be dependent on activation of CRF1 receptor in the central nucleus of the amygdala, however we have recently observed that there is a strong activation of neurons in the central nucleus of the amygdala during withdrawal from alcohol binge drinking in non-dependent rats. Here we test the causal relationship between inactivation of CRF neurons in the central nucleus of the amygdala and alcohol drinking in non-dependent rats using optogenetic (halorhodopsin-specific inactivation in CRF-CRE transgenic rats) and pharmacogenetic (Daun02 inactivation in Fos-LacZ transgenic rats). Rats were tested for alcohol drinking using alcohol self-administration in 30min daily session (optogenetic) or using the intermittent access to two-bottle choice (pharmacogenetic). We found that both optogenetic inactivation of CRF neurons and pharmacogenetic inactivation of Fos-activated neurons in the central nucleus of the amygdala significantly decreased alcohol drinking in non-dependent rats. These results demonstrate that while activation of CRF1 receptor in the central nucleus of the amygdala is not required for alcohol drinking in non-dependent rats, activation of CRF neurons in the central nucleus of the amygdala contributes to alcohol drinking.

**P125****MT-7716 effectively blocks ethanol-induced increase in GABAergic transmission in the central amygdala**Marsida Kallupi<sup>1,2</sup>, Christopher S. Oleata<sup>1</sup>, George Luu<sup>1</sup>, Koji Teshima<sup>3</sup>, Roberto Ciccocioppo<sup>2</sup> and Marisa Roberto<sup>1\*</sup><sup>1</sup>Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, California, USA; <sup>2</sup>School of Pharmacy, Pharmacology Unit, University of Camerino, Via Madonna delle Carceri, 62032 Camerino, ITALY; <sup>3</sup>Research Laboratory I (CNS), Pharmaceutical Research Unit, R&D Division, Mitsubishi Pharma Corporation, 3-7-25, Koyata, Iruma, Saitama 358-0026, Japan

The GABAergic system in the central amygdala (CeA) plays a major role in ethanol dependence and the anxiogenic-like response to ethanol withdrawal. A large body of evidence shows that nociceptin/orphanin FQ regulates ethanol intake and anxiety-like behavior. In the rat, ethanol significantly augments CeA GABA release, whereas nociceptin diminishes it. Using electrophysiological techniques in an *in vitro* slice preparation, in this study we investigated the effects of a nonpeptidergic agonist of the NOP system, MT-7716, and its interaction with ethanol on GABAergic transmission in CeA slices of naïve rats. We found that MT-7716 dose-dependently (100nM-1mM) diminished evoked GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic potentials (IPSPs) and increased paired-pulse facilitation ratio (PPF) of these evoked IPSPs, suggesting a presynaptic site of action of the MT-7716 by decreasing GABA release at CeA synapses. The presynaptic action of MT-7716 was also supported by the significant decrease in the frequency of miniature inhibitory postsynaptic currents (mIPSCs) induced by the NOP agonist. Notably, MT-7716 prevented the ethanol-induced augmentation of evoked IPSPs. These data provide new evidence for an interaction between the nociceptin and GABAergic systems in the CeA and for the anti-alcohol properties of the NOP activation. The development of a synthetic non-peptidergic NOP receptor agonist such as MT-7716 may represent a useful therapeutic target for alcoholism. Funding for this study was supported by grants AA016895 and AA006420 to Marisa Roberto.

**P126****Association and replication study of *NPY2R* promoter variant rs6857715 with drinking and smoking behavior in patients and the general population**Jana Strohmaier<sup>1\*</sup>, Josef Frank<sup>1\*</sup>, Jens Treutlein<sup>1</sup>, Stephanie H. Witt<sup>1</sup>, Ludwig A. Hothorn<sup>2</sup>, Frank Schaarschmidt<sup>2</sup>, Thomas G. Schulze<sup>3</sup>, Til Stürmer<sup>4,5</sup>, Manfred Amelang<sup>6</sup>, Gu Zhu<sup>10</sup>, John B. Whitfield<sup>10</sup>, Markus M. Nöthen<sup>7,8</sup>, Marleen H. M. de Moor<sup>9</sup>, Nicholas G. Martin<sup>10</sup>, Marcella Rietschel<sup>1\*</sup>

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Animal studies have shown that the neuropeptide Y (NPY)-system plays an important role in stress-triggered substance abuse. Association studies in humans reported significant associations between alcohol dependence (AD) and cocaine dependence and the functional neuropeptide Y 2 receptor (*NPY2R*) promoter variant rs6857715. In the present study, we replicated the association with AD in a case-control sample of German descent. To examine the relevance of rs6857715 in the general population, we investigated its association with alcohol consumption and smoking quantity - both prevalent addictive behaviors - in a German population-based cohort. Rs6857715 was not associated with alcohol consumption, however, with smoking quantity at age 20. We also tested a possible influence of the personality trait neuroticism on this association. Neuroticism is correlated with smoking and individuals with high scores are known to be very stress-

sensitive. Neuroticism was significantly associated with rs6857715 and we observed a significant interaction between rs6857715, neuroticism, and sex on smoking quantity. In a replication sample of European descent from the general population of Australia, no association with drinking measures was observed. A marginally significant association was found with nicotine dependence phenotypes. In women, there was a nominally significant association between rs6857715 and neuroticism. We present independent replication for association between AD and rs6857715. Our findings in the general population suggest that the variant confers a risk for increased smoking quantity. While the findings in the German population indicate a possible interaction with stress sensitivity, this could only be replicated in women of the larger Australian sample.

**P127****The Role Of DNA Methylation In The Aetiology Of Alcohol Dependence**

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The genetic component of alcohol dependence (AD) is substantial but monozygotic twin discordance indicate a role for non-heritable differences that can be mediated by epigenetic processes. Because MZ twins share an identical DNA sequence, disease-discordant MZ twin pairs provide an ideal model for examining the contribution of environmentally driven epigenetic factors in disease. We carried out a genome-wide analysis of DNA methylation of 18 MZ twin pairs discordant for AD or alcohol abuse (AA), using peripheral blood DNA and differentially methylated CpG sites were validated with an independent method in 36 MZ AD/AA-discordant twin pairs. We further investigated the behavioural and physiological processes related to the most significant differential methylation, using functional magnetic resonance and personality traits assessment. We demonstrated that AD-related differential methylation is associated with risk personality traits as impulsiveness and novelty seeking as well as lower STN BOLD assessed during the stop signal task in adolescents. Overall our data provide evidence for the role for DNA methylation differences in mediating aetiology of AD and AA in MZ twins. We also characterised the mechanism of action of differential methylation that could lead to alcohol dependence by altering personality traits such as impulse control, impulsivity and novelty seeking that are risk factors in adolescence for future alcohol abuse.

**P128****Nf1 regulates compulsive-like drinking, GABA release in the central amygdala, and is associated with alcoholism in humans**Vez Repunte-Canonigo<sup>a\*</sup>, Melissa Herman<sup>b\*</sup>, Henry R. Kranzler<sup>c</sup>, Richard Sherva<sup>d</sup>, Joel Gelernter<sup>e</sup>, Lindsay A. Farrer<sup>d,f,#</sup>, Marisa Roberto<sup>b,h</sup>, Pietro Paolo Sanna<sup>#</sup>

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NF1, the causative gene of neurofibromatosis type 1, encodes a large multifunctional protein with Ras-GTPase activity termed neurofibromin. Neurofibromin acts as a negative regulator for Ras family small G proteins and the Ras-ERK signal transduction pathway. Alcohol acutely increases presynaptic GABA release in the central nucleus of the amygdala (CeA), and the transition to compulsive-like drinking associated with alcohol dependence is characterized by increased presynaptic GABA release in this brain region. Here, we show that Nf1 heterozygous null mice (Nf1<sup>+/−</sup>) do not

differ from wild-type mice in nondependent drinking behavior, including in a 24 hr 2-bottle choice (2BC) ethanol preference test and the drinking in dark (DID) binge drinking paradigm. However, following chronic intermittent ethanol vapor exposure (CIE), *Nfl*<sup>+/-</sup> mice did not display the escalation in drinking that was observed in WT mice. Since the transition to alcohol dependence is associated with increased presynaptic GABA release in the CeA, we investigated GABA release in the CeA of *Nfl*<sup>+/-</sup> and WT mice and found that *Nfl*<sup>+/-</sup> mice displayed an augmented baseline GABA release that was not increased by alcohol application like in wild-type mice. These data suggest that *Nfl* activity regulate compulsive-like drinking and spontaneous and ethanol-stimulated GABA release. Additionally, we observed that multiple variants in the human *NF1* gene are associated with a quantitative measure of alcohol dependence in both African Americans (AAs) and European Americans (EAs). The present results implicate *Nfl* as an important gene in the cellular and behavioral effects of alcohol and in the increased drinking associated with alcohol dependence in mice. In addition, genetic variations in *NF1* may confer an inherent susceptibility to the emergence of compulsive (dependent) drinking in humans.

### P129

#### Hypocretin-1 and -2 receptor antagonism reduces compulsive-like ethanol intake in rats

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Alcoholism is associated with increased stress sensitivity and negative emotional states, including dysphoria, anxiety, and depression that emerge during withdrawal. Hypocretin/orexin (HCRT) is a neuropeptide that has recently been associated with both stress and drug seeking behavior. However, the role of HCRT-receptor subtypes 1 and 2 (-1R and 2R, respectively) on escalated alcohol self-administration during alcohol dependence has yet to be examined. This study investigated the effects of HCRT-1R and -2R antagonism on alcohol intake in both dependent and non-dependent rats. Wistar rats were trained to self-administer ethanol (10% w/v) and then split into two groups: one group was made dependent by chronic, intermittent alcohol vapor exposure (i.e. dependent); the other group was not exposed to alcohol vapor (i.e. non-dependent, control). Upon stable post-vapor responding, rats were given the HCRT-1R antagonist SB-408124 (0, 7.5, 15, and 30 mg/kg; i.p.) or the HCRT-2R antagonist NBI-80713 (0, 3, 10, and 30 mg/kg; i.p.) in a within-subject Latin-square design and tested for ethanol self-administration. Results show that antagonism of both HCRT-1R and HCRT-2R elicited a dose-dependent reduction in ethanol self-administration in dependent rats, whereas ethanol intake in nondependent rats was unaffected. These findings demonstrate a functional role for HCRT signaling in compulsive-like alcohol drinking during dependence. Ongoing studies are also examining the effects of a mixed HCRT-1/2R antagonist, NBI-87571, on compulsive-like ethanol self-administration. Furthermore, additional molecular studies are investigating the anatomical underpinnings for HCRT actions in stress-related brain regions, including the extended amygdala, in alcohol dependence.

### P130

#### Simultaneous exposure to $\Delta^9$ -tetrahydrocannabinol and intoxicating doses of alcohol leads to marked neurotoxicity in young rats

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Epidemiological data indicate that alcohol and THC are largely co-abused, especially among young people, however very little is known about the potential neurodegenerative effects associated with their co-administration. Binge alcohol drinking has been shown to induce neuronal damage in young rats in discrete brain areas.  $\Delta^9$ -tetrahydrocannabinol (THC), the main active ingredient of cannabis, has been shown to impair working memory and cognition, which may suggest that neuronal damage may also occur

following THC exposure. The present study, carried out in young rats, explored the neurodegenerative effects of combined administration of intoxicating doses of THC (5 mg/kg, 10 mg/kg and an escalating dose, twice a day for 4 days) and alcohol (10-12 g/kg day). Fluorojade B staining was used for histological analysis of dead neuronal cells. Results showed high level of neurodegeneration in the group of rats exposed to alcohol especially in the dentate gyrus, entorhinal cortex, perirhinal cortex, olfactory bulb and infralimbic area. At the dose tested here, THC alone did not lead to evident neurodegenerative effects. However, combination of alcohol and THC led to the highest level of cell death and neurodegeneration. Notably, neuronal death in the medial orbital cortex, the ventrolateral orbital cortex and lateral orbital cortex were observed only in rats exposed to combined treatment with THC and alcohol. Altogether these findings indicate that simultaneous exposure to THC and alcohol may be particularly detrimental and may result in significant neurodegeneration and possibly impairment of cognitive functions. This study was supported by IMPACT PROJECT from DIPARTIMENTO POLITICHE ANTIDROGA (to RC).

### P131

#### Absence of LPA<sub>1</sub> signaling results in increased alcohol consumption

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The lysophosphatidic acid (LPA) is a bioactive molecule that belongs to the family of lysophospholipids. LPA<sub>1</sub> receptor is one of the six characterized G protein-coupled receptors (LPA<sub>1-6</sub>) through which LPA acts as an intercellular signaling molecule. Several studies have demonstrated that mice lacking the LPA<sub>1</sub> receptor display motor, emotional and cognitive alterations. In the present study we aimed to investigate the effects of the absence of LPA<sub>1</sub> signaling on ethanol consumption. To address this issue, male and female mice lacking the LPA<sub>1</sub> receptor and their littermate controls were given free access to water or an ascending series of ethanol concentration (3, 6, 9, 12 and 15%). Each ethanol concentration was offered for 4 days, with bottle position changed every day. Our data indicated that the absence of the LPA<sub>1</sub> receptor in male significantly increased ethanol consumption and the preference. However, this effect was only observed at lower concentrations in female mice. These results suggest that LPA<sub>1</sub> receptor or its absence along development might be involved in the regulation of alcohol intake. However, further studies are necessary in order to determine which mechanisms are involved in these effects as well as a better understanding of the sexual dimorphism observed in these animals. Supported by Instituto de Salud Carlos III, Red de Trastornos Adictivos UE-FEDER 2006 (RD06/0001/0000 and RD06/0001/1009) and 2012 (RD12/0028/0001 and RD12/0028/0009); Consejería de Salud, Junta de Andalucía (PI-0823/2012).

### P132

#### Activation of basolateral amygdala $\beta_3$ adrenoreceptors blocks fear conditioning, extinction learning, and the induction of synaptic plasticity

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We have previously demonstrated that  $\beta_3$ -adrenoreceptor activation selectively enhances lateral paracapsular (LPC) feed-forward inhibition in the basolateral amygdala (BLA) and that BLA microinfusion of a  $\beta_3$ -adrenoreceptor (AR) agonist reduces anxiety measures and ethanol drinking behaviors. As the BLA plays an integral role in the acquisition and extinction of conditioned fear, we hypothesized that  $\beta_3$ -AR enhancement of LPC inhibition might likewise decrease fear learning and extinction. To test this, we microinjected  $\beta_3$ -AR agonists into the BLA of adult male Long-Evans rats immediately prior to the acquisition or extinction of fear learning. As hypothesized, intra-BLA infusion of a  $\beta_3$ -AR agonist blocked fear learning and extinction. We next used ex vivo electrophysiology to

determine whether fear conditioning alters inhibitory plasticity at LPC synapses, and found that low-frequency stimulation of the external capsule induces a long-lasting depression of LPC IPSCs in BLA slices from naive animals, while having no significant effect on IPSCs recorded from fear conditioned animals. To examine whether  $\beta$ 3-AR potentiation of LPCs was sufficient to occlude BLA plasticity, we then examined the effect of low frequency stimulation of the external capsule on evoked field potentials. Initial results suggest that this stimulation protocol induces long-term potentiation of BLA field potentials, which is blocked by  $\beta$ 3-AR agonist pretreatment. These data imply that LPC inhibition plays a previously unrecognized role in fear learning. Moreover, given that chronic ethanol increases anxiety and disrupts fear conditioning, lateral paracapsular inputs may provide a novel target for treating some of the negative affective symptoms associated with alcohol use disorders. These studies were supported by National Institutes of Health grants R01 AA017531, P01 AA21099, and F31 AA022275

### P133

#### A selective NOP receptor agonist MT-7716 is a potent inhibitor of alcohol drinking and relapse to alcohol seeking in msP rats

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Even though some medications to treat alcohol addiction are currently available, only a minority of patients respond to these treatments and alcoholism remains to a large extent an unmet medical need. The development of new medications is highly desirable. From its discovery in 1995, the Nociceptin/orphanin FQ (N/OFQ) NOP receptor system has been extensively investigated as a promising target for new drugs in addiction. The encouraging results obtained with the endogenous ligand N/OFQ, burst research towards the development of novel brain penetrant NOP receptor agonists with a pharmacological and toxicological profile compatible with clinical development. Here we describe a novel NOP receptor agonist, named MT-7716, and its *in vivo* activity in preclinical models of excessive alcohol drinking and relapse. MT-7716 has high affinity for human NOP receptors expressed in HEK293 cells with a  $K_i$  value of 0.21 nM suggesting a full agonist activity. Chronic treatment (14 days) with MT-7716 (0.3-3 mg/kg) dose-dependently decreased voluntary alcohol intake in Marchigian Sardinian alcohol-preferring rats (msP). The effect became gradually stronger following repeated administrations, and was still significant at one week after discontinuation of the drug. Naltrexone (30 mg/kg) that was used as a comparator, also reduced ethanol intake, however the effect was limited to the 14-day treatment period. MT-7716 blocked cue- and yohimbine-induced reinstatement of alcohol seeking, suggesting that this drug is also effective on relapse. Finally, using alcohol dependent Wistar rats we demonstrated that MT-7716 significantly attenuated the expression of somatic alcohol withdrawal. Altogether, these findings indicate that MT-7716 is a promising drug for treatment alcohol abuse. Work supported by Mitsubishi Tanabe by grant AA014351 to FW and RC and by grant AA017447 to MR and RC.

### P134

#### Quantification of 10 neurosteroids in plasma following acute and chronic ethanol exposure in Withdrawal Seizure-Prone and -Resistant male mice

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Neurosteroids have rapid potent effects on  $\gamma$ -aminobutyric acid<sub>A</sub> receptor (GABA<sub>A</sub>)-mediated inhibition. With assistance from Dr. Leslie Morrow and her laboratory, we validated a gas chromatography mass spectrometry (GCMS) assay for simultaneous quantification of 10 neurosteroids in plasma and brain. In Withdrawal Seizure-Control (WSC) mice, basal hippocampal

allopregnanolone (ALLO) levels were increased 3.65 fold at 30 minutes following acute injection of 2 g/kg ethanol (EtOH). Current data in Withdrawal Seizure-Prone (WSP-1) and -Resistant (WSR-1) mice, which were selectively bred from WSC mice for high (WSP) and low (WSR) chronic ethanol withdrawal, quantified 10 neurosteroids in plasma following acute (2 g/kg) and chronic (72 hr vapor) EtOH exposure. Neurosteroid levels did not differ between the lines following saline injection, but corticosterone values suggested a basal level of stress. EtOH injection increased corticosterone by 44% (WSP) and by 99% (WSR). In WSP mice, acute EtOH decreased androstadiol, DHEA, and tetrahydrocorticosterone (THDOC) by 33-45% while pregnenolone and ALLO were unchanged. In WSR mice, there were similar decreases in androstadiol and THDOC while DHEA and pregnenolone were unchanged and ALLO was increased by 82%. Basal neurosteroid levels were higher in WSP versus WSR mice exposed to 72 hr air, but withdrawal from 72 hr ethanol vapor produced a similar pattern of changes in neurosteroid levels (decreases from 38-87%), suggestive of a subtle imbalance in neurosteroid synthesis. Analysis of hippocampal neurosteroid levels is underway, which we predict will reveal a larger imbalance in neurosteroid levels during withdrawal. Supported by AA012439 and VA Research.

### P135

#### Effect of early life stress and ethanol consumption on Pomc and Avp expression in rat hypothalamus

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Alcohol use disorder is a complex phenotype, for which both genetic and environmental factors contribute and interplay. Epigenetic mechanisms, such as DNA methylation, can serve to transform effects of environmental cues, such as early life stress and ethanol exposure, into stable changes in gene expression. Proopiomelanocortin (Pomc), arginine vasopressin (Avp) and oxytocin (Oxt) are key-players in the hypothalamus-pituitary-adrenal (HPA) axis response to early life stress and alcohol. Wistar rats were exposed to daily 15 or 360 minutes of maternal separation during postnatal day 1-21 or reared under normal animal facility conditions. In early adulthood, the rats had a free choice between water and ethanol for 2h three days/week. After 7 weeks of ethanol drinking the rats were sacrificed. Pomc, Avp and Oxt gene expression was analyzed using Q-PCR, and DNA methylation at the CpG island in the Pomc promoter region was assessed with restriction digestion and Q-PCR assay. Pomc gene expression but not DNA methylation in hypothalamus was negatively associated with early life stress, with voluntary ethanol intake and with the combination of early-life stress and voluntary ethanol drinking. A non-significant trend was observed for Avp with higher expression in the group of animals exposed to early life stress and ethanol, but no differences between groups for Oxt. Pomc gene expression is modulated by early life stress and ethanol. DNA methylation at the CpG island could not explain this effect indicating changes at other sites or other epigenetic regulation. Further analyses on epigenetic-related genes and the pituitary are on-going.

### P136

#### Amygdala dopamine D1 and corticotropin releasing hormone receptor 1 interactions in alcohol dependence

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Up-regulated corticotropin releasing hormone (CRH) and CRH receptor subtype 1 (CRHR1) signaling within the amygdala is critically involved in alcohol dependence. Within the amygdala there exist dopamine (DA) D1 and CRHR1 receptor rich neuronal populations - the intercalating cell

masses (ITC) - with important gating functions for intra-amygdala signal flow. Increasing evidence also indicates that amygdala DA receptors play a role in the modulation of emotional responses. Besides an up-regulation of CRHR1, D1 receptors are strongly and significantly down-regulated within ITCs of alcohol-dependent animals. Our experiments aimed 1) to establish an interaction between D1 and CRHR1 receptors in regulating emotional responses and 2) to demonstrate a maladaptive functional interaction between the two receptors in dependence. Stimulation of CRHR1 via intracerebroventricular and intra-amygdala injection of the highly selective agonist stressin I strongly upregulates D1 binding sites in all amygdala regions, especially within the ITCs. Such effect was not found outside the amygdala. This pharmacological interaction was accompanied by an expected anxiety-like response on the elevated plus maze test after CRHR1 activation that was fully blocked by pretreatment with D1 antagonist SCH23390. The functional consequences of these interactions on alcohol-related behavior became evident in knockout mice lacking CRHR1 expression in D1 neurons. While knockout mice and their litter mate controls did not differ in voluntary alcohol consumption, they are less vulnerable towards stress and stress-induced drinking in the post-dependent state. Together, these findings suggest that CRHR1 is synergistically acting with D1 in mediating anxiogenic responses, and thus point to a novel mechanism of amygdala physiology. In dependence, D1-CRHR1 receptor interactions seem to be involved in the hyper-responsivity to stress in abstinence. Our data give important mechanistic insight into amygdala functioning under normal conditions and in alcohol dependence. The nature of D1-CRHR1 receptor interaction is currently under investigation.

#### P137

##### **Yohimbine induces acute stress response and assesses the specific effect of mifepristone in ethanol self-administration**

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Stress has long established to play a critical role in mediating excessive alcohol intake and relapse in both animals and humans. Repeated alcohol use leads to stimulation of the hypothalamic–pituitary–adrenal (HPA) axis and the abrupt cessation of chronic use leads to significant changes in glucocorticoid release. The objective of this study was to determine whether the pharmacological stressor yohimbine will induce acute stress response to

assess the specific effect of mifepristone, a glucocorticoid receptor antagonist, in ethanol self-administration. Male, Long–Evans rats were tested for yohimbine-induced ethanol reinstatement and corticosterone (CORT) release. Both systemic and intra-CeA mifepristone administration suppressed yohimbine-induced reinstatement of ethanol-seeking, while only systemic injections attenuated sucrose seeking and circulating CORT levels were unaffected. We also did not observe adverse effects administering yohimbine together with mifepristone and ethanol. Regulating stress outcomes by acting on the glucocorticoid release may offer the possibility to develop a novel therapeutic approach that aim to diminish the central effect initiated in the HPA-axis transmissions. The use of mifepristone would provide a pharmacotherapeutic opportunity in promoting resilience and protecting the body from the dysregulation effects of acute stress in alcohol use disorders (AUDs). Our group is now translating these results in humans by evaluated if yohimbine can be a candidate for a stress-induced human laboratory study when administered with mifepristone and alcohol. *We would like to acknowledge the 5T32AA007459-28 grant from the National Institute Alcohol Abuse Alcoholism (NIDAAA) supports the work of Dr. Haass-Koffler, State California Medical Research UCSF, DoD:W81XWH-08-0016, W81XWH-10-1-0247, NIH:1R01AA017924-01 (SEB).*

#### P138

##### **Binge alcohol exposure during the 3<sup>rd</sup> trimester-equivalent increases levels of the anti-inflammatory cytokine IL-10 in the rat hippocampus**

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The neuroimmune system has been shown to play an important role in the refinement of developing neuronal circuits. Studies have linked alterations in the neuroimmune system to the pathophysiology of fetal alcohol spectrum disorder (FASD). This study aimed to determine changes in the neuroimmune system after postnatal alcohol exposure (PAE), a model of human 3<sup>rd</sup> trimester ethanol exposure. Rat pups were exposed to alcohol vapor from postnatal day (PD) 3 until PD 5 for four hours daily. The average pup blood ethanol concentration was  $90 \pm 18.2$  mM ( $n = 6$ ). At the end of the 4 hr exposure on PD 5, whole hippocampi were dissected from male rats and used for mRNA collection. We found that PAE significantly increased mRNA levels of the anti-inflammatory cytokine IL10 ( $p = 0.029$  by Mann Whitney U test, average  $2^{-CT}$  values for Air =  $0.0033 \pm 0.0009$ , PAE =  $0.016 \pm 0.008$ ,  $n = 4$  litters). In contrast, Levels of TNF and IL-1 $\beta$  were not significantly affected ( $p = 0.200$ , average  $2^{-CT}$  values for Air =  $0.0038 \pm 0.002$ , and for PAE =  $0.012 \pm 0.010$ , and  $p = 0.349$  average  $2^{-CT}$  values for Air =  $0.005 \pm 0.0037$ , and for PAE =  $0.011 \pm 0.0077$ , respectively;  $n = 3-4$ ). These findings suggest that PAE elicits a neuroprotective microglial response in the hippocampus that may help mitigate the damage induced by ethanol exposure. Ongoing studies are characterizing the impact of PAE on the neuroimmune system in other brain regions and are also assessing the possibility that levels of other cytokines are altered in ethanol-exposed animals



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