



8TH BIENNIAL MEETING

of the

***INTERNATIONAL DRUG
ABUSE RESEARCH SOCIETY***

Nice, France

September 26-30, 2022



8TH
BIENNIAL INTERNATIONAL DRUG ABUSE
RESEARCH SOCIETY

PROGRAM AND ABSTRACTS

Recent Advances in Drug Addiction

September 26 - 30, 2022
Hyatt Regency Nice Palais de la Mediterranee Hotel
Nice, France

Conference Organizers:
Syed Ali, Nick Gilpin, Olivier Deschaux, Barbara Mason, Sulie L. Chang,
Emmanuel Onaivi, Michael Kuhar, Antonio Noronha and
George Koob

The International Drug Abuse Research Society (IDARS) would like to thank the following organizations for their generous financial support of the meeting:



**SPECIAL THANKS TO
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El-Sholey Inc.**

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PROGRAM SCHECULE

Sunday, September 25, 2022

REGISTRATION AND WELCOME

3rd Floor

3:00 – 6:00 PM

Monday, September 26, 2022

- 8:00 – 2:00 PM Registration in 3rd Floor Conference Area
- 8:00 – 8:20 AM Welcome & Opening Remarks in Cotton Conference Room
Travel Award Presentations
George Koob/Syed Ali/Nick Gilpin
- 8:20 – 9:00 AM Plenary Lecture
Closing the Treatment Gap, and Alcohol Use Disorder Perspective
George Koob, NIAAA, Baltimore, Maryland, USA
- Moderator: Michael Kuhar, Emory University, Atlanta, Georgia, USA

SESSION I: THE STRESS-SIDE OF ALCOHOL AND OPIOID USE DISORDERS: INSIGHT FROM RODENT AND HUMAN STUDIES
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- Moderators: Leandro Vendruscolo (USA) and Barbara Mason (USA)
- 9:20 – 9:40 AM Acute effects of Glucocorticoid receptor antagonist Mifepristone on GABA signaling in the central nucleus of amygdala: Impact of alcohol history, Strain and Sex.
Sophia Khom, Department of Pharmaceutical Sciences, University of Vienna, Vienna, Austria.
- 9:40 – 10:00 AM Double-blind placebo-controlled trial of Glucocorticoid receptor antagonist treatment of alcohol use disorder.
Barbara Mason, Department of Molecular Medicine, SCRIPPS Reserach, La Jolla, California, USA
- 10:00 – 10:20 AM Converging evidence across species of a role for corticoosteroid sensitization in opioid dependence.
Leandro Vendruscolo, NIDA-IRP, Baltimore, Maryland, USA.
- 10:20 – 10:40 AM Stress-testing decision making in opioid addiction.
Silvia Lopez-Guzman, NIDA-IRP, Baltimore, Maryland, USA
- 10:40 – 11:00 AM COFFEE/TEA BREAK

SESSION II: BRAIN ADAPTATION TO CHRONIC OPIOID USE: FROM RODENTS TO HUMAN

Moderators: Renata Marchette (USA) and Daniele Caprioli (Italy)

11:00 – 11:20 PM **A role of dynorphin and k-opioid receptor systems on hyperalgesia, hyperkatifeia and opioid addiction-like behaviors.**
Renata Marchette, NIDA-IRP, Baltimore, Maryland, USA

11:20 – 11:40 PM **The Impact of continuously high versus intermittently high brain levels of heroin on incubation of craving.**
Daniele Caprioli, University of Rome, Rome, ITALY

11:40 – 12:00 PM **Acute and chronic effects of opioids on stress and reward processes in the human brain.**
Merie Eikemo, University of Oslo, OSLO, NORWAY

12:00 – 12:20 PM **The brain dopamine system in individuals taking medications for opioid use disorder.**
Peter Manza, NIAAA, Baltimore, Maryland, USA.

12:30 – 2:00 PM **LUNCH - ATRIUM**

SESSION III: FLASH TLK- STUDENT & POST-DOC

Moderator: Nick Gilpin (USA)

2:00 – 2:10 PM **Increased mechanical sensitivity following alcohol or sucrose forced abstinence in mice.**
Gaëlle Awad, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), Université de Strasbourg, Centre de La Recherche Nationale Scientifique, Strasbourg, FRANCE.

2:10 – 2:20 PM **Andrographolide, an NFκ inhibitor, increase cocaine self-administration behavior in rats.**
Hildenbrand Cecile, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), Université de Strasbourg, Centre de La Recherche Nationale Scientifique, Strasbourg, FRANCE

2:20 – 2:30 PM **Conditioned place preference in Zebrafish: beyond measuring drug reward.**
Hwei-Hsien Chen, Center for Neuropsychiatric Research, national Health Research Institute, Miaoli, TAIWAN

SESSION IV: THE ROLE OF STRESS AND STRESS SYSTEMS IN ALCOHOL DRINKING

Moderators: Marcus Weera (USA) and Roberto Ciccocioppo (Italy)

2:30 – 2:50 PM Central amygdala-lateral hypothalamus circuitry mediates stress-enhanced alcohol drinking in rats.

Marcus Weera*, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA. *Dr. Michael Kuhar Young Investigator Awardee

2:50 – 3:10 PM Exercise reduces stress-enhanced drinking in alcohol dependent mice: role of BDNF.

Howard Becker, Medical University of South Carolina, Charleston, South Carolina, USA.

3:10 – 3:30 PM Corticolimbic activity and the role of endocannabinoids in modulating stress reactive behaviors and subsequent alcohol drinking.

Laura Ornelas, University of North Carolina, Chapel Hill, North Carolina, USA.

3:30 – 3:50 PM Individual vulnerability to stress and development of excessive drinking and seeking in the rats.

Esi Domi, University of Camerino, Camerino, ITALY

4:00 – 4:20 PM COFFEE/TEA BREAK

SESSION V: PRECLINICAL MODELS OF STIMULANT AND OPIOID USE DISORDERS: MOLECULAR SUBSTRATE AND POTENTIAL TREATMENT STRATEGIES

Moderators: Jayanthi Sankar (USA) and Jean Lud Cadet (USA)

4:20 – 4:40 PM Modeling oxycodone use disorder in rats: biochemical and epigenetics consequences.

Jean Lud Cadet, NIDA-IRP, Baltimore, Maryland, USA.

4:40 – 5:00 PM The Dopamine D1/5 antagonist attenuates compulsive methamphetamine intake: involvement of dopaminergic systems and transcriptional mechanisms.

Jayanthi Sankar, National Institute on Drug Abuse-IRP, National Institutes of Health, Baltimore, Maryland USA

5:00 – 5:20 PM Effects of environmental enrichment and environmental mimetics on drug seeking behavior in rodents.

Nathalie Thiriet, Universite de Poitiers, Poitiers, FRANCE

5:20 – 5:40 PM **Sex differences in the effects of abstinence from oxycodone self-administration on PVT to NAc glutamatergic transmission.**
Yanaira Alfonso-Caraballo, *University of Minnesota, Minneapolis, Minnesota, USA.*

Tuesday, September 27, 2022

8:00 – 11:00 AM Registration desk open

SESSION VI: CANNABINOIDS AND ALCOHOL: IN-VIVO, EX-VIVO AND IN VITRO EFFECTS IN MICE, RATS AND ZEBRAFISH MODELS

Moderators: **Anna Bukiya (USA) and Emmanuel Onaivi (USA)**

8:30 – 8:50 AM Effects of simultaneous alcohol and tetrahydrocannabinol on cerebral artery.

Anna Bukiya, *University of Tennessee Health Science Center, Memphis, Tennessee, USA*

8:50 – 9:10 AM (-)THC exposure in zebrafish embryos results in altered behavior, physiology and gene expression in next generation animals.

Declan Ali, *University of Alberta, Alberta, Canada*

9:10 – 9:30 AM Unraveling the role of CB2 receptors on midbrain dopaminergic neurons on behavior.

Ana Canseco-Alba, *Institute National de Neurologia y Neurocirugia, México City, México*

9:30 – 9:50 AM Cannabinoids CB2 receptor neuro-immune crosstalk in alcohol consumption.

Emmanuel Onaivi, *NIDA-NIH and William Patterson University, Wayne, New Jersey, USA*

9:50 – 10:10 AM COFFEE/TEA BREAK

SESSION VII: CANNABINOIDS: microRNAs, INFLAMMATION, ADDICTION AND EXTRACELLULAR VESICLES

Moderators: **Mitzi Nagarkatti (USA) and Chemio Okoma (USA)**

10:10 – 10:30 AM scRNASeq and transcriptomic analysis of the role of cannabidiol in neuro- and intestinal inflammation in experimental multiple sclerosis.

Prakash Nagarkatti, *Department of Pathology, Microbiology and Immunology, University of South Carolina, Columbia, South Carolina, USA*

10:30 – 10:50 AM Cannabinoids decrease the lncRNA AW112010 that promotes the differentiation of inflammatory T cells by suppressing IL-10 expression through histone demethylation.

Mitzi Nagarkatti, Department of Pathology, Microbiology and Immunology,
University of South Carolina, Columbia, South Carolina, USA

10:50 – 11:10 AM Broad-spectrum antiemetic efficacy of a large dose of temsirolimus against diverse emetogens including the cannabinoid CB1 receptor inverse agonist/antagonist SR141716A.

Nissar Darmani, Western University of Health Sciences, Pomona, California
USA

11:10 – 11:30 AM Hippocampal mu opioids and cannabinoid 1 receptors are modulated following cocaine self-administration in male rats.

Katia Befort, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA),
CNRS, Université de Strasbourg, Strasbourg, France

11:30 – 11:50 AM SIV and delta-9-THC induced alterations in host miRNAome: insights from extracellular Vesicles.

Chioma Okeoma, Department of Pathology, Microbiology and Immunology,
New York Medical College, Valhalla, New York, USA

12:00 – 2:00 PM LUNCH BREAK - ATRIUM

SESSION VIII: Alcohol, Opioid, Nicotine and Cocaine: New Mechanistic Complexities and abundant opportunities for improving Therapy

Moderators: Eliot Gardner (USA) and Alex Dopico (USA)

2:00 – 2:20 PM A novel skin cell-based therapy for alcohol and/or cocaine use disorder.

Ming Xu, Department of Anesthesia and Critical Care, The University of
Chicago, Chicago, Illinois, USA

2:20 – 2:40 PM Is Brain Ghrelin a New Target for Anti-Addiction Medication?

Eliot Gardner, NIRP, NIDA-NIH, Baltimore, Maryland, USA.

2:40 – 3:00 PM Alcohol and pregnenolone interaction on cerebrovascular potassium channels of the BK type: a tale of two ligands and two channel subunits.

Alex Dopico, Department of Pharmacology, Addiction Science, and Toxicology,
The University of Tennessee Health Science Center, Memphis Tennessee, USA

3:00 – 3:20 PM Pharmacological properties of opioids are dependent on the G subunit mediating opioid receptor signaling.

Jean Bidlack, Department of Pharmacology and Physiology, University of
Rochester, School of Medicine and Dentistry, Rochester, New York, USA.

3:20 – 3:40 PM Boosting nicotine replacement efficacy with monoaminergic co-treatments.

Ed Levin, Department of Psychiatry and Behavioral Sciences, Duke University
Medical Center, Durham, North Carolina, USA.

3:40 – 4:00 PM COFFEE/TEA BREAK

4:00 – 5:00 PM IDARS Business Meeting

George Koob, Michael Kuhar, Nicolas Gilpin, Emmanuel Onaivi, Syed Ali

Wednesday, September 28, 2022

Conference Tour of Nice & Monaco

No Scheduled Conference activities

Thursday, September 29, 2022

SESSION IX: FROM MOLECULE TO CIRCUITRY IN DRUG ADDICTION

Moderators: Marisela Morales (USA) and Alban de Kerchove (Belgium)

8:30 - 8:50 AM Dorsal Raphe glutamatergic inputs to VTA and cocaine seeking behavior.
Marisela Morales, NIDA-IRP, Baltimore, Maryland, USA

8:50 - 9:10 AM VTA dopamine neuronal heterogeneity and morphine.
Barbara Juarez, University of Maryland, Baltimore, Maryland, USA

9:10 - 9:30 AM Mesolimbic dopamine signatures of relapse
Gavan McNally, School of Psychology, University of New South Wales, AUSTRALIA

9:30 – 9:50 AM Histone H2A monoubiquitination in the thalamus regulates cocaine use disorder.
Alban de Kerchove d’Exaerde, Directeur de Recherche, Fund for Scientific Research, FRS-FNRS, WELBIO, Neuroscience Institute, Universite Libre de Bruxelles, Brussel, BELGIUM

10:00 - 10:20 AM COFFEE/TEA BREAK

SESSION X: HOW REINFORCEMENT LEARNING SHAPES ADDICTION BEHAVIOR

Moderators: Celine Nicolas (FRANCE) and Brendan Tunstall (USA)

10:30 – 10:50 AM Fos-expressing neuronal ensembles in rat infralimbic cortex encode initial and maintained oxycodone seeking in rats.
Brandon Warren, Department of Pharmacodynamics, University of Florida, City, USA.

10:50 – 11:10 AM Intermittent access to operant self-administered alcohol promotes more “binge-like” alcohol consumption in rats.
Brendan Tunstall*, Department of Pharmacology, Addiction Science, and Toxicology, The University of Tennessee Health Science Center, Memphis Tennessee, USA ***Dr. George Koob Young Investigator Awardee**

11:10 – 11:30 AM Sex differences on incubation of cocaine craving after continuous and intermittent cocaine self-administration.
Celine Nicolas, University of Bordeaux, INSERM, Bordeaux, FRANCE

11:30 – 11:50 AM Evidence for Heroin-induced social isolation in the rats.

Daniele Caprioli, Department of Physiology and Pharmacology, Sapienza, University of Rome, Rome, ITALY.

11:50 – 12:10 PM **Backtranslation of human cocaine use patterns in rats.**

Morgan James, Department of Psychiatry, Robert Wood Johnson Medical School, Rutgers University, Rutgers, New Jersey, USA.

12:20 – 2:00 PM LUNCH

SESSION XI: CLINICAL AND PRE-CLINICAL VIEWS ON THE CONTRIBUTION OF THE INSULAR CORTEX TO ADDICTION

Moderators: **Anna Beyeler (France) and Wolfgang Sommer (Germany)**

2:00 - 2:20 PM **Insular mechanisms distinguishing flexibility phenotypes.**
Donna Calu, University of Baltimore, Baltimore, Maryland, USA

2:20 - 2:40 PM **Network analysis of brain activity in humans and rats implicates the insula in alcohol addiction.**
Wolfgang Sommer, University of Heidelberg, Heidelberg, GERMANY

2:40 – 3:00 PM **Anterior insula cortex inputs to the dorsolateral striatum govern the maintenance of binge alcohol drinking.**
Brady Atwood, Indiana University School of Medicine, Indianapolis, Indiana, USA.

3:00 - 3:20 PM **Asexual dimorphism of the posterior insular cortex in alcohol binge and compulsive drinking in mice.**
Celine Nicolas, University of Bordeaux, INSERM, Bordeaux, FRANCE

3:20 - 3:40 PM COFFEE/TEA BREAK

SESSION XII: INTERORGAN AND INTER CELLULAR CROSS TALK IN HIV AND DRUG ABUSE MEDIATED NEUROPATHOGENESIS

Moderators: Sabita Roy (USA) and Shilpa Buch (USA)

3:40 – 4:00 PM **Morphine mediated Neuroinflammations: Role of Astrocyte derived extracellular Vesicles.**

Shilpa Buch, *University of Nebraska Medical Center, Omaha, Nebraska USA*

4:00 – 4:20 PM **Brain and lung injury caused by alcohol and electronic cigarettes: Mechanisms of Deleterious effects on the blood brain and alveolar endothelial barriers.**

Yuri Persidsky, *Department of Pathology and Laboratory Medicine, Temple University Health Science Center, Philadelphia, Pennsylvania, USA*

4:20 – 4:40 PM **Opioid Induced Dysregulation of Gut Microbiota and Systemic Inflammation Accelerate HIV-Associated Premature Aging in a Mouse Model of HIV.**

Umakant Sharma, *Department of Surgery, University of Miami, Miami, Florida, USA*

4:40 – 5:00 PM **Morphine mediated Neuroinflammation involves astrocyte-specific activation of NLRP6 inflammasome signaling via miR-152.**

Palsamy Periyassamy, *University of Nebraska Medical Center, Omaha, Nebraska USA*

5:00 – 5:20 PM **Methamphetamine impairs neurogenesis of neural progenitor cells via activation of the FOXO3 signaling and induction of inflammatory reactions.**

Michael Toborek, *Department of Biochemistry and Molecular Biology, University of Miami Scholl of Medicine, Miami, Florida, USA*

5:20 – 5:40 PM **Microbiome implications in opioid withdrawal: Consequences of HIV infection.**

Sabita Roy, *Department of Surgery, University of Miami, Miami, Florida, USA.*

Friday, September 30, 2022

SESSION XIII: EXTRACELLULAR VESICLES, EPIGENETICS, NEUROIMMUNE SIGNALING, AND ALCOHOL
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Moderators: Antonio Noronha (USA) and Fulton Crews (USA)

8:30 - 8:50 AM **Epigenetics shifts in microglial and neuronal phenotype following adolescent intermittent ethanol (AIE) exposure in human AUD.**
Fulton T. Crews, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel, North Carolina, USA

8:50 - 9:10 AM **Identifications of exosomal cargo molecules involved in microglia induced neuronal death during ethanol-induced pathogenesis in the hypothalamus of fetal rats.**
Dipak Sarkar, Rutgers Endocrine Research Program, Department of Animal Sciences, Rutgers University School of Environmental and Biological Sciences, New Brunswick, New Jersey, USA

9:10 - 9:30 AM **Network meta-analysis on the mechanisms underlying alcohol-induced antinociception and pain.**
Sulie L. Chang, Institute of NeuroImmune Pharmacology, Department of Biological Sciences, Seton Hall University, South Orange, New Jersey, USA

9:30 – 9:50 AM **Extracellular vesicles, microglia, and epigenetics as potential therapeutic targets for AUD pathology.**
Leon Coleman, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel, North Carolina, USA.

9:50 - 10:10 AM **Inflammatory pain induces alcohol intake increase after forced abstinence in female rats: unravelling the role of the mu opioid receptor and neuroinflammation crosstalk.**
Lucia Hipolito*, Department of Pharmacy and Pharmaceutical Technology and Parasitology, University of Valencia, Burjassot, Spain. ***Institution of Neuroimmuno Pharmacology, Setton Hall University Young Investigator Awardee**

10:10 – 10:30 AM COFFEE/TEA BREAK

SESSION XIV: SIGMA-1 RECEPTOR REGULATION AND ADDICTION

Moderator: Hsian-en Wu (USA) and Yuko Yasui (USA)

10:30 – 10:50 AM **Sigma-1 receptor confers protein translational regulation after neuropathic pain.**

Hsian-en Wu, Cellular Pathobiology Section, INRB, IR, NIDA, NIH, Baltimore, Maryland, USA

10:50 – 11:10 AM **Sigma-1 receptor regulation of steroid hormones at the adrenal gland.**

Nino Sharikadze, Cellular Pathobiology Section, INRB, IR, NIDA, NIH, Baltimore, Maryland, USA

11:10 – 11:30 AM **Sigma-1 receptor regulates energy metabolism by impacting the NAD/NADH ratio: Potential relation to addiction.**

Simon Couly, Cellular Pathobiology Section, INRB, IR, NIDA, NIH, Baltimore, Maryland, USA

11:30 – 11:50 AM **Cocaine-induced functional deficit in orbitofrontal cortex is prevented by systemic administration of a sigma-1 receptor antagonist.**

Yuriko Kimura, Cellular Pathobiology Section, INRB, IR, NIDA, NIH, Baltimore, Maryland, USA

11:50 – 12:10 PM **Sigma-1 receptor is involved in cocaine-induced AMPA receptor synaptic plasticity in the VTA dopamine neurons.**

Yuko Yasui, Cellular Pathobiology Section, INRB, IR, NIDA, NIH, Baltimore, Maryland, USA

12:20 – 2:00 PM LUNCH

SESSION XV: ALCOHOL DEPENDENCE AND TOXICITY IN ADOLESCENT AND ADULTHOOD.

Moderators: Yousef Tizabi (USA) and Youssef Sari (USA)

2:00 – 2:20 PM **Overview of behavioral and neurobiological effects of alcohol.**

Yousef Tizabi, Department of Pharmacology, Howard University School of Medicine, Washington DC, USA.

2:20 – 2:40 PM **Adolescent ethanol exposure alters the adult response to drug of abuse.**

Sheketha Hauser, Indiana university School of Medicine, Indianapolis, Indiana, USA.

2:40 – 3:00 PM **Neurocircuits involved in alcohol dependence: neuropharmacological studies.**

Youssef Sari, College of Pharmacy, University of Toledo, Toledo, Ohio, USA.

3:00 – 3:20 PM **Alcohol consumption in adolescents vs adults: differences in attitudes, consequences and treatment.**
Bruk Getachew, Department of Pharmacology, Howard University Scholl of Medicine, Washington DC, USA.

3:20 – 3:40 PM **COFFEE/TEA BREAK**

SESSION XVI: IDENTIFICATION OF NOVEL MECHANISMS UNDERLYING ALCOHOL, OPIOID, AND NICOTINE ADDICTION USING SINGLE-CELL WHOLE-BRAIN IMAGING

Moderators: Olivier George (USA)

3:40 – 4:00 PM **Normalization of the functional connectome in alcohol dependent mice following treatment with a CFR-1 antagonist.**
Lieselot Carrette, Preclinical Addiction Research Consortium, Department of Psychiatry, UC San Diego, La Jolla, California, USA.

4:00 – 4:20 PM **Whole Brain mapping of neuronal ensembles of oxycodone seeking.**
Alexander Smith, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, New York, USA.

4:20 – 4:40 PM **Delineating the insula-centric negative affective circuitry engaged by stress and alcohol exposure.**
Samuel Centanni, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA.

4:40 – 5:00 PM **Hyperconnectivity of long-range cholinergic regions contributes to the reorganization of the brain functional connectivity during nicotine withdrawal.**
Olivier George, Preclinical Addiction Research Consortium, Department of Psychiatry, UC San Diego, La Jolla, California, USA.

SESSION XVII: 5:00 – 6:00 PM

PANEL DISCUSSION, SUMMARY & RECOMMENDATION

Barbara Mason, Nick Gilpin, Fulton Crews, Sulie L. Chang, Alex Dopico, Mitzi Nagarkatti, Eliot Gardner Marisela Morales, Yuri Persidsky, Anna Beyeler, Antonio Noronha, Olivier George and Syed Ali

7:00 - 10:00 PM
FAREWELL DINNER
Poolside Terrace

ABSTRACTS

PRELIMINARY LECTURE

Speaker: Goerge Koob

1. Closing the Treatment Gap, and Alcohol Use Disorder Perspective

George Koob

*National Institute on Alcohol Abuse and Alcoholism
Baltimore, Maryland USA*

Alcohol misuse and alcohol use disorder (AUD) cause an enormous amount of human suffering, loss of productivity and cost to our medical care system and the nation's economy all of which have been exacerbated by the COVID-19 pandemic. Recent developments include a 26% increase in alcohol-related deaths, 34% increase in the prevalence of hospital visits for alcohol withdrawal, and a 51% increase in hospitalizations for alcohol-associated hepatitis during the COVID-19 pandemic in the United States. In addition, there continues to be a significant treatment gap where less than 10% of individuals in need of treatment receive treatment for AUD and less than 2% receive one of the 3 FDA approved medications for treatment of AUD. Ongoing challenges include the increased drinking to cope with stress exacerbated by the COVID-19 pandemic, interaction of alcohol with mental health, the role of alcohol in women's health, alcohol and health in older adults, and research on recovery from AUD. Current priorities and challenges for closing the treatment gap include promoting medications development, expanding NIAAA resources for the public, development of a heuristic definition of recovery, expanding the uptake of screening, brief intervention and referral to treatment (SBIRT), exploring and expanding a role for telehealth in treatment, addressing stigma, addressing diversity, equity and health disparities in the alcohol field, and supporting the next generation of alcohol researchers. Addressing such challenges will facilitate the implementation of evidence-based treatment for AUD in primary care, mental health, and other health care settings.

SESSION I: THE STRESS-SIDE OF ALCOHOL AND OPIOID USE DISORDERS: INSIGHTS FROM RODENT AND HUMAN STUDIES

Co-Chairs: Dr. Leandro F. Vendruscolo and Dr. Barbara Mason

Speakers: Sophia Khom, Barbara Mason, Leandro Vendruscolo, Silvia Lopez-Guzman

2. Acute Effects of Glucocorticoid Receptor Antagonist Mifepristone on GABA Signaling in the Central Nucleus of the Amygdala: Impact of Alcohol History, Strain, and Sex

Sophia Khom

Department of Pharmaceutical Sciences, University of Vienna, Vienna, Austria

Elevated γ -aminobutyric acid (GABA) transmission in the central nucleus of the amygdala (CeA) is a key characteristic of chronic alcohol consumption across species. Recent studies have shown enhanced glucocorticoid receptor (GR) signaling and changes in GR-mediated transcriptional activity in the CeA that is associated with acute alcohol withdrawal and protracted abstinence. Whether and how these changes in CeA GR activity contribute to heightened GABAergic tone, however, are unknown. We used *ex vivo* slice electrophysiology and the GR antagonist mifepristone in rodent models of alcohol use disorder (AUD). We found that acute application of the GR antagonist mifepristone decreased GABA transmission more effectively in male Sprague-Dawley (SD) rats after chronic intermittent exposure (CIE) to alcohol vapor compared with alcohol-naive controls. Mifepristone also prevented the augmentation of GABA transmission in response to an acute alcohol challenge, suggesting that GR antagonism counteracts CeA neuroadaptations in response to chronic alcohol and blocks its acute effects. Interestingly, preliminary data showed that mifepristone decreased CeA GABA activity in female ethanol-naive rats but did not exert a significant effect in female rats after chronic alcohol exposure, suggesting different neuroadaptations in the GR system in response to alcohol in female rats. In contrast, we found that mifepristone decreased CeA GABA transmission in alcohol-preferring Marchigian-Sardinian (msP) rats of both sexes with similar efficacy, but we did not observe an effect in naive Wistar controls, suggesting greater mifepristone efficacy in animals that exhibit elevated CeA GABA tone. Altogether, these findings reveal the complex regulation of CeA GABA transmission by GRs under basal conditions and the recruitment of GRs with chronic alcohol exposure, providing critical mechanistic insights into the therapeutic potential of mifepristone for the treatment of AUD.

3. Double-Blind, Placebo-Controlled Trial of Glucocorticoid Receptor Antagonist Treatment of Alcohol Use Disorder

Barbara Mason

Department of Molecular Medicine, Scripps Research, La Jolla, CA, USA

A blunted cortisol response in alcohol use disorder (AUD) is associated with increase in alcohol craving and drinking. We previously found that mifepristone, a potent glucocorticoid receptor antagonist, increased blood cortisol levels and decreased responsivity to alcohol in preclinical and human laboratory models of AUD. The present study extended these findings to a treatment-seeking sample to learn how mifepristone could optimize AUD treatment outcome. We conducted a single-site, parallel-group, placebo-controlled study with random assignment to 1-week treatment with double-blind mifepristone or matched placebo (2:1 ratio) in 103 male and female (~40% female) outpatients with current AUD that was greater than or equal to moderate severity. All subjects received 8 weeks of AUD counseling. The number of drinks per day was the primary outcome measure and analyzed using latent growth models (LGMs). The LGM for drinks per day showed that mifepristone was most effective for decreasing drinking in individuals who did not have abstinence as a treatment goal, and effects increased as plasma drug levels increased. The effectiveness of mifepristone was apparent during the week on drug and for the following 2 weeks, consistent with the half-life of mifepristone. Effects were not detected thereafter. Individuals who entered treatment with abstinence as a treatment goal decreased drinking with counseling, regardless of whether they were assigned to mifepristone or placebo. Mifepristone significantly ($p < 0.05$) improved liver function test values and Pittsburgh Sleep Quality Index scores relative to placebo. Mifepristone's effects on drinking were equivalent between men and women. Future studies of pulsed dosing with 1-week of mifepristone at 3-week intervals may optimize long-term AUD treatment outcome. The significant effects of mifepristone on decreasing drinking while improving sleep and hepatic function are particularly of interest in the AUD population. Funding: NIAAA R01AA023152

4. Converging Evidence Across Species of a Role for Corticosteroid Sensitization in Opioid Dependence

Leandro F. Vendruscolo

Neurobiology of Addiction Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA

The global crisis of opioid overdose fatalities has led to an urgent search to discover neurobiological mechanisms of opioid use disorder (OUD). A driving force of OUD is the dysphoric and emotionally painful state (hyperkatifeia) that is produced during acute and protracted opioid withdrawal. Our hypothesis was that the sensitization of glucocorticoid receptor (GR) activity plays a functional role in addiction-like behavior. We investigated a mechanistic role for GR systems in the central nucleus of the amygdala (CeA) in driving opioid addiction using multiple preclinical models. We found that acute and chronic GR antagonism with mifepristone reduced opioid addiction-like behaviors in male and female opioid-dependent rats, without affecting behavior in nondependent rats. The increase in addiction-like behavior in dependent rats was accompanied by an increase in the firing of corticotropin-releasing factor neurons in the rat CeA (i.e., a marker of brain stress system activation), an effect that was decreased by mifepristone. An intra-CeA infusion of an antisense oligonucleotide that blocked the transcriptional activity of GR also reduced addiction-like behavior. Finally, we identified transcriptional adaptations of GR signaling in the CeA in humans with OUD. Thus, GRs, their coregulators, and downstream systems are functionally involved in opioid dependence and may be viable targets for treating the “stress side” of OUD. Funding: National Institute on Drug Abuse, Intramural Research Program

5. Stress-Testing Decision Making in Opioid Addiction

Silvia Lopez-Guzman

*Unit on Computational Decision Neuroscience, National Institute of Mental Health,
Intramural Research Program, National Institutes of Health, Bethesda, MD, USA*

Individuals with opioid use disorder (OUD) who are committed to treatment struggle to maintain their long-term goals of abstinence and protracted psychosocial wellbeing while facing acute stressors that can elicit relapse. For example, day-to-day fluctuations of stress have been shown to increase craving, which in turn were predictive of next-day drinking in a group of individuals with alcohol use disorder. One way that stress and craving could lead to relapse is by biasing decision-making away from long-term rewards in favor of immediate rewards, a computational algorithm that is known as delay discounting. Acute stress has been shown to increase delay discounting but primarily in individuals who are highly reactive to it. To explore the liability of delay discounting to these acute states in OUD, we stress-tested decision-making in a group of individuals with OUD who received methadone in an outpatient treatment service. Participants completed a delay discounting task under high and low levels of opioid craving. Heart-rate variability, arousal, and emotional valence were assessed during decision-making to capture physiological and emotional manifestations of low and high craving states. Thirty-one individuals with OUD who endorsed recent craving for heroin or other opioids were recruited to participate in two sessions: one session that was conducted before the participant received their medication (methadone) and one session that was conducted afterward. The order of the two sessions was randomized across subjects. In each session, we employed validated instruments to assess craving, subjective withdrawal symptom severity, and current levels of anxiety. The participants then completed a 12-min delay discounting task. We estimated a discount rate parameter for each session. Heart rate variability, skin conductance, and corrugator (necessary for frowning) and zygomatic (necessary for smiling) surface electromyography were measured during the task. The participants reported higher craving for opioids (heroin and methadone) in the session before the medication than in the session after the medication ($t = 3.66$, $p = 0.001$). Interestingly, physiological, and emotional responses were distinct as a function of choices that were made in the task and as a function of state. In low craving states, facial expression and arousal were sensitive to the type of choices that the participants made (immediate or delayed). However, in high craving states, emotional responses were desensitized, and heart rate variability was lower. Altogether, these results indicate that opioid users' decision making is affected by acute craving induction. Funding: National Institute of Mental Health, Intramural Research Program

SESSION II: BRAIN ADAPTATION TO CHRONIC OPIOID USE: FROM RODENTS TO HUMAN

MODERATOR: Renata Marchette

Speakers: Renata Marchette, Daniele Caprioli, Peter Manza, & Merie Eikemo

6. A role of dynorphin and κ -opioid receptor systems on hyperalgesia, hyperkatifeia and opioid addiction-like behaviors

Renata Marchette

National Institute on Drug Abuse Intramural Research Program, Baltimore, MD, USA

Chronic opioid intake leads to a negative emotional state during withdrawal (e.g., dysphoria, anxiety, sleep disturbances, irritability, and physical (hyperalgesia) and emotional pain), also known as hyperkatifeia, that is hypothesized to be the result of opponent processes and contribute to drug taking and seeking. Emerging evidence suggests that the dynorphin/ κ -opioid receptor (DYN/KOR) system plays a key role in hyperkatifeia and addiction-like behaviors. Here, we sought to further our understanding of the role of the DYN/KOR system in hyperalgesia and opioid addiction-like behavior in mice. We found that mice allowed extended access (6 h) to fentanyl vapor self-administration escalated their intake, were more motivated to work to obtain the drug, show greater hyperalgesia, and greater signs of precipitated withdrawal. A principal component analysis indicated the emergence of two independent behavioral constructs: “intake/motivation” and “hyperalgesia/punished seeking.” We then used these constructs to assess the role of DYN deletion and KOR antagonism on addiction-like opioid behaviors. Male and female C57BL/6J mice were allowed to self-administer vaporized fentanyl under long-access (LgA, 6 h) conditions. Mice escalated their fentanyl intake and showed increase motivation for fentanyl. The short-acting KOR antagonist, aticaprant (0-30 mg/kg, PO), failed to reduce fentanyl self-administration. Following three weeks of abstinence, a single treatment with the long-acting KOR antagonist norBNI (0-10 mg/kg, IP), 24 h before a self-administration session, significantly reduced the re-escalation of fentanyl intake. In another experiment, we trained male and female prodynorphin knockout (KO) and wildtype mice to self-administer vaporized fentanyl in LgA conditions. They escalated their fentanyl intake, but female pDyn KO mice escalated their intake faster and self-administered more fentanyl than wildtype (WT) mice. Although there was no difference on withdrawal-induced hyperalgesia in female mice, the male pDyn KO mice showed no hyperalgesia compared with WT mice. These data suggest that extended blockade of KORs is necessary to decrease opioid self-administration in dependent mice and the lack of the prodynorphin gene modulates intake and hyperalgesia constructs in a sex dependent manner.

7. The impact of continuously high versus intermittently high brain levels of heroin on incubation of craving

Daniele Caprioli

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Drug-seeking progressively increases during abstinence (incubation of drug craving). Most studies of this phenomenon used continuous access drug self-administration procedures. Recently, studies using intermittent access drug self-administration procedures showed increased motivation to self-administer and seek psychostimulants. In this talk we will discuss the similarities and differences between the intermittent and continuous access heroin self-administration procedures on heroin intake, patterns of heroin self-administration (and related modeled heroin brain concentrations) and incubation of craving after forced or voluntary abstinence. Our results suggests that intermittent heroin access in rats mimic critical features of heroin addiction: binge-like intake and high relapse vulnerability during early abstinence

8. The brain dopamine system in individuals taking medications for opioid use disorder

Peter Manza

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Individuals with chronic use of drugs, including opioids, have significant changes to the brain dopamine system, including reduction in striatal dopamine D₂ receptors. Indeed, prior positron emission tomography (PET) studies found that individuals actively using heroin have lower striatal dopamine D_{2/3} receptor availability and stimulant-induced dopamine release than controls. However, it remains unclear whether these deficits resolve after individuals reduce their illicit opioid use and are maintained on medications for opioid use disorder, including methadone and buprenorphine. This is an important area of study because these medications are underutilized, in part due to stigma driving the notion that they are just as unhealthy as heroin. Further, little is known about the dopamine D₁-like receptors in opioid use disorder, which are crucial for drug reward. Here, I will discuss novel results from an ongoing study that collects three PET scans in people with opioid use disorder maintained on either methadone or buprenorphine, and healthy controls: a D₁ receptor scan at baseline, and two sets of D₂ receptor PET scans after administration of either 60 mg oral methylphenidate or placebo (two separate days, blinded, order counterbalanced). We computed baseline striatal D₁ and D_{2/3} receptor availability as well as methylphenidate-induced striatal dopamine increases. Interim analyses of the data showed that in contrast to prior studies with individuals actively using heroin, patients maintained on methadone and buprenorphine did not have significantly lower striatal D_{2/3} receptor availability nor stimulant-induced dopamine release than controls. However, patients had a trend for higher striatal D₁ receptor availability than controls. The sample size was insufficient to compare sex differences or differences between buprenorphine and methadone. This study provides preliminary evidence that individuals maintained on opioid agonist or partial agonist treatment do not appear to have the deficits in D_{2/3} receptor availability nor in stimulant-induced dopamine increases previously reported in untreated patients. By documenting evidence of dopamine recovery with medication-based treatment for opioid use disorder, these findings could help reduce the stigma around the use of these medications. *Funding: National Institute on Alcohol Abuse and Alcoholism (ZIAAA000550). Conflict of interest: None.*

9. Acute and chronic effects of opioids on stress and reward processes in the human brain

Marie Eikemo

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The addiction potential of opioids is often ascribed to their ability to produce pleasure or euphoria. We and others have found that the human opioid system, parallel to rodent findings, modulates reward responsiveness. However, this modulation typically occurs in the *absence* of opioid-induced mood improvement, which is rare in experimental studies with healthy participants. The context and affective state of an animal can have profound effects on the rewarding effects of opioids. In humans, opioid drug effects are typically studied in a “neutral” laboratory context. Here, I will discuss novel results from several projects where we have tested how context and affective state shape opioid reward. I will present data on opioid effects in patients about to undergo surgery, from experiments using stress-manipulations in healthy participants and from outpatients receiving chronic opioid treatment. In a sample of day surgery patients, we unexpectedly found robust evidence against mood improvement following intravenous oxycodone or remifentanyl administered open-label in the minutes before surgery. However, the probability of a mood improvement following opioids was increased in patients with high pre-surgery anxiety and particularly for those with a history of prolonged opioid use. In an experimental placebo-controlled double-blind cross-over study, stress induction caused a robust increase in opioid self-administration in men, but not women. The effect of stress on abuse liability was independent of changes in mood and drug ‘liking’ and indicates that men may preferentially turn to opioid drugs in response to stress. These findings will be discussed together with results from studies on reward responsiveness in patients with chronic opioid use. Most people will receive an opioid drug for pain during their lifetime. The findings have implications for understanding how affective state and prior experiences influence abuse liability of commonly used opioid drugs. *Funding: ERC starting grant no. 802885 and the South-Eastern Norway Regional Health Authority project no. 2018035. Conflict of interest: None.*

SESSION III: STUDENT & POST-DOC FLASH TALK

MODERATOR: Nick Gilpin

SPEAKERS: Gaëll Awad, Cecile Hildenbrand, & Hwei-Hsien Chen

10. Increased mechanical sensitivity following alcohol or sucrose forced abstinence in mice

Gaëlle Awad, Anne-Sophie Aubry, Paul Chu Sin Chung, Isabelle Décosterd, Mary C. Olmstead, Katia Befort

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Alcoholic neuropathy is a devastating condition since it affects 65% of alcoholic use disorder (AUD) patients in the USA and is largely resistant to treatment. Alcoholic neuropathy develops following long term excessive alcohol intake with patients usually complaining of pain in the extremities. Preclinical animal models of alcoholic neuropathy have shown possible mechanisms in the periphery such as reduced density of unmyelinated or small myelinated fibers, decreased nerve conduction, and increased number of glial cells in the spinal cord. A few studies focused on brain mechanisms in pain related regions, identifying an increase of microglia in these sites. Neuropathic pain involves a sensory experience as well as emotional and cognitive components, confirming involvement of both peripheral and central nervous systems. The latter may occur through the mesocorticolimbic reward network such as the nucleus accumbens, the ventral tegmental area and the prefrontal cortex. In parallel, the reward system is also implicated in AUD. Therefore, the reward network may play a major role in neuropathic pain comorbidities. The biological intersection between neuropathic pain and AUD may involve neuroinflammatory signaling, as suggested by preclinical studies showing neuroimmune signaling in brain reward structures in spare nerve injury animal models, in preclinical experiments of ethanol consumption, binge drinking and preference. The goal of our study was first to model binge drinking in male and female mice, allowing us to investigate sex difference in the progression of ethanol intake. In parallel, we measured mechanical and thermal sensitivity to identify any dysregulation in nociceptive responses due to alcohol intake in each sex. Finally, we looked at alcohol deprivation effects on thermal and mechanical nociception and assessed the impact of withdrawal on nociceptive responses.

11. Andrographolide, an NFκB inhibitor, increase cocaine self-administration behavior in rats

Cecile Hildenbrand

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It is well established since several years that cocaine consumption induces many brain adaptations, which, at least partially, contribute to the development of addiction. It has been demonstrated that exposure to cocaine can increase, in different brain areas, several markers of neuroinflammation, such as the transcription factor nuclear factor kappa-light-chain enhancer of activated B cells (NFκB). However, the role of these regulations in cocaine consumption is still unclear. In this study, using cocaine intravenous self-administration (ivSA) in rats, we assessed the effect of long-term cocaine consumption on NFκB and, in turn, the effect of NFκB pharmacological inhibition on cocaine consumption. Using immunohistofluorescence, we measured, in different brain regions, the level of the phosphorylated form of NFκBp65 subunit on serine 536, a post-transductional modification strongly associated with NFκB activation, after one-month of daily cocaine ivSA sessions. Then, we tested the influence of systemic administration of andrographolide (AGL), an inhibitor of NFκB DNA binding, on cocaine intake and motivation for the drug. We show that cocaine consumption can increase levels of phosphorylated NFκBp65 in striatal regions and that AGL further increases both cocaine consumption and motivation for the drug. These results suggest that cocaine induced NFκB activation could be a mechanism involved in the limitation of cocaine consumption.

12. Conditioned place preference in Zebrafish: beyond measuring drug reward

Liao-Chen Chen, Ming-Huan Chan, Hwei-Hsien Chen

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Conditioned place preference (CPP) has been well demonstrated in zebrafish to investigate the drug reinforcement. However, extinction and reinstatement of CPP remains poorly applied in zebrafish. The present study described a bias CPP procedure in zebrafish, which can be used to produce extinction and reinstatement of drug-seeking behavior. Using this CPP paradigm, methamphetamine (MA) via oral gavage dose-dependently induced CPP. After confined and non-confined extinction training, CPP was extinguished. CPP could be robustly reinstated by MA priming after extinction. When CPP was re-tested after 14 days, no preference was observed, while a priming dose of MA still significantly could reinstate the extinguished CPP, indicating the unrelenting propensity to elicit MA-associated memory in zebrafish. Furthermore, the extinguished MA CPP could be reinstated by an acute stressor (spoon chasing) and the pharmacological stressor yohimbine. As shown in rodents, the dopamine D1 receptor antagonist SCH 23390 blocked the acquisition of MA CPP and the opioid antagonist naltrexone suppressed the priming-induced reinstatement of MA CPP in zebrafish, implying the similar CPP behavioral phenotypes and pharmacological responses between rodents and zebrafish. Finally, three synthetic cathinones, 3, 4-methylenedioxypyrovalerone (MDPV) and N-ethylpentylone (NEP), and pentylone, were applied in this CPP paradigm to compare their minimal effective dose for CPP, the number of training sessions to reach extinction, and the priming dose to induce reinstatement. The results suggest that the order of abused potential was MDPV > NEP > pentylone. This work demonstrates an incremental value of using CPP in zebrafish to study drug addiction.

SESSION IV: THE ROLE OF STRESS AND STRESS SYSTEMS IN ALCOHOL DRINKING

MODERATOR: Marcus Weera & Roberto Ciccocioppo

SPEAKERS: Marcus Weera, Howard Becker, Laura Ornelas, & Esi Domi

13. Central Amygdala-Lateral Hypothalamus Circuitry Mediates Stress – Enhanced Alcohol Drinking in Rats

Marcus Weera

Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA

In humans, avoidance symptomatology (i.e., persistent avoidance of stress-associated stimuli) following traumatic stress is associated with post-traumatic stress disorder (PTSD) and increased alcohol drinking. Using rats, our lab has shown that predator odor (i.e., bobcat urine) stress produces persistent avoidance of stress-associated stimuli in a subset of subjects, termed 'Avoiders', mirroring avoidance symptomatology in humans. Interestingly, Avoider rats display long-lasting increases in alcohol self-administration following stress exposure, a phenomenon that is absent in stress-exposed Non-Avoiders. Here, using a combination of retrograde tracing and RNAscope in situ hybridization, we found that a population of CeA CRF1 receptor-expressing neurons project to the lateral hypothalamus (LH), a brain region that modulates motivated behaviors. Using a combination of ex vivo optogenetics and electrophysiology, we showed that CeA-LH projections form functional GABAergic synapses with LH neurons. Using immunohistochemistry, we found that Avoider rats have more c-Fos+ CeA-LH neurons than Non-Avoiders after stress exposure. Using chemogenetics, we found that inhibition of CeA-LH neurons attenuates avoidance behavior in Avoiders, and that stimulation of CeA-LH neurons recapitulates avoidance in stress-naïve rats. Using slice electrophysiology, we found that CeA-LH neurons in Avoiders have greater intrinsic excitability than in Non-Avoiders. To delineate the role of the CRF1-expressing subpopulation of CeA-LH neurons in stress-enhanced alcohol drinking and avoidance behavior, we conducted a separate set of studies using a novel CRF1-Cre-tdTomato rat that we generated. In these studies, we found that stress produces c-Fos activation in CRF1+ CeA-LH neurons of Avoiders only, and that chemogenetic inhibition of these neurons during post-stress alcohol self-administration and elevated plus maze tests rescues the escalated alcohol self-administration and anxiety-like behavior in Avoider rats. However, inhibition of CRF1+ CeA-LH neurons did not affect conditioned avoidance of a stress-associated context in Avoider rats. Collectively, these results suggest that recruitment of CeA-LH neurons, including those that express CRF1 receptors, by stress supports an Avoider phenotypic profile, and that inhibition of CRF1+ CeA-LH neurons is sufficient for rescuing stress-induced escalation of alcohol drinking and anxiety-like behavior in Avoiders. Current work is focused on elucidating the role of subpopulations of LH neurons in supporting an Avoider phenotype. This work was supported by grants from NIAAA (R01 AA023305, R01 AA026531, R21 AA026022, and F32 AA027145) and the VA (BX003451).

14. Exercise Reduces Stress-Enhanced Escalated Drinking in Alcohol Dependent Mice: Role of BDNF

Howard C. Becker

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Stress is a significant factor in promoting alcohol intake and triggering relapse. We previously showed that forced swim stress (FSS) further enhances escalated drinking in mice with a history of chronic intermittent ethanol (CIE) exposure. This effect is accompanied by reduced BDNF expression in brain. Exercise is known to elevate BDNF levels in brain and recently we showed that exercise can reduce escalated drinking in CIE-exposed mice. This study was designed to examine whether exercise (wheel-running) attenuates stress-enhanced drinking in CIE-exposed mice. Mice were given 1-hr access to alcohol (15% v/v) vs. water and then 1-hr later, wheels were placed in the home-cage of wheel-running mice for 2-hr. After 6 weeks, wheel-running (WH) and no-wheel-running (NWH) mice were divided into CTL, CIE, FSS, and CIE+FSS groups (N= 7-10/group). Weekly cycles of alcohol vapor (CIE) or air (CTL) exposure (16-hr/day x 4 days) were alternated with weekly test drinking sessions, where half the CTL and CIE mice received FSS (10-min) 4-hr prior to the drinking sessions (FSS and CIE+FSS groups, respectively). Wheel-running mice had daily 2-hr access to a wheel 1-hr following the test drinking sessions. Results indicated that CIE exposure increased alcohol intake over baseline and this was attenuated by wheel-running during Test-3 (NWH-CIE: 62.3±22.4% vs. WH-CIE: 30.1±16.6%) and Test-4 (NWH-CIE: 79.9±20.7% vs. WH-CIE: 49.9±16.0%). Exercise (wheel- running) also attenuated stress (FSS)-enhancement of CIE-induced drinking during Test-3 (NWH-CIE+FSS: 86.0±9.6%: vs. WH-CIE+FSS: 28.7±6.1%) and Test-4 (NWH-CIE+FSS: 102.5±14.9% vs. WH-CIE: 51.1±10.7%). FSS did not alter alcohol intake relative to CTL regardless of access to wheel-running. Taken together, these data show that exercise (wheel- running) can not only attenuate CIE-induced escalated alcohol intake, but also block the ability of stress (FSS) to further enhance CIE-induced alcohol intake. Supported by NIAAA grants (U01 AA014095, U24 AA020929, R01 AA026536, P50 AA010761) and VA Medical Research (BX000813).

15. Corticolimbic Activity and the Role of Endocannabinoids in Modulating Stress Reactive Behaviors and Subsequent Alcohol Drinking

Laura C. Ornelas

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Individual differences in response to stress suggest resilient and susceptible populations, which may be important for understanding the high comorbidity of post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD). We have previously shown sex and individual differences in stress-reactive behaviors during exposure to the synthetically produced predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT). Female rats that engaged in greater active coping behaviors (digging) during TMT exposure showed increases in alcohol self-administration, indicating a lasting consequence of stress. In contrast, males and females that engaged in greater passive coping behavior (immobility) during the TMT exposure showed decreased or no increases in alcohol drinking. Therefore, the present work begins to determine underlying neural circuitry and mechanisms that may modulate these coping behaviors. We examined 1) the relation between neuronal activation in corticolimbic regions (prelimbic, PrL; basolateral amygdala, BLA; central amygdala, CeA) and stress-reactive behaviors during TMT exposure, and 2) the role of endocannabinoids in the PrL in modulating stress-reactive behaviors during TMT exposure to prevent alcohol drinking after stress. To examine the association between neuronal activation in the PrL, BLA and CeA and stress-reactive behaviors during TMT exposure, female Long-Evans (n=20) rats were exposed to TMT. Brains were collected and c-Fos expression was quantified. Rats that engaged in greater digging behavior compared to immobility showed decreased c-Fos IR in the PrL, BLA and CeA. Next, we aimed to examine a role of the endocannabinoids in stress-reactivity and alcohol self-administration. In a separate experiment, female Long-Evans rats were implanted with bilateral cannulae aimed at the PrL. After 1 week recovery from surgery, rats were trained on operant alcohol self-administration (SA). After the last day of alcohol SA training, rats received intra-PrL injection of 0 or 2.5 µg/µL JZL-184 (MAGL inhibitor) 30 min prior to water or TMT exposure; (N=60). Rats returned to alcohol self-administration 2 weeks after TMT exposure, for 30 sessions. During TMT exposure, all rats treated with JZL-184 prior to TMT exposure engaged in greater immobility behavior and less digging behavior, suggesting that increasing 2-AG regulates adaptive responses to TMT and can prevent engagement in maladaptive responses to TMT. Together, these data suggest reduced neuronal activation in key corticolimbic regions may lead to engagement in maladaptive coping responses to TMT. In addition, these data implicate an important role of the eCB system in the PrL in promoting adaptive responses to stress and a potential in mitigating against later increases in alcohol self-administration.

16. Sex Differences in Stress Response and Alcohol Abuse Vulnerability in Wistar and Marchigian Sardinian Alcohol-Preferring Rats

Esi Domi

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Alcohol Use Disorder (AUD) is a chronic, relapsing disease associated with a significant burden of medical consequences and high socioeconomic costs. Although AUD is more prevalent in men, the number of women with AUD is rapidly increasing and many of the harmful health effects occur more rapidly and severely in women. In the majority of preclinical studies, sex differences are still poorly considered, leading to a gender gap in understanding the mechanisms that drive excessive drinking linked to stress in women. Here we evaluated the influence of stress on alcohol-related behaviors in male and female Wistar and Marchigian Sardinian alcohol-preferring (msP) rats. Results demonstrated that msP rats consume larger amounts of alcohol compared to Wistars with females drinking more than males. No sex differences in alcohol consumption were observed in Wistars. In the msP line we also found that in male rats alcohol alleviated generalized anxiety while in females it reduced the expression of post traumatic (PTSD)-like symptoms in a fear conditioning paradigm. Next, using a model of maternal separation, we found that repeated mild social deprivations experienced during the third postnatal week did not affect the motivation for alcohol later in life in male and female msP and Wistar rats. In response to administration of the pharmacological stressor yohimbine (0.312, 0.625, 1.25 mg/kg) msP and Wistar rats enhanced their alcohol drinking independently from sex. Yohimbine-induced reinstatement of alcohol seeking was observed in all groups of rats. The maternal separation experience enhanced this effect of yohimbine only in female msPs. In basal condition, females of both rat lines had higher blood levels of corticosterone (CORT) compared to males; alcohol consumption reduced CORT in females but not in males. Finally, female rats were more sensitive to the selective blockade of glucocorticoid receptor by mifepristone and CORT113176. More in general, msP rats were less sensitive to this pharmacological manipulation compared to Wistars. Overall, these findings show a different sensitivity to stress in male and female alcohol drinking rats. In females, alcohol intake seems to be associated with stress and PTSD-like conditions, while in males it is more likely linked to generalized anxiety. Overall, the data suggest that the impact of stress on alcohol drinking and seeking is more pronounced in msP rats compared to Wistars. Support grant AA017447 (to MR).

SESSION V: PRECLINICAL MODELS OF STIMULANT AND OPIOID USE DISORDERS: MOLECULAR SUBSTRATE AND POTENTIAL TREATMENT STRATEGIES

MODERATOR: Jayanthi Sankar & Jean Lud Cadet

SPEAKERS: Jean Lud Cadet, Jayanthi Sankar, Nathalie Thiriet, & Yanaira Alfonso-Caraballo

17. Modeling oxycodone use disorder in rats: biochemical and epigenetic consequences

Jean Lud Cadet

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The molecular and biochemical signatures of oxycodone on the brain remain to be fully characterized despite being in use for many decades. My laboratory has used the drug self-administration models in rats to identify oxycodone-induced changes in the rat striatum. Male Sprague-Dawley rats were trained to press a lever to self-administer oxycodone or saline for 3h (short access, ShA) or 9h (long access, LgA) training sessions. At various intervals after the end of oxycodone SA, we isolated tissues from the dorsal striatum to perform postmortem biochemical and molecular analyses. Rats in the LgA, but not the ShA, group exhibited escalation of oxycodone SA. The LgA, but not the ShA, phenotype showed time-dependent increases in oxycodone seeking during 31 days of forced abstinence. Rats from both LgA also exhibited decreased levels of striatal mu opioid receptor protein levels in comparison to saline and ShA rats. LgA rats showed increased striatal protein phosphorylation of mitogen and stress activated kinases 1 and 2 (MSK1/2). Histone H3, phosphorylated at serine 10 and acetylated at lysine 14 (H3S10pK14Ac), a MSK1/2 target, showed increased abundance only in LgA rats. RT-qPCR analyses revealed increased AMPA receptor subunits, GluA2 and GluA3 mRNAs, in the LgA-H rats. GluA3 mRNA expression correlated positively with changes in pMSK1/2 and H3S10pK14Ac. These findings also suggest that escalated oxycodone-induced MSK1/2-dependent histone phosphorylation might serve to regulate striatal AMPA gene expression. These observations offer potential avenues for interventions against oxycodone addiction.

18. The dopamine D1/5 antagonist attenuates compulsive methamphetamine intake: involvement of dopaminergic systems and transcriptional mechanisms

Jayanthi Sankar

National Institute on Drug Abuse, Intramural Research Program, Baltimore, Maryland USA

Methamphetamine (METH) use disorder (MUD) remains a persistent public health menace. Perturbations in striatal dopamine (DA) homeostasis are thought to underlie the behavioral and pathobiological consequences of METH use disorder in humans. To identify potential consequences of long-term METH exposure, we modeled the adverse consequence DSM criterion of substance use disorders by giving footshock to rats that had escalated their intake of METH during a drug self-administration procedure. Next, DA D1 receptor antagonist, SCH23390 were injected. Footshocks split the METH rats into two phenotypes: (i) shock-sensitive that decreased their METH-intake and (ii) shock-resistant that continued their METH intake. SCH23390 caused substantial dose-dependent reduction of METH taking in both groups. Stopping SCH23390 caused re-emergence of compulsive METH taking in shock-resistant rats. Compulsive METH takers also exhibited greater incubation of METH seeking than non-compulsive rats during withdrawal from METH SA. Analyses of DA metabolism revealed non-significant decreases (about 35%) in DA levels in resistant and sensitive rats. However, striatal contents of the deaminated metabolites, DOPAL and DOPAC, were significantly increased in sensitive rats. Cysteinyl-DA, a byproduct in the nonenzymatic formation of DA quinone was also increased in the sensitive rats. VMAT2 and DAT protein levels were decreased in both phenotypes. Moreover, protein expression levels of the D1-like DA receptor, D5R, and D2-like DA receptors, D3R and D4R, were significantly decreased in the compulsive METH takers. RNA sequencing studies also revealed substantial differences in the expression of genes between shock-resistant and sensitive rats. We identified 492 significantly differentially expressed genes that are involved in p13-AKT, cAMP and MAPK signaling pathways. Other differentially expressed genes participate in glutamatergic signaling. Our results parallel findings in post-mortem striatal tissues of human METH users who develop Parkinsonism after long-term METH intake and support the use of this model to investigate potential therapeutic interventions for METH use disorder.

19. Environmental enrichment and relapse to addiction: focus on stress mechanisms

Nathalie Thiriet

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Accumulating evidence indicates that environmental enrichment (EE) has powerful beneficial effects on drug addiction. In fact, exposing animals to EE during adolescence decreases the rewarding effects of drugs in conditioned place preference procedures and reduces cocaine self-administration at adulthood. In addition, exposure to EE during periods of abstinence from drugs eliminates already developed addiction-like behaviors and reduces the risks of relapse. These preventive and curative effects of EE are associated with neuroadaptations such as alteration of neurotransmitter levels, changes in gene expression and transcription factors, chromatin rearrangement, and stimulation hippocampal neurogenesis in several brain areas. These effects could be a result of EE-induced modulation of the activity of the stress system. Consistent with this hypothesis, we have recently demonstrated that the effects of EE on relapse to methamphetamine are associated with changes in the levels of glucocorticoid receptors (GR) in the brain and that GR antagonists mimic the effects of EE.

20. Sex differences in the effects of abstinence from oxycodone self-administration on PVT to NAc glutamatergic transmission

Yanaira Alonso-Caraballo

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Biological sex is an important factor in risk of misuse and development of opioid use disorder (OUD), but the neurobiological mechanisms are not known. In OUD, craving and relapse are often triggered by exposure to the drug itself or to drug-associated cues. Glutamatergic synaptic transmission within the nucleus accumbens (NAc) underlies cue-driven reward-seeking behaviors, and recent evidence shows that synaptic plasticity within projections from the paraventricular nucleus of the thalamus (PVT) to the NAc shell (NAcSh) is required for the expression of morphine withdrawal in male mice. However, it is unknown whether abstinence from opioid self-administration (SA) results in similar PVT-NAcSh plasticity and if sex differences in synaptic mechanisms exist in rats. Here, we determined the effects of short and long abstinence from oxycodone SA in male and female rats. Our goal was to determine sex differences in the PVT-NAcSh pathway and how it is affected by various timepoints of opioid abstinence, incubation of craving, and ovarian hormones. We hypothesized that glutamatergic transmission from PVT-NAcSh will be enhanced in both male and female rats after prolonged abstinence from oxycodone SA. Our preliminary findings suggest that the effects of abstinence from oxycodone SA on PVT-NAcSh synaptic transmission are time-dependent. Collectively, these data suggest that prolonged abstinence from oxycodone self-administration enhances PVT-NAcSh synaptic plasticity in male and female rats, but it is stronger in females.

SESSION VI: CANNABINOIDS AND ALCOHOL: IN-VIVO, EX-VIVO AND IN-VITRO EFFECTS IN MICE, RATS, AND ZEBRAFISH MODELS

MODERATOR: Anna Bukiya & Emmanuel Onaivi

SPEAKERS: Anna Bukiya, Declan Ali, Ana Canseco-Alba, Emmanuel Onaivi

21. Effect of simultaneous alcohol and delta-9-tetrahydrocannabinol administration on cerebral artery diameter

Alexandria Slayden, Steven Mysiewicz, Kelsey North, Alex Dopico, **Anna Bukiya**

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Alcohol (ethanol, ethyl alcohol) and cannabis (marijuana plant, *Cannabis sativa*) are among the most widely consumed recreational substances in the world. With increased efforts towards legalization of marijuana and expansion of cannabis preparations for medical use, there is an alarming trend towards the simultaneous use of ethanol and marijuana. While each drug alters distinct physiological functions, the consequences of their simultaneous use are often unique and distinct from mere summation of effects from each drug. While there are reports of individual effects of alcohol and cannabis on brain perfusion and cerebral artery function, consequences of simultaneous use on cerebral artery diameter have never been studied. Thus, we set to address the effect of simultaneous application of ethanol and Δ -9-tetrahydrocannabinol (THC) on cerebral artery diameter. We chose the middle cerebral artery (MCA), which irrigates temporal and parietal cortical areas, and several critical deep brain structures. Indeed, MCA irrigates the largest brain territory when compared to any other cerebral artery stemming from the circle of Willis. Moreover, MCA and its collaterals are often targets of drug-induced ischemic events. In males, ethanol mixed with THC resulted in greater constriction of *ex vivo* pressurized MCA when compared to the effects exerted by separate application of each drug at toxicologically relevant concentrations of 50 mM and 42 nM, respectively. In females, ethanol, THC, or their mixture failed to elicit measurable effects despite that female and male arteries exhibited similar degree of constriction by 60 mM potassium chloride, a depolarizing and vasoconstricting agent. Vasoconstriction by ethanol mixture with THC in arteries from males was abolished by endothelium removal, pharmacological block of calcium/voltage-gated potassium channels (BK-type), and either cannabinoid receptor 1 or cannabinoid receptor 2 blockers. Inhibiting either prostaglandin production or endothelin receptors blunted constriction by THC-ethanol mixture in arteries from males. Additionally, cranial window data showed that *in vivo* constriction of male MCA by the THC-ethanol mixture did not differ from ethanol alone. In females, the MCA *in vivo* constriction by ethanol alone was significantly smaller than that in males. However, difference in artery constriction by the THC-ethanol mixture in females versus males did not reach statistical significance. Our data demonstrate that simultaneous application of a THC-ethanol mixture elicits complex effects on cerebral artery diameter, which include both local and systemic components.

22. (-)THC exposure in zebrafish embryos results in altered behavior, physiology and gene expression in next generation animals

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Cannabis is one of the most commonly used illicit recreational drugs. Up to 14% of pregnant females aged between 12-44 have used cannabis during their first trimester. Research in our lab has focused on understanding the effects of cannabinoids on developing organisms. In this study we asked whether the primary isoform of THC found in cannabis ((-) trans δ^9 THC (referred as (-) THC afterwards)) has long-term effects on animals following a brief exposure during early development. Additionally, we asked whether the next generation of animals are also impacted by early exposure to (-) THC. In this study we exposed zebrafish embryos to (-) THC during gastrulation (5.5-hour exposure from 5.25 hours post fertilization to 10-75 hours post fertilization). We found that (-) THC alters the gross morphology, heart rate, MN branching and locomotion similar to other cannabinoids we have previously examined. Interestingly, (-) THC (>0.5 mg/L)-treated zebrafish did not survive past 15 days of development, possibly due to improper development of the swimbladder or a reduced ability to swim. RNAseq analysis revealed a significant downregulation of key developmental genes in (-) THC-treated animals including hedgehog genes such as *ptch2*, *smo*, *gli1* and *gili2b*. In fact, more than 1000 and 1400 genes were downregulated and upregulated respectively in (-) THC-treated embryos. We asked whether the brief exposure of cannabinoids had persistent effects into later life stages. (-) THC-treated embryos were reared to adulthood and behavioral tests such as the open field, novel object approach and shoaling tests were performed. (-) THC-treated adults exhibited anxiety-like behavior that was largely similar to control animals. However, when we examined the next generation of animals (F1 generation), we found that they exhibited reduced locomotion compared with untreated controls. These findings suggest that brief exposure to (-) THC has effects that persist into adulthood and even into the next generation of animals.

23. Unraveling the role of CB2 receptors on midbrain dopaminergic neurons on behavior

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The generation of the conditional knockout (cKO) mice with specific deletion of CB2R in dopamine midbrain neurons has been crucial for the understanding of this receptor in the modulation of neuronal function and behavior. This is providing further insight on the emerging and expanding endocannabinoid system (ECS) to endocannabinoidome (eCBome). In these studies, we investigated the neurodevelopmental and behavioral characteristics of the DAT-*Cnr2* mice in basal condition and in response to drugs of abuse. One of the most compelling results is that DAT-*Cnr2* mice are highly hyperactive through their life span in comparison to the wild type (WT) controls. It turns out that this hyperactivity is susceptible to be reduced following the administration of a low dose of amphetamine. This result, along with other experiments, allowed us to conclude that DAT-*Cnr2* cKO mice may be a possible model for studying ADHD. Another striking result is that DAT-*Cnr2* cKO mice are insensitive to the rewarding properties of ethanol but not cocaine, implicating a differential role of this receptor in the rewarding properties of drugs of abuse. Specifically, the exposure to cocaine and ethanol prenatally altered some of the neurodevelopmental features. For example, DAT-*Cnr2* mice started walking before the WT mice. This feature is consistent with impulsivity, supporting the hypothesis that CB2R acts as a “break” in dopaminergic transmission, resulting in impairments in motor function regulation. DAT-*Cnr2* mice also exhibit a reduction in anxiety like behavior, implicating CB2R with emotional regulation as well. Thus, cannabinoid type 2 receptors may be possible targets in CNS disorders associated with dopaminergic dysregulation.

24. Cannabinoid CB2 receptor neuro-immune crosstalk in alcohol consumption

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This is a collaborative effort using multidisciplinary approaches between several laboratories. The emerging endocannabinoid system (ECS) provides a platform for an expanded endocannabinoidome (eCBome), with renewed interest in targeting components of the eCBome in Alcohol Use Disorders (AUDs), addictions, and CNS disturbances associated with neuroimmune dysregulation. In this study, we generated two types of conditional knockout (cKO) mice – those with cell type specific deletion of CB2Rs in dopamine (DA) neurons (DAT-*Cnr2*) and those with deletion of CB2Rs in microglia (CX3CR1-*Cnr2*). DAT-*Cnr2* cKO mice displayed exaggerated hyper-psychomotor responses and were insensitive to the rewarding effects of alcohol but not cocaine; whereas CX3CR1-*Cnr2* cKO mice failed to display hyperactivity but were sensitive to the rewarding properties of alcohol and psychostimulants and exhibited, increased weight gain compared to the wild type (WT) controls. This implicated differential phenotypic effects of CB2Rs. We therefore next examined the neuro-immuno-eCBome basis for the observed differential changes and the opposing CB2R roles in neurons and microglia cells in the context of alcohol induced behavioral effects. CB2R mediated neuro-immuno-eCBome alterations in the brain were determined by flow cytometry. Our findings demonstrated that CB2Rs in DA neurons and microglia upregulated the expression of NLRP3 inflammasome pathway including NLRP3, cleaved – caspase 1, and mature IL 1 β in the striatal region compared with the WT C57BL/6J mice. Brains of CX3CR1-*Cnr2* cKO and WT mice were examined for activation of microglia and for immune infiltration after lipopolysaccharide (LPS - 0-48hrs) exposure. Microglia activation using markers for M1 (pro-inflammatory) and M2 (anti-inflammatory) were higher in CX3CR1-*Cnr2* cKO mice while there were differential immune infiltration cells based on CD45hi and LY6C expression. We also observed increased expression of pro-inflammatory cytokines TNF- α , IL-6, IL-1 α and IL-1 β in the frontal cortices of the cKO mice, following subacute treatment with 8% alcohol compared to vehicle treated mice. In summary, we demonstrate that selective deletion of CB2Rs from either DA neurons or microglia differentially modifies alcohol behavioral effects, thus implicating a possible role of neuro-immuno-eCBome signaling in alcohol-mediated cognition. CB2R involvement in neuroimmune crosstalk could thus be exploited as a therapeutic target in alcohol and psychostimulant addiction.

SESSION VII: CANNABINOIDS: MicroRNAs, INFLAMMATION, ADDICTION, AND EXTRACELLULAR VESICLES

MODERATOR: Mitzi Nagarkatti & Chemio Okama

SPEAKERS: Prakash Nagarkatti, Mitzi Nagarkatti, Nissar Darmani, Katia Befort, & Chioma Okeoma

25. scRNASeq and Transcriptomic Analysis of the Role of Cannabidiol in Neuro- and Intestinal Inflammation in Experimental Multiple Sclerosis

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Previous studies from our lab have shown that Cannabidiol (CBD), the nonpsychoactive component in marijuana, attenuates inflammation, based on which the FDA has approved the use of CBD to treat autoimmune hepatitis as an orphan drug. We have also shown that CBD can prevent neuroinflammation and thus attenuate a murine model of multiple sclerosis (MS) known as experimental autoimmune encephalomyelitis (EAE). More recently, we have used single cell RNA sequencing (scRNA Seq) and array-based transcriptomics, to investigate the mechanisms through which cannabinoids limit excessive inflammation in the central nervous system (CNS) as well as within the intestinal lining in EAE. scRNA Seq analysis of CNS tissue demonstrated that CBD reduced the expression of CXCL9, CXCL10 and IL-1 β expression within the CNS, leading to suppression of inflammatory migration of immune cells. CBD inhibited IL-1 β production, which was not dependent on the classical cannabinoid receptors, CB1 and CB2. CBD treatment also led to induction of myeloid-derived suppressor cells (MDSCs) in the CNS. Interestingly, CBD treatment of EAE mice revealed significant suppression of inflammation in the gastrointestinal (GI) tract. Combination of THC+CBD was also highly effective in preventing neuroinflammation and EAE. This effect was regulated by miRNA inasmuch as microarray analysis of brain-derived CD4+ T cells revealed that THC+CBD treatment significantly down-regulated miR-21a-5p, miR-31-5p, miR-122-5p, miR-146a-5p, miR-150-5p, miR-155-5p, and miR-27b-5p while upregulating miR-706-5p and miR-7116 that targeted inflammatory pathways. These findings demonstrate the beneficial effect of CBD treatment on autoimmune neuroinflammation by ablating expression of pro-inflammatory chemoattractants, regulating inflammatory macrophage activity, promoting MDSC expansion, and limiting the systemic low-grade inflammation in the GI tract, culminating in the attenuation of EAE (Supported in part by NIH grant P01AT003961, P20GM103641, R01ES030144, R01AI129788, R01AI160896 and R01AI123947).

26. Cannabinoids Decrease the lncRNA AW112010 That Promotes the Differentiation of Inflammatory T Cells by Suppressing IL-10 Expression Through Histone Demethylation

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Long noncoding RNAs (lncRNAs) have been demonstrated to play important regulatory roles in gene expression, from histone modification to protein stability. However, the functions of most identified lncRNAs are not known. In this study, we investigated the role of a lncRNA called AW112010. The expression of AW112010 was significantly increased in CD4⁺ T cells from C57BL/6J mice activated in vivo with myelin oligodendrocyte glycoprotein, *Staphylococcal* enterotoxin B, or in vitro with anti-CD3 anti-CD28 mAbs, thereby demonstrating that activation of T cells leads to induction of AW112010. In contrast, anti-inflammatory cannabinoids such as cannabidiol or δ -9-tetrahydrocannabinol decreased the expression of AW112010 in T cells. Interestingly, the expression of AW112010 was high in in vitro-polarized Th1 and Th17 cells but low in Th2 cells, suggesting that this lncRNA may regulate inflammation. To identify genes that might be regulated by AW112010, we used chromatin isolation by RNA purification, followed by sequencing. This approach demonstrated that AW112010 regulated the transcription of IL-10. Additionally, the level of IL-10 in activated T cells was low when the expression of AW112010 was increased. Use of small interfering RNA to knock down AW112010 expression in activated T cells led to increased IL-10 expression and a decrease in the expression of IFN- γ . Further studies showed that AW112010 interacted with histone demethylase KDM5A, which led to decreased H3K4 methylation in IL-10 gene locus. Together, these studies demonstrate that lncRNA AW112010 promotes the differentiation of inflammatory T cells by suppressing IL-10 expression through histone demethylation. (Supported in part by NIH grant P01AT003961, P20GM103641, R01ES030144, R01AI129788, R01AI160896 and R01AI123947)

27. Broad-spectrum antiemetic efficacy of a large dose of temsirolimus against diverse emetogens including the cannabinoid CB₁ receptor inverse agonist/antagonist SR141716A

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Δ^9 -THC and related cannabinoid CB_{1/2} receptor agonists suppress emesis evoked by diverse emetogens including cisplatin-evoked vomiting via activation of central and peripheral cannabinoid CB₁ receptors.

Temsirolimus and other anticancer agents that block the activity of one of the two mammalian targets of rapamycin (mTOR) complexes, mTORC1. We investigated the emetic potential of varying doses (0, 0.5, 1, 2.5, 5, 10, 20, and 40 mg/kg, i.p.) of temsirolimus in the least shrew. Temsirolimus caused a bell-shaped and dose-dependent increases in both the mean vomit frequency and the number of shrews vomiting with maximal efficacy at 10 mg/kg. Its larger doses (20 or 40 mg/kg) had no significant emetic effect. We also evaluated the emetic potential of its analogs (5, 10 and 20 mg/kg, i.p.), all of which exhibited a similar emetic profile. Our observational studies indicated that temsirolimus can reduce shrew motor activity at 40 mg/kg, and subsequently we examined the motor effects of its lower doses. At 10 and 20 mg/kg, it did not affect the spontaneous locomotor activity (distance moved) but attenuated the mean rearing frequency in a U-shaped manner at 10 mg/kg. We then determined the broad-spectrum antiemetic potential of a 20 mg/kg (i.p.) dose of temsirolimus against diverse emetogens including SR141716A, as well as selective and non-selective agonists of: i) dopaminergic D_{2/3} receptors (apomorphine and quinpirole); ii) serotonergic 5-HT₃ receptors [5-HT (serotonin) and 2-Methyl-5-HT]; iii) cholinergic M₁ receptors (pilocarpine and McN-A-343); iv) substance P neurokinin NK₁ receptors (GR73632); v) the L-type calcium (Ca²⁺) channel (LTCC) (FPL64176); as well as the sarcoplasmic endoplasmic reticulum Ca²⁺ ATPase inhibitor, thapsigargin; and the chemotherapeutic cisplatin. Temsirolimus prevented vomiting evoked by the above emetogens with varying degrees. The mechanisms underlying pro- and antiemetic effects of temsirolimus evaluated by immunohistochemistry for c-fos expression demonstrated a c-fos induction in the AP and NTS, but not DMNX with the 10 mg/kg emetic dose of temsirolimus, whereas its larger antiemetic dose (20 mg/kg) had no significant effect. Our study is the first to provide pre-clinical evidence demonstrating promising antiemetic potential of high doses of temsirolimus and possibly its analogs in least shrews. This Study was supported in part by NIH RO1 grant # CA207287.

28. Hippocampal mu opioid and cannabinoid 1 receptors are modulated following cocaine self- administration in male rats

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Cocaine addiction is a complex pathology inducing long-term brain changes that contribute to maladaptive behaviors. Adaptations include transcriptional reprogramming in specific brain reward regions, although the mechanisms underlying this modulation are not fully understood. Both endogenous opioid and cannabinoid systems play a major role in reward and contribute to cocaine-induced neural adaptations. In this study, we investigated whether intravenous cocaine- self administration in rats induces transcriptional, epigenetic and functional changes of the mu opioid and the cannabinoid 1 (CB1) receptors in reward-related brain regions. Interestingly, gene expression of both receptors was increased and associated with a potentiation of their functionality in the hippocampus of cocaine self-administering animals, compared to saline controls. Chromatin immunoprecipitation followed by qPCR in the hippocampus revealed that two activating histone marks, H3K4Me3 and H3K27Ac, were enriched at specific endocannabinoid genes following cocaine intake. We further investigated epigenetic modifications using chromosome conformation capture targeting CB1 receptors. Our results highlighted spatial chromatin re-organization in the hippocampus, as well as in the nucleus accumbens, suggesting that destabilization of the chromatin may contribute to neuronal responses to cocaine. Our study highlights the hippocampus as an important target to further investigate neuroadaptive processes leading to cocaine addiction.

29. SIV and Δ^9 -THC induced alterations in host miRNAome: Insights from Extracellular Vesicles

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Human immunodeficiency virus 1 (HIV-1) invades the central nervous system (CNS) early in infection by crossing the blood–brain barrier (BBB). HIV infection of the CNS impairs the integrity of the basal ganglia (BG) and is associated with inflammation-related increase in BG white matter, secretion of proinflammatory and neuromodulatory host factors that dysregulate CNS cells (neurons, microglia, astrocytes). How HIV-induced proinflammatory and neuromodulatory molecules bring about CNS dysfunction is unclear but may be mediated by extracellular vesicles (EVs), which have been linked to viral pathogenesis. Confirming a direct causal link between HIV, THC, and EVs in humans is difficult, hence the need to use simian immunodeficiency virus (SIV)/rhesus macaque (RM) as an in vivo model to elucidate the cellular/extracellular events in HIV/SIV infection and possible counteraction by THC. Nine age and weight-matched Mamu-A0*1/B08/B17⁻ specific-pathogen-free (free of SIV, D retrovirus, STLV and Herpes B) male Indian RMs were randomly assigned to three experimental groups. Group 1 (n=3) received twice daily injections of vehicle (SIV). Group 2 (n=3) received twice-daily injections of THC (THC/SIV) started four weeks prior to SIV infection until 5 months post-SIV infection. Group 3 (n=3) served as uninfected controls (VEH). EVs are present in BGs. There were no significant differences in the physical properties of BG-EVs across the groups. However, SIV infection and THC treatment are associated with significant changes in BG-EV associated miRNA. BG-EVs from SIV infected RMs (SIV EVs) contained 11 significantly downregulated miRNAs. Remarkably, treatment with THC is associated with significant upregulation of 37 miRNAs in BG-EVs (SIV-THC EVs). Most of these miRNAs are predicted to regulate inflammation/immune regulation, TLR signaling, Neurotrophin TRK receptor signaling, and cell death/response pathways. Furthermore, BG-EVs were internalized by primary mouse astrocytes, activated astrocytes by increasing the expression of astrocyte marker GFAP, and altered the expression of astrocyte CD40, TNF α , MMP2, and MMP9 gene products. Our findings reveal a role for BG-EVs as a vehicle with potential to disseminate HIV and THC induced changes within the CNS.

**SESSION VIII: ALCOHOL, OPIOID, NICOTINE, AND
DRUG ABUSE: NEW MECHANISTIC
COMPLEXITIES AND ABUNDANT
OPPORTUNITIES FOR IMPROVING THERAPY**

MODERATOR: Eliot Gardner & Alex Dopico

SPEAKERS: Ming Xu, Eliot Gardner, Alex Dopico, Jean Bidlack, Ed Levin, & Hwei-Hsien Chen

30. A novel skin cell-based therapy for alcohol and/or cocaine use disorder

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Alcohol and cocaine are commonly misused and frequently co-misused drugs. Available medications do not meet the needs for treating ongoing alcohol and cocaine use disorders (AUD and CUD), relapse and co-use. The glucagon-like peptide 1 (GLP1) receptor agonists can attenuate the reinforcing properties of alcohol and cocaine as well as reinstatement induced by these two drugs and cues in rodents. The modified human butyrylcholinesterase (hBChE) exhibits great catalytic potency and substrate specificity for cocaine hydrolysis and is effective in reducing the behavioral and toxic effects of cocaine in rodents. Both GLP1 and hBChE have very short half-lives *in vivo*, however, limiting their potential in treating alcohol abuse and co-abuse with cocaine. We have developed a skin cell-based gene delivery platform that is capable of delivering GLP1, hBChE or both to address AUD and/or CUD. This approach is effective in preventing mice from alcohol- or cocaine- taking or seeking behaviors, reducing ongoing alcohol drinking and protecting mice from cocaine overdose, respectively. Co-grafting *GLP1* and *hBChE* cells attenuated drug-seeking and lethality induced by alcohol and cocaine co-administration. To start testing the potential usability of this approach in humans, we have targeted both *GLP1* and *hBChE* genes into human keratinocytes and grafted the genetically modified cells using nude mice as recipients. High levels of GLP1 or hBChE were detectable in the grafted mice. We will assess the efficacy of the grafted cells in reducing behaviors induced by alcohol or cocaine in these mice. This work will lay key groundwork for the development of a highly personalized and long-lasting approach for combating AUD and/or CUD. Supported by NIH R21AA027172 and RO1DA047785.

31. Is Brain Ghrelin a New Target for Anti-Addiction Medications?

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We have found – somewhat unexpectedly – that the orexigenic hormone ghrelin is located in portions of the brain’s reward, craving, and relapse circuitry. This raises the obvious question of a possible role for brain ghrelin in opioid-motivated behaviors and in opioid self-administration. We therefore studied the involvement of the endogenous ghrelin system in oxycodone self-administration behavior in laboratory rats. Oxycodone self-administration significantly elevated plasma ghrelin, des-acyl ghrelin, and growth hormone. Acquisition of oxycodone self-administration significantly upregulated endogenous ghrelin receptor (GHS-R1a) mRNA levels in dopamine neurons in the ventral tegmental area (VTA) – a brain locus critical to addictive drug reward. Pretreatment with JMV2959, a selective GHS-R1a antagonist, dose-dependently reduced oxycodone self-administration and decreased the breakpoint for oxycodone under progressive ratio reinforcement in Long-Evans rats. The inhibitory effects of JMV2959 on oxycodone self-administration is selectively mediated by GHS-R1a as JMV2959 showed a similar effect in Wistar wildtype but not in GHS-R knockout rats. These findings suggest that elevation of ghrelin signaling by oxycodone or oxycodone-associated stimuli may underlie at least a portion of the neural process(es) by which oxycodone motivates oxycodone drug-taking and incentive motivation for opioid use. Targeting the ghrelin system may be a viable treatment approach for opioid use disorders.

32. Alcohol and pregnenolone interaction on cerebrovascular potassium channels of the BK type: a tale of two ligands...and two channel subunits –

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In rat and mouse models, acute exposure to alcohol at toxicologically relevant concentrations (10-100 mM) has been reported to constrict middle cerebral arteries (MCA) both *in vitro* and *in vivo*. This ethanol action is independent of circulating, metabolic and endothelial factors but mediated by drug inhibition of potassium channels of the BK type present in vascular smooth muscle (SM). In turn, pregnenolone (PREG) is a neurosteroid that constricts MCA (North et al., 2021), regulates several brain functions, and may play a role in alcohol use disorders. Despite the critical dependence of brain functions on cerebral artery diameter, and its modification by ethanol or PREG, there have been no studies aimed at addressing the combined effects of PREG and ethanol on cerebral arteries and their underlying molecular mechanisms. To cover this gap in knowledge, we first obtained concentration response curves to PREG in the presence and absence of 50 mM ethanol on the diameter of de-endothelialized, *in vitro*-pressurized middle cerebral arteries (MCA) from C57BL/6 adult mice. PREG (1 nM-10 μ M) constricted MCA in a concentration-dependent and reversible fashion. Ethanol potentiated PREG action, with this potentiation progressively decreasing as PREG reached EC_{max} (\sim 10 μ M). A similar lack of additivity was observed with 10 μ M PREG+75 mM EtOH, suggesting that these MCA constrictors shared a SM target(s). Indeed, MCA constriction by PREG+ethanol was abolished by 1 μ M paxilline indicating BK channel involvement. Likewise, injection of 50 mM ethanol+10 μ M PREG to anesthetized mice evoked an MCA constriction similar to those produced by each ligand. BK channel recordings from mouse MCA SM cells showed that PREG and ethanol, separately or in combination, inhibited BK channels at concentrations that constricted de-endothelialized MCA. Ethanol-induced inhibition of SM BK channels and eventual MCA constriction under physiological Ca^{2+}_i and voltage conditions requires BK regulatory β_1 subunits. Moreover, PREG inhibition of BK channels was blunted by the Y450F substitution in the CRAC4 domain identified within the cbv1 cytosolic tail. In conclusion, PREG primarily constricts MCA through SM BK channels; while this action is shared with ethanol, these agents inhibit BK channels via different sites and subunits. Support: R37 AA11560 and R01-HL147315 (AMD); F31-HL-156290 (KCN).

33. Pharmacological properties of opioids are dependent on the G subunit mediating opioid receptor signaling -

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To produce a wide variety of cellular responses, opioid receptors couple to heterotrimeric G proteins to serve as the intermediaries between the receptor and downstream effectors. Opioid receptors couple to the Gai/o family of G α subunits. Members of the Gai/o family include Gai1, Gai2, Gai3, GaoA, GaoB, and Gaz. Using bioluminescence resonance energy transfer (BRET) and HEK 293T cells transfected with either the κ opioid receptor (KOR) or μ opioid receptor (MOR), differential potency and efficacy of opioid agonists were observed when the OR signaled through different G α subunits. While the full KOR agonists U50,488, salvinorin A, nalfurafine, and dynorphin peptides were equally efficacious regardless of the G α subunit present, the concentration-response curves were leftward shifted when the KOR was signaling through Gaz compared to other Gai/o subunits. In contrast, the G α subunit distinctly affected both the efficacy and potency of partial κ agonists, such as the benzomorphans, and the classical μ opioid antagonists, naloxone, naltrexone, and nalmefene, which act as partial agonists at the KOR. Likewise, the full μ -selective agonist DAMGO and the endogenous μ -preferring opioid peptide β -endorphin were more potent when the MOR signaled through Gaz than other G α subunits. Morphine, a partial agonist at the MOR, had EC₅₀ values of 11 ± 5.8 nM and 180 ± 17 nM when the MOR signaled through Gaz and GaoA, respectively. As observed with the KOR, morphine had a greater E_{max} value when the MOR signaled through Gaz in comparison to GaoA. The morphine E_{max} values were $87 \pm 4.1\%$ and $71 \pm 6.5\%$ when the MOR signaled through Gaz and GaoA, respectively. Buprenorphine was the most potent and efficacious when the MOR signaled through the Gaz subunit in comparison to other Gai/o subunits. Among the G α subunits besides Gaz, variability in potency and efficacy was observed with partial agonists. This study demonstrates that the observed opioid pharmacology is dependent on the G α subunit transducing the signal from the receptor. The synthesis of biased agonists for activating a particular G α subunit may lead to new therapeutics. Supported by NIH grant DA046817, Alkermes, Inc. and the Margo Cleveland Fund. No conflict of interest.

34. Boosting Nicotine Replacement Efficacy with Monoaminergic Co-treatments

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The brain is an organ of communication that embodies multiple interacting neural systems, and no brain disease or dysfunction involves only one neural system. Even with addiction to a drug that has its principal mechanism of action on a particular neurotransmitter receptor, like nicotine effects on nicotinic acetylcholine receptors, there are also consequent effects mediated via interacting neural systems. These interactions certainly complicate understanding of the addictive processes, but they also could provide additional avenues for developing more effective therapeutic treatments to combat tobacco smoking addiction. Nicotine replacement therapy is the most commonly used therapeutic treatment to aid tobacco smoking cessation. It does help increase smoking abstinence rates, but abstinence rates with nicotine replacement therapy are still quite low. Given that tobacco addiction involves a variety of neural systems beyond nicotinic acetylcholine receptors, co-treatments with monoaminergic compounds might boost the efficacy of nicotine replacement therapy. In a series of studies with a rat model of IV nicotine self-administration, we have evaluated combinations of nicotine treatment with co-treatments affecting of dopamine D₁, serotonin 5HT_{2c} and histamine H1 receptors as well as a monoamine transmitter reuptake inhibitor. As with humans, chronic nicotine administration significantly reduces nicotine self-administration. The dopamine D₁ antagonist SCH23390, the serotonin 5HT_{2c} agonist lorcaserin and the histamine H1 antagonist pyrilamine when given in combination with chronic nicotine infusions significantly improved the magnitude and duration of effect for reducing nicotine self-administration. The triple dopamine, serotonin and norepinephrine reuptake inhibitor amitifadine when given in combination with chronic nicotine infusions also significantly improved the duration of effect for reducing nicotine self-administration. There appear to be a variety of neural systems interacting with nicotinic acetylcholine systems that can provide effective avenues for therapeutic co-treatments that can boost the efficacy of nicotine replacement therapy. Research supported by P50-DA027840.

SESSION IX: FROM MOLECULE TO CIRCUITRY IN DRUG ADDICTION

MODERATOR: Marilesa Morales & Alban de Kerchove

SPEAKERS: Marilesa Morales, Barbara Juarez, Gavan McNally, Alban de Kerchove

35. Dorsal Raphe glutamatergic inputs to VTA and cocaine-seeking behavior

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Findings from clinical studies and animal models have provided evidence supporting the crucial role that dopamine neurons from the Ventral Tegmental Area (VTA) play in the relapse to use of drugs, such as cocaine. It is unclear how different inputs to the VTA participate in regulating the activity of VTA-dopamine neurons and reinstatement of cocaine seeking behavior. By a multidisciplinary approach, we had found that Dorsal Raphe (DR)-glutamatergic neurons (expressing vesicular glutamate transporter type 3, VGluT3) and dual DR-serotonergic-glutamatergic neurons (expressing VGluT3 and serotonergic markers) induced activation of VTA dopamine neurons, resulting in the release of dopamine in the nucleus accumbens, and mice preference for a place associated with the activation of this pathway; suggesting that both DR-glutamatergic and dual DR-glutamatergic-serotonergic neurons regulate the function of VTA dopamine neurons. In a follow up study, we determined the extent to which DR inputs to VTA participate in the reinstatement of cocaine-seeking behavior, measured by a conditioned place preference task. In an initial study, we used *vglut3-cre* mice in which we injected a viral vector for the selective expression of Channelrhodopsin tethered to eYFP in DR-VGluT3-glutamatergic neurons and a control cohort for the expression of eYFP without Channelrhodopsin. We implanted an optic fiber in VTA to induce local release of glutamate from the DR-VGluT3-glutamatergic fibers and found that release of glutamate induced reinstatement of previously extinguished cocaine-seeking behavior. We next determined the extent to which DR-serotonergic inputs to the VTA play a role in cocaine-seeking behavior. For these studies, we used serotonin transporter (*sert*)-*cre* mice to induce the expression of Channelrhodopsin in the total population of DR-serotonergic neurons. We found that, in contrast to VTA glutamate release from DR-VGluT3-fibers, VTA-serotonin release from DR-serotonergic fibers did not induce reinstatement of cocaine-seeking behavior, although VTA release of glutamate or serotonin from DR-fibers induced reward. In summary, we found that while VTA release of glutamate or serotonin from DR axons induces reward, VTA release of glutamate from DR neurons, but not release of serotonin, induces reinstatement of cocaine-seeking behavior.

36. VTA dopamine neuronal heterogeneity and morphine

Dr. Barbara Juarez

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The progression into opioid-use disorders (OUD) is based on pathological adaptations following cycles of opioid exposure, withdrawal, and abstinence. This opioid-exposure cycle is thought to drive dysregulation of associative learning, an adaptive learning process that helps an organism link environmental cues or actions with rewarding or aversive outcomes to coordinate behavioral responses ideal for survival. In OUD, increased tolerance to opioids and potentiated contextual cue-opioid associations have been linked to increases in opioid seeking. It is important to understand how the opioid-sensitive neural circuits that control associative learning and reward processing are regulated across the opioid-exposure cycle. Dopamine neurons of the ventral tegmental area (VTA) and their projections to subdivisions of the nucleus accumbens (NAc), such as the NAc core or NAc shell, have been demonstrated to be critical for environmental associations and reward attribution. Through foundational studies, VTA dopamine neuron activity has also been shown to be regulated indirectly by opioids through disinhibitory mechanisms of local and distal GABA signaling. However, there is an increasing recognition of VTA dopamine neuron diversity from a genetic, anatomic, circuit, and functional level. Whether opioids uniformly or distinctly modulate these dopamine subpopulations remains largely unknown. It was previously established that VTA dopamine neurons that express the corticotrophin releasing hormone receptor-1 ($Crhr1_{VTA}$) project to the NAc Core and impact cue-food associations while cholecystokinin-expressing VTA (Cck_{VTA}) dopamine neurons project to the NAc shell to impact motivation and performance of conditioned behaviors. Here, we use a genetic strategy to isolate functionally distinct dopamine subpopulations that project to the NAc core or NAc shell to determine whether opioids distinctly regulate the heterogeneous midbrain dopamine system. We found that a single injection of morphine selectively activates cFos in Cck_{VTA} dopamine neurons, suggesting distinct intrinsic or extrinsic neurophysiological regulation of dopamine subpopulations by opioids. Next, we profiled baseline neurophysiological properties of $Crhr1_{VTA}$ and Cck_{VTA} neurons to determine whether there are differences in intrinsic excitability and synaptic connectivity in VTA dopamine subpopulations. Finally, we are using fiber photometry to measure calcium dynamics in these subpopulations to define their responses to opioid exposure and during morphine associative learning

37. Mesolimbic dopamine signatures of relapse

Dr. Gavan McNally

School of Psychology. University of New South Wales

The mesolimbic dopamine system comprises distinct compartments supporting different functions in learning and motivation. Less well understood is how complex addiction-related behaviors emerge from activity patterns across these compartments. I will show how signatures of relapse can be identified from heterogeneous activity profiles across the mesolimbic dopamine system and that these signatures are unique for different forms of relapse.

38. Histone H2A monoubiquitination in the thalamus regulates cocaine use disorder

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Drug addiction, defined as uncontrollable drug intake despite harmful consequences, depends on genetic and environmental factors and involves epigenetic changes. Identification of the mechanisms leading to an addictive state is mandatory for new therapeutic development. Taking advantage of the importance of Maged1 in cocaine addiction, we demonstrate, in mice, a specific increase in H2A monoubiquitination in the thalamus mediated through the Maged1/USP7 interaction after cocaine exposure. Moreover, Maged1 inactivation and USP7 activity inhibition in vGluT2 neurons jointly impaired thalamic H2A monoubiquitination and cocaine sensitization. Finally, we confirmed the Maged1 and USP7 interaction in human neuronal cells and identified single nuclear polymorphism (SNP) mutations in Maged1 and USP7 in polydrug users and found that these SNPs mediated the transition to cocaine addiction and aggressive behavior in cocaine users. These findings pave the way for the development of new treatments for cocaine use disorder (CUD).

SESSION X: HOW REINFORCEMENT LEARNING SHAPES ADDICTION BEHAVIOR

MODERATOR: Celine Nicolas & Brendan Tunstall

SPEAKERS: Brandon Warren, Brendan Tunstall, Celine Nicolas, & Daniele Caprioli

39. Fos-expressing neuronal ensembles in rat infralimbic cortex encode initial and maintained oxycodone seeking in rats

Brandon L Warren, Christina Gobin, Bo Sortman, Samantha Rakela

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Neuronal ensembles within the infralimbic cortex (IL) as well as their projections to the nucleus accumbens (NAc) have been shown to mediate opiate seeking in well-trained rats. However, it is unclear if this circuitry is recruited during initial oxycodone self-administration. Here, we tested the necessity of IL neuronal ensembles in initial and maintained oxycodone seeking behavior using the Daun02 inactivation procedure. We trained male and female transgenic *Fos-LacZ* rats to self-administer oxycodone for 3hr daily sessions until rats met acquisition criteria (>30 active lever presses, >75% responding on active lever). We then infused Daun02 to selectively inactivate IL Fos expressing ensembles associated with initial oxycodone self-administration. We then tested the rats' oxycodone seeking behavior 2 days later. We then repeated the experiment using a longer training period to determine the role of IL neuronal ensembles in oxycodone seeking after prolonged training. Here, we trained male and female transgenic *Fos-LacZ* rats to self-administer oxycodone in 3hr daily sessions under an increasing schedule of reinforcement for 9 days. After 1 week of oxycodone abstinence, we put animals through a 30 min induction test to reactivate neuronal ensembles associated with recall of oxycodone self-administration and infused Daun02 into the IL. We measured the rats' oxycodone-seeking behavior 2 days later. We found that inactivation of IL neuronal ensembles reduced oxycodone-seeking after initial oxycodone self-administration on test day ($t_{23}=2.5$, $p=0.02$). Next, we found that Daun02 attenuated oxycodone seeking behavior in well-trained rats ($t_{17}=2.6$, $p=0.02$). In both experiments, Daun02 infusions decreased Fos-expression after the test, indicating ablation of Fos-expressing neuronal ensembles by Daun02. These results suggest that IL neuronal ensembles are formed during initial learning of oxycodone self-administration and required for initial and maintained oxycodone seeking behavior.

40. Intermittent access to operant self- administered alcohol promotes more "binge-like" alcohol consumption in rats

Brendan Tunstall

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The gold standard animal model to study substance use disorders is operant drug self-administration. For decades, the long-access model has been used to study the intensification of drug self-administration. In this model, rats allowed long- vs. short- sessions of continuous access to self-administered drug are compared. However, the pattern of drug intake has been shown to influence the development of addictive behaviors, and human studies indicate that people often use drugs intermittently, particularly during drug "binges". Brendan Tunstall will demonstrate how manipulating the access to self-administered alcohol within long-access alcohol self-administration can alter the pattern of alcohol drinking, alcohol seeking, and alcohol intake despite negative consequences.

41. Sex differences on incubation of cocaine craving after continuous and intermittent cocaine self-administration

Nicolas Céline

Neurocentre Magendie, University of Bordeaux, Inserm, France

Studies using continuous-access drug self-administration showed that cocaine seeking increases during abstinence (incubation of cocaine craving). Recently, studies using intermittent-access self-administration, a procedure mimicking humans drug intake pattern, showed increased motivation to self-administer and seek cocaine. Céline Nicolas will discuss how the pattern of cocaine self-administration learning influences sex differences on incubation of craving and will emphasize the role of the estrous cycle in this effect.

42. Evidence for heroin-induced social isolation in the rat

Daniele Caprioli

Department of Physiology and Pharmacology, Sapienza University of Rome, Santa Lucia Foundation, Italy

A critical feature of opioid use disorder (OUD) is the progressive loss of interest and decreased investment in social relationships. State of the art OUD preclinical choice models do not capture this aspect. Daniele Caprioli will discuss how social and schedule determinants of opioid learning will influence rat choice between heroin infusion and social interaction.

43. Backtranslation of human cocaine use patterns in rats

Morgan James

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New Jersey, USA*

The intermittent access model of drug self-administration has received significant recent attention as a means of more faithfully recapitulating human drug use patterns in laboratory animals. Despite this, little work has been done to investigate the temporal profile of drug use in persons with substance use disorders. To this end, Morgan James will describe results from a study of examining drug use patterns in men and women with cocaine use disorder, which revealed that cocaine use was both intermittent and episodic. He will then show that this pattern of cocaine intake in laboratory rats promotes a stronger addiction phenotype, and recruits motivational brain systems to a greater extent, compared to other self-administration paradigms.

SESSION XI: CLINICAL AND PRE-CLINICAL VIEWS ON THE CONTRIBUTION OF THE INSULAR CORTEX TO ADDICTION

MODERATOR: Anna Beyeler & Wolfgang Sommer

SPEAKERS: Donna Calu, Wolfgang Sommer, Brady Atwood, & Celine Nicolas

44. Insular mechanisms distinguishing flexibility phenotypes

Donna Calu

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While not all individuals who try recreational drugs develop Substance Use Disorder (SUD), those that do are vulnerable to specific triggers that drive drug seeking even in the face of negative consequences. Preclinical evidence in rats suggests that sign- and goal-tracking individual differences predict differences in drug relapse vulnerability to discrete and contextual cues. These unique relapse vulnerabilities persist despite negative consequences of drug seeking actions. We focus on behavioral flexibility differences of sign- and goal-tracking rats, which are evident both before and after drug experience. We have established that discrete cue-triggered relapse vulnerable sign-tracking rats are less flexible than goal-tracking rats even before drug experience. Prior work demonstrated disconnection of the basolateral amygdala (BLA) and anterior insular cortex (aIC) decreased both goal- and sign-tracking behaviors. Given the role of these regions in appetitive motivation and behavioral flexibility we predicted that disrupting BLA to aIC pathway during outcome devaluation would reduce flexibility in GT rats and reduce rigid appetitive motivation in ST rats. We inhibited the BLA to aIC pathway by infusing inhibitory DREADDs (hM4Di-mcherry) or control (mCherry) virus into the BLA and implanted cannulae into the aIC to inhibit BLA terminals using intracranial injections of clozapine N-oxide (CNO). After training, we used a within-subject satiety-induced outcome devaluation procedure in which we sated rats on training pellets (devalued condition) or homecage chow (valued condition). All rats received bilateral CNO infusions into the aIC prior to brief non-reinforced test sessions. Contrary to our hypothesis, BLA-IC inhibition did not interfere with devaluation sensitivity in GT rats but did make ST behaviors sensitive to devaluation. Intermediate rats showed the opposite effect, showing rigid in responding to cues with BLA-aIC pathway inactivation. Together, these results demonstrate BLA-IC projections mediate tracking-specific Pavlovian devaluation sensitivity and highlights the importance of considering individual differences in Pavlovian approach when evaluating circuitry contributions to behavioral flexibility. These approaches inform our understanding of the brain circuits driving sign- and goal-tracking differences before drug experience, which may aid in circuit investigation of sign- and goal-trackers' distinct relapse vulnerabilities observed after drug experience.

45. Network analysis of brain activity in humans and rats implicates the insula in alcohol addiction

Wolfgang Sommer

University of Heidelberg, Germany

Excessive use of alcohol promotes the development of alcohol addiction, but the understanding of how alcohol-induced brain alterations lead to addiction remains limited. To further this understanding, we adopted an unbiased discovery strategy based on the principles of systems medicine. We used functional magnetic resonance imaging data from patients and animal models of alcohol use disorder (AUD) and developed mathematical models of the 'relapse-prone' network states to identify brain sites and functional networks that can be selectively targeted by therapeutic interventions. Our systems level, non-local, and largely unbiased analyses converged on a few well-defined brain regions, with the **insula** emerging as one of the most consistent findings across studies. In proof-of-concept experiments we were able to demonstrate that it is possible to guide network dynamics towards increased resilience in animals but an initial translation into a clinical trial targeting the **insula** failed. Here, I will highlight a few key experiments that established a role of the insula in 'relapse-prone' network states in AUD patients and animals to illustrate the complexity of the findings. Future concerted efforts are necessary to gain a deeper understanding of **insula** function in a state-dependent, circuit-specific and cell population perspective, and to develop the means for **insula**-directed interventions before therapeutic targeting of this structure may become possible.

46. Anterior insular cortex inputs to the dorsolateral striatum govern the maintenance of binge alcohol drinking

Brady Atwood

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How does binge drinking alcohol change synaptic function, and do these changes maintain binge consumption? The anterior insular cortex (AIC) and dorsolateral striatum (DLS) are brain regions implicated in alcohol use disorder. In male, but not female mice, we found that binge drinking alcohol produced glutamatergic synaptic adaptations selective to AIC inputs within the DLS. Photoexciting AIC→DLS circuitry in male mice during binge drinking decreased alcohol, but not water consumption and altered alcohol drinking mechanics. Further, drinking mechanics alone from drinking session data predicted alcohol-related circuit changes. AIC→DLS manipulation did not alter operant, valence, or anxiety-related behaviors. These findings suggest that alcohol-mediated changes at AIC inputs govern behavioral sequences that maintain binge drinking.

47. Sexual dimorphism of the posterior insular cortex in alcohol binge drinking and drinking despite negative consequences in mice

Céline Nicolas

University of Bordeaux, INSERM, Bordeaux, France

Sex differences may play a critical role in modulating how chronic or heavy alcohol use impacts the brain. Understanding whether and how the underlying mechanisms that drive alcohol drinking vary by sex could provide novel and more targeted therapeutic treatments. The insular cortex has been shown to play an important role in alcohol drinking and dependence in both humans and rodents. Using an adapted model of drinking in the dark (DID), we investigated sexual dimorphism of posterior insular cortex functions in ethanol binge drinking and drinking despite negative consequences in mice. Behaviorally, we confirmed that in mice, females drink more ethanol than males relative to their body weight. At the neural level, we found that chemogenetic inhibition of glutamatergic neurons in the posterior insular cortex (pIC) reduces alcohol drinking despite negative consequences specifically in females without changing the level of binge drinking. We then characterized the coding properties of pIC glutamatergic neurons during binge and drinking despite negative consequences using fiber photometry recordings and found that ethanol drinking increases pIC excitatory neuron activity. These findings show a specific role of pIC glutamatergic neurons in alcohol drinking despite negative consequences in female but not male mice and provide a starting point in our understanding of sexual dimorphism neurobiology in alcohol drinking and dependence.

SESSION XII: INTERORGAN AND INTER CELLULAR CROSS TALK IN HIV AND DRUG ABUSE MEDIATED NEUROPATHOGENESIS

MODERATOR: Sabita Roy & Shilpa Buch

SPEAKERS: Shilpa Buch, Yuri Persidsky, Umakant Sharma, Palsamy Periyassamy, Michael Toborek, & Sabita Roy

48. Role of lncRNA BACE1AS in morphine-mediated Senescence leading to synaptodendritic injury

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Heroin abuse can accelerate the process of aging, specifically impacting age-sensitive brain functional networks. Extracellular vesicles (EVs) have been recognized as conduits for transferring the cellular cargo across the cells and mediating neuronal synaptodendritic injury. These EVs can also carry the senescence associated secretory phenotype (SASP) cargoes. While the direct role of morphine-induced neuronal synaptodendritic injury has been well demonstrated, the role of other central nervous system (CNS) cell types such as the astrocytes in contributing to morphine-mediated neuronal injury remains an enigma. This study aims to focus on the cellular cross-talk mediated by astrocyte-derived extracellular vesicles (ADEVs) in inducing synaptodendritic injury. We assessed the novel role of lncRNA BACE1AS by qPCR. Synaptic density and dendritic arborization was assessed in presence of Mor-ADEVs. Long and short-term memory tasks and anxiety-like behavioral assays were monitored in morphine-dependent mice. Morphine induced senescence phenotype (p16, p21, ROS, cell cycle arrest, β -gal activity, cytokines) in human astrocytes *in vitro*. Using silencing approach, we identified lncRNA BACE1AS as a regulator of this process. EVs isolated from morphine-exposed human astrocytes were shown to carry the SASP cargoes with EV numbers regulated by lncRNA BACE1AS. EVs upon being taken up by the neurons induced neuronal senescence & synaptodendritic injury. Interestingly, morphine administration in mice resulted in upregulation of aging markers in the frontal cortex and hippocampus. Behavioral assays demonstrated ageing phenotype and cognitive deficits in these mice. This study underscores the role of lncRNA BACE1AS in astrocytic senescence and the role of ADEVs in mediating synaptodegeneration leading to cognitive impairments associated with ageing. This work was supported by RO1DA044586 (S. Buch) from National Institute of Health.

49. Brain and Lung Injury Caused by Alcohol and Electronic Cigarettes: Mechanisms of Deleterious Effects on Blood Brain and Alveolar - Endothelial Barriers

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Polydrug abuse (especially alcohol use disorder, AUD, and smoking) are known individually to compromise the lung alveolar-endothelial barrier (AEB) and the blood brain barrier (BBB). Very limited knowledge exists regarding damage in lung and brain due to electronic cigarettes (e-cig) in combination with AUD. Very limited data indicate that e-cig adversely affect innate immune responses, cause endothelial dysfunction and result in a pro-inflammatory phenotype in macrophages and endothelium in lungs. While e-cig are known to be addictive, their effects on the brain and cognition are unknown. Our data show that chronic e-cig exposure in mice enhanced permeability of the BBB, increased neuroinflammation, diminished expression of a key glucose transporter and tight junction protein on brain endothelium, and impaired cognition. We discovered that the combination of alcohol/e-cig exposure in an animal model caused enhanced AEB permeability and signs of neuroinflammation/BBB compromise. We found that e-cig and alcohol cause mitochondrial dysfunction and oxidative stress, leading to pro-inflammatory phenotype of cellular components of AEB/BBB pointing to potential synergistic effects of e-cig/AUD on end-organ pathology. Using primary human brain endothelial cells, lung epithelial and endothelial cells, we found that alcohol and e-cig share similar mechanisms of injury. E-cig and alcohol caused mitochondrial impairment [spared respiration measured by Seahorse, diminished expression of Complex-II (succinate dehydrogenase) and Complex-IV (cytochrome c oxidase)] and signs of endoplasmic reticulum stress leading to intracellular Ca^{2+} accumulation and extracellular ATP releases. Further, we discovered a role of purinergic receptor P2X7r in these harmful effects of alcohol and e-cig suggesting novel therapeutic interventions. Funding: R37AA015913, U01AA023552 Conflicts of interests: None

50. Opioid Induced Dysregulation of Gut Microbiota and Systemic Inflammation Accelerate HIV-Associated Premature Aging in a Mouse Model of HIV

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Premature aging is a comorbidity in HIV-infected adults despite ART therapy. Both laboratory and epidemiological studies strongly indicate that drug dependence exacerbates HIV progression and accelerates neurocognitive decline, however its impact on premature aging has not been fully explored yet. Microbial dysbiosis and systemic inflammation as a potential contributor for accelerated aging has not been previously investigated in a HIV mouse model in the context of opioid abuse. To our knowledge, this is the first study describing the association among gut microbiota, sustained inflammation, and accelerated aging in HIV infected mice in the context of opioid. Here we investigated how inflammatory responses are induced and the mechanisms by which these responses ultimately contribute to age associated pathology. Therefore, we have investigated if restoration of normal gut microbiota using synbiotics and reducing inflammation delay aging in opioid treated HIV infected animals. In this study, the young, middle, and old aged wild-type and germ-free mice were used, which were treated with morphine and EcoHIV alone and in combination. All the treatment groups were treated with ART. The data was analyzed for gut bacterial composition, systemic inflammation, and aging markers. The results show exacerbated microbial translocation and disrupted gut barrier morphology in morphine treated HIV infected mice compared to all other treatment groups of mice. Furthermore, we show a significantly distinct microbial composition in the opioid treated HIV infected animals compared to control and HIV treatment alone. Next, we found decreased macrophage phagocytosis by HIV infection and worsened in combination with morphine, which may contribute to lack of bacterial clearance and sustained systemic inflammation that contribute to premature aging. Delineating the mechanisms underlying premature aging in opioid treated HIV infected animals allow us for the development of therapeutic strategies to ameliorate factors that contribute to premature aging. This study postulates that accelerated aging in HIV infected people who use opioids is a consequence of microbial dysbiosis, thus paving the way toward synbiotic therapies for delaying premature aging and improve health.

51. Morphine-mediated neuroinflammation involves astrocyte-specific activation of NLRP6 inflammasome signaling via miR-152

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To determine the molecular mechanism(s) by which morphine alters the NLR family pyrin domain containing 6 (NLRP6) inflammasome signaling via miR-152, leading, in turn, to astrocyte activation. For *in vitro* experiments, human primary astrocytes were exposed to morphine to determine the expression of miR-152, NLRP6 inflammasome signaling proteins, astrocyte activation markers, and proinflammatory cytokines by qPCR, Western blotting (WB), and ELISA. We also employed both pharmacological inhibition and gene silencing approaches for determining the critical roles of NLRP6 inflammasome in morphine-exposed astrocytes. We further specifically used dual luciferase assay and argonaute immunoprecipitation (Ago IP) for miR-152 target validation. For *in vivo* experiments, 8-weeks old mice were administered with morphine to determine the expression of NLRP6 inflammasome signaling, miR-152 expression, astrocyte activation, and proinflammatory cytokines in the brain tissues by qPCR, WB, ELISA, RNAscope, and confocal microscopy. Exposure of human primary astrocytes to morphine decreased expression of miR-152 with concomitant upregulation of NLRP6 inflammasome signaling and astrocyte activation. We also identified NLRP6 as a novel target regulated by miR-152 using dual luciferase assay and Ago IP. We also found that pharmacological inhibition of μ -opioid receptor, overexpression of miR-152 using mimic, and gene silencing using NLRP6 siRNA further validated the NLRP6 activation followed by caspase1 cleavage and proinflammatory cytokines production in morphine-exposed human primary astrocytes. *In vitro* findings were also confirmed in the brain tissues of mice administered with morphine. **Conclusion:** These findings demonstrated the involvement of miR-152-mediated activation of NLRP6 inflammasome signaling in the morphine-exposed astrocytes. R01DA052266 (Periyasamy)

52. Methamphetamine impairs neurogenesis of neural progenitor cells via activation of the FOXO3 signaling and induction of inflammatory reactions

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Maintaining an intact pool of neural progenitor cells (NPCs) is crucial for generating new and functionally active neurons. Methamphetamine (METH) can affect adult neurogenesis; however, potential mechanisms of this influence are still poorly understood. Using mice chronically exposed to METH, we present evidence that induction of inflammatory responses and overproduction of IL-1 β are critical components of METH-induced aberrant neurogenesis and the development of cognitive dysfunction. Interestingly, this impact can be reversed, at least in part, by enhanced physical activity. Moreover, chronic exposure to METH combined with brain infection by EcoHIV results in enhanced proliferation of NPCs in the subventricular zone (SVZ) in mice. This effect is long-lasting as it is preserved *ex vivo* in NPCs isolated from the exposed mice over several passages. Interestingly, this effect is associated with dysregulation of Cyclin B1 and Cyclin D. Transcriptomic studies indicate that the majority of differentially expressed genes in response to the employed treatment are targets of the Forkhead box O transcriptional factor (FOXO), and primarily FOXO3. Additional *ex vivo* studies and *in vitro* experiments revealed upregulation of the CXCL12-CXCR4 axis, leading to activation of downstream pAkt and pErk, the pathways that can phosphorylate FOXO3 and force its exports from the nuclei into the cytoplasm. These results provide novel information that exposure to METH combined with HIV infection can induce aberrant proliferation of SVZ-derived NPCs and identifies CXCL12-CXCR4-Akt-1-mediated phosphorylation of FOXO3 as the mechanism responsible for this effect. Supported by the National Science Centre (NSC) grants 2015/17/B/NZ7/02985 and 2019/35/B/NZ7/03155 and the National Institutes of Health (NIH) grants MH128022, MH122235, MH072567, HL126559, DA044579, DA039576, DA040537, DA050528, and DA047157.

53. Microbiome Implications in Opioid Withdrawal: Consequence of HIV infection

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The ongoing opioid epidemic has caused numerous deaths, increasing rates of hospitalizations, and left millions of people struggling with opioid use disorder. Opioids are commonly prescribed for pain management, however chronic exposure can result in dependence, which requires the presence of the drug to prevent withdrawal. Additionally, the negative symptoms of withdrawal can drive people to seek out the substance again to find relief, often contributing to the development and continuation of addiction. Even though opioid use disorder is a growing problem, there are few therapeutic strategies to combat addiction. This study investigated the microbiome as a potential therapeutic target for morphine withdrawal, since chronic morphine treatment causes dysbiosis of the microbiome, and this microbial shift has been shown to contribute to the development of morphine tolerance. Results showed that depleting the microbiome with an antibiotic cocktail caused a shift in the timing of peak withdrawal severity, with antibiotic treated mice displaying the most severe withdrawal symptoms at 6hrs after morphine pellet removal, as compared to 12hrs for water treated controls. This also resulted in shorter duration of withdrawal for antibiotic treated mice. Additionally, 16sRNA sequencing of fecal samples during the withdrawal process reveal a unique microbiome composition that occurs during peak withdrawal symptoms, suggesting that there are key bacteria contributing to the behavioral symptoms of morphine withdrawal that could be future therapeutic targets.

SESSION XIII: EXTRACELLULAR VESICLES, EPIGENETICS, NEUROIMMUNE SIGNALING, AND ALCOHOL

MODERATOR: Antonio Noronha & Fulton Crews

SPEAKERS: Fulton Crews, Dipak Sarkar, Sulie Chang, & Leon Coleman

54. Epigenetic shifts in microglial and neuronal phenotype following Adolescent Intermittent ethanol (AIE) exposure

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Emerging studies find that long-lasting changes in gene expression in brain following ethanol exposure are linked to neuroimmune signaling. Adolescent intermittent ethanol exposure (AIE) increases adult brain proinflammatory genes including HMGB1 and Toll-like receptors; reduces cholinergic and serotonergic neurons, hippocampal neurogenesis, and rsfMRI connectivity; and increases drinking and other behaviors associated with risks for alcohol use disorder (AUD). Increased expression of adult brain HMGB1, a cytokine-like protein released by ethanol that stimulates Toll-like receptors and RAGE receptors, is a key proinflammatory signal that crosses cell types. Proinflammatory signals have been linked to loss of neurons, neurogenesis, and increased tolerance to alcohol. A newly developed ethanol response battery finds AIE causes long-lasting reductions in adult acute alcohol hypothermia, intoxication, and balance, i.e. alcohol tolerance. To determine if HMGB1, TLR, and RAGE signaling contributed to these adult AIE pathologies, we allowed rats to exercise or treated them with anti-inflammatory indomethacin and galantamine in experiments to test whether these treatments could prevent or reverse AIE pathology. All anti-inflammatory treatments prevented and reversed AIE-increased HMGB1 neuroimmune signaling as well as the loss of adult forebrain cholinergic, dorsal raphe serotonergic, and hippocampal loss of neurogenesis. Changes in neurons and glia were associated with increases in histone and CpG methylation gene-silencing markers and upregulation of RE-1 silencing transcript (REST). *Ex vivo* brain slice studies indicate epigenetic modifications shift to proinflammatory genes and reduced neurotransmitter gene expression, altering brain circuitry. Taken together, these findings suggest AIE alters glial and neuronal phenotypes by silencing genes, not neuronal death. Reversal of HMGB1 signaling restores both neurons and cognitive dysfunction. AIE increases risks for AUD that persist to adulthood but are reversible. (Supported by NIAAA NADIA).

55. Identifications of exosomal cargo molecules involved in microglia induced neuronal death during ethanol-induced pathogenesis in the hypothalamus of fetal rats

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Using primary cultures of hypothalamic microglia and pharmacological approaches, we have recently obtained evidence that microglial derived exosomes, small vesicles with a diameter of 40–100 nm that are liberated from these cells into the extracellular space, participate in ethanol neurotoxic action on proopiomelanocortin neurons by delivering apoptotic molecules in developing rat brain. The types of cargo molecules that exosome carries from microglia to use for induction of neuronal apoptosis are not well defined. We have recently provided evidence that C1 complement system released from exosome might be one of the candidate apoptosis inducers. Using proteomic analysis, we also found that chemokine levels were upregulated in the exosomes derived from ethanol-activated microglia. We determined whether chemokines carried by exosome from ethanol-activated microglia are also involved in proopiomelanocortin neuronal death. We found that several chemokines, especially MIP1 alpha and MCP1, were increased significantly in exosomes from the ethanol-activated microglia. Whereas, the blockers of these chemokine lowered the apoptosis of proopiomelanocortin neurons, which was caused by ethanol-activated microglial exosomes. PCR array measurements of exosome-deposited proopiomelanocortin neurons provided additional support for the activation of apoptotic signaling molecules. Hence, these data suggest that C1 complement system and chemokines are major exosome cargos involved in the communication between microglia and proopiomelanocortin neurons during ethanol-induced neuronal death in fetal brain.

56. Meta-analysis of the mechanisms underlying alcohol elevation of amyloid precursor protein expression upon SARS-CoV-2 infection

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SARS-CoV-2 infection is responsible for COVID-19 in over 120 million people. Alcohol consumption has significantly increased during the COVID-19 pandemic. Despite the sparse drinking records of COVID-19 patients, our in-silico network meta-analysis studies found that that IL-1 β , IL-6, JUN, NR3C1 (GR), TNF, IFNG, STAT, NFKB, HIF1A, PPARG and PPARG-RXT may play an important role in mediating alcohol augmentation of SARS-CoV-2-induced systemic inflammation and COVID-19 pathologies. Neuroinflammation signaling pathways may be involved in alcohol modulation of amyloid precursor protein (APP) production, and TNF, IFNG, STAT1, IL-1 β , IL6, and STAT3 may act as upstream regulators to increase APP production during COVID-19. With these premises, we hypothesized that exposure to ethanol (EtOH) enhances buildup of APP expression induced by SARS-CoV-2 infection. Meta-analysis using QIAGEN IPA was conducted. Applying both IPA networking and molecule activation predictor (MAP) tools on each of the 11 common inflammatory mediators between alcohol and COVID-19, we found that IL-1 β , IL-6, JUN, NR3C1 (GR), TNF and IFNG would activate APP expression, PPARG and PPARG-RXT would reduce APP expression, while STAT, NFKB and HIF1A would have no effect on APP expression. However, the overall effects of concurrent activation of these 11 genes would result in strong activation of APP expression holistically. IPA Core Analysis of these 11 genes revealed a series of canonical pathways, of which the neuroinflammation signaling and IL-10 signaling pathways were among the top five. Currently, we have examined how exposure to EtOH could augment APP expression leading to development of Alzheimer's disease (AD) in the course of each of 12 diseases with inflammation including COVID-19. By showing EtOH augmentation on APP expression upon SARS-CoV-2 infection, our studies call attention to long-term complications of alcohol and COVID-19 on AD and other possible neurodegenerative complications.

57. Presenter. Extracellular Vesicles, Microglia, and Epigenetics as Potential Therapeutic Targets for AUD Pathology

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Proinflammatory pathways are involved in AUD, however underlying drivers of immune activation remain unclear. A loss in adult hippocampal neurogenesis (AHN) is found across neuroimmune disorders and is associated with negative affective states and depression. Extracellular vesicles (EVs) have emerged as drivers of immune dysfunction across a range of inflammatory disorders. We find that ethanol changes the cargo and function of EVs (EtOH-EVs). Using 3D organotypic brain slice culture (OBSC) we investigated the role of EVs in proinflammatory activation by ethanol as well as ethanol-induced loss of AHN. Ethanol was found to cause the release of proinflammatory EVs that phenocopied ethanol immune activation (e.g. TNF α and IL-1 β) in ethanol-naïve OBSCs. Inhibition of EV secretion with imipramine blocked ethanol-induction of proinflammatory genes. Next, we investigated the impact of proinflammatory EtOH-EVs on AHN. EtOH-EVs caused a reduction in AHN. This was found to involve EV-induced epigenetic changes, with EtOH-EVs increasing the level of the repressive histone methylation mark H3K9me2. Inhibitors of H3K9me2 forming enzymes (UNC and BIX) prevented the loss of AHN caused by EtOH-EVs and ethanol alone. Lastly, we discuss the cellular origin of EtOH-EVs. Microglia were found to be necessary for the secretion of EtOH-EVs, with microglial depletion preventing proinflammatory activity of EtOH-EVs, and microglial repopulation normalizing persistent proinflammatory activation caused by ethanol. Together, this work implicates EVs and microglia in proinflammatory signaling caused by ethanol and the resulting loss in AHN.

SESSION XIV: SIGMA-1 RECEPTOR REGULATION AND ADDICTION

MODERATOR: Hsian-en Wu & Yuko Yasui

SPEAKERS: Hsian-En Wu, Nino Sharikadze, Simon Couly, Yuriko Kimura, & Yuko Yasui

58. Sigma-1 receptor confers protein translational regulation after neuropathic pain

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Much evidence indicates that primary sensory neurons at the dorsal root ganglions are important sites for pathophysiologic changes leading to neuronal hyperexcitability, which makes them a key target for identifying pain mechanisms amenable to treatment. The chaperone protein sigma-1 receptor (S1R), its roles in the pathogenesis of a wide range of diseases have only recently become apparent. Direct involvement of S1R in pain processing is revealed by prevention of nerve injury-induced hyperalgesia with S1R blockade and in mice lacking S1R. Yet, the molecular event that may link S1R to the modulation of neuropathic pain is largely unknown. Since voltage-gated Ca channels, particularly Cav2.2, regulate sensory neuron excitability and neurotransmission, their regulation may be a key site of S1R action. We therefore examined the effect of S1R on Cav2.2 in the context of neuropathic pain following spared nerve injury (SNI). In behavioral study, direct ganglionic injection of S1R agonist (+)Pentazocine elicited hyperalgesia in naive animals, while antagonist BD1047 attenuated painful responses in injured animals. EP studies revealed that (+)Pentazocine increased neuronal firings by shorting afterhyperpolarization duration in control sensory neurons. These data suggest that S1R modulates neuropathic pain by regulating neuronal excitability. We found that Cav2.2 protein, but not mRNA, was downregulated indicating a post-transcription modification; thus, we tested whether Sig-1R regulates VGCCs via a translational control mechanism. In S1R knockout HEK cells, binding of Cav2.2 mRNA and eIF4E was increased using RNA IP; 4E-BP1, an eIF4E inhibitory protein of eIF4E to repress protein translation, reduced Cav2.2 protein expression; and 4E-BP1 protein and mRNA were both downregulated. On the other hand, 4E-BP1 expression and its interaction with eIF4E were increased after SNI. With Bioinformatic analysis prediction software, the transcription factor cFOS can bind to 4E-BP1 promoter region. Since S1R has been shown to translocate to nuclear envelop upon cocaine activation, we found that S1R interacted directly with cFOS to regulate 4E-BP1 transcription and expression using coimmunoprecipitation and ChIP assay. We conclude that S1R hampers Cav2.2 protein expression through eIF4E/4E-BP1 translational regulation axis and provides a novel mechanistic link between s1R and painful neuropathy and may generate new translational opportunities for pain treatment. (Supported by the IRP, NIDA, NIH).

59. Sigma-1 Receptor regulation of steroid hormones at the adrenal gland

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A classic physiological stress response is the activation of HPA axis, which in turn regulates circulating levels of endogenous glucocorticoid hormones and provides a rapid response and defense against stress. The sigma-1 receptor (SIGMAR1) is a transmembrane protein residing in the ER-mitochondria interface and is highly expressed in adrenal gland and the brain regions involved in emotion and neuropsychiatric disorders. Some of SIGMAR1 agonists are a class of drugs for the treatment of depression and anxiety. The aim of our study is to investigate the role of SIGMAR1 in regulation of steroid hormones at the level of adrenal gland. We performed immunohistochemical studies and found that the SIGMAR1 is mainly localized in adrenal gland cortex zone which produces glucocorticoids. We used male and female wild type and SIGMAR1 knockout (KO) mice in different housing conditions and analyzed the plasma level of corticosterone as well as their metabolite as a non-invasive estimate of circadian glucocorticoid production. In multiple housing mice, the SIGMAR1 KO males have higher corticosterone than the WT males. Same effect was not seen in female mice. Single housing female mice exhibits much higher corticosterone concentrations compared to males in their fecal and serum samples. However, across the 4-day comparison the SIGMAR1 KO male mice show a tendency of increases in corticosterone. These results suggest that the SIGMAR1 is inhibiting the release or synthesis of corticosterone in male mice. We performed western blot analysis to evaluate SIGMAR1 expression under purportedly stressful single housing conditions. SIGMAR1 protein expression increases in female mouse adrenal glands after 4 days of single housing, suggesting a critical role of SIGMAR1 in female stress response system. We also examined corticosterone release per ACTH stimulation on either primary adrenal gland cells or the ex vivo intact adrenal glands from WT vs SIGMAR1 KO mice. SIGMAR1 KO primary adrenal gland cells showed higher corticosterone release with 1nM ACTH stimulation. Same results were seen in ex vivo adrenal gland stimulation with 20nM ACTH. Both results thus confirm the SIGMAR1 inhibitory effect on corticosterone release in adrenal gland. We propose that SIGMAR1 receptor is involved in stress response through a regulation on adrenal corticosterone. This effect of SIGMAR1 however was seen only in male mice, suggesting a role of sex hormones in the SIGMAR1 regulation of stress response at the level of adrenal steroid hormones. This research was supported by the Intramural Research Program, NIDA, NIH.

60. Sigma-1 Receptor Regulates Energy Metabolism by Impacting the NAD/NADH ratio: Potential Relation to addiction

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Sigma-1 receptor (S1R) is a protein located at the junction of two organelles: endoplasmic reticulum (ER) and mitochondria. Upon activation by ER calcium depletion or ligand binding, S1R can increase calcium efflux from ER to mitochondria by chaperoning IP3 receptor type3. Thus, S1R ligands have been shown to be effective to treat numerous neurodegenerative disorder models where mitochondrial functions are impaired. Interestingly, it is known that affecting mitochondria impact glycolysis, the other source of energy for neurons. For example, compensatory glycolysis is observed when oxidative phosphorylation in the mitochondria is pharmacologically inhibited. However, despite the facts that the S1R regulates calcium entries into mitochondria, the consequences of S1R actions on glycolysis and on the overall cellular energy metabolism are not yet elucidated. This study utilizes Wild type (Wt) or S1R knockout (S1R-KO) Neuro2a (N2a) cells created by CRISPR-CAS9, and primary cortical neurons from Wt or S1R-KO mice to investigate the fundamental functions of S1R on the glycolysis, mitochondrial activity and on the NAD/NADH metabolism which is a key player on the homeostasis of cellular energy production. In S1R-KO N2a cells and cortical neurons we observed a reduced glycolytic activity, a decreased enolase and an increased mitochondria complex I protein. All three changes were successfully rescued by overexpression of S1R. To examine the underlying mechanisms behind those alterations, we first hypothesized that S1R could chaperone glycolytic proteins. Yet, we did not find any colocalization between those proteins and S1R. Interestingly, using extracellular flux analysis assay we observed that the compensatory glycolysis, induced by inhibitors of oxidative phosphorylation chain, is reduced in S1R-KO primary neurons, suggesting a lack of communication between mitochondria and the glycolysis system. Moreover, we measured the NAD⁺/NADH concentrations, key coenzyme essential for glycolysis and for mitochondrial complex I activity and found that the ratio is modified in S1R-KO conditions. Altogether, those data show for the first time that the S1R modulates the NAD metabolism. This new insight on the S1R function may lead to new therapeutic value of S1R ligands in diseases in which the cellular NAD⁺/NADH ratio is implicated such as addiction through effects on Sirtuins, CD38 or adenosine levels. (Supported by IRP NIDA NIH).

61. Cocaine-induced functional deficit in orbitofrontal cortex is prevented by systemic administration of a sigma-1 receptor antagonist

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The orbitofrontal cortex (OFC) is necessary for inferring expected outcomes to guide appropriate responding. This function can be shown in a sensory preconditioning task, in which behavior to the preconditioned but not the directly conditioned cue is sensitive to inactivation of the OFC after learning. Self-administration of cocaine causes similar deficits in preconditioning, suggesting drug-induced problems in model-based inference that might complicate treatment. Here, we investigated the potential role of the Sig-1R in cocaine-induced functional alterations of OFC. Sig-1Rs were localized in the rat OFC by immunohistochemistry and western blot. Rats were given the opportunity to spontaneously acquire self-administration of cocaine or sucrose, with a prior injection of either BD1063 or saline of cocaine self-administration session for 14 days. After four weeks of cocaine withdrawal, rats were trained in a sensory preconditioning task. As reported previously, rats withdrawn from cocaine self-administration exhibited a deficit in sensory preconditioning performance, failing to respond appropriately to the preconditioned cue. This deficit was not present in rats that had received BD1063 injection prior to each session. After the behavioral test, mEPSC and mIPSC of lateral OFC neurons were recorded. We found that neurons of cocaine self-administration significantly reduced the mEPSC frequency but not that of mIPSC. This reduction was diminished in the neurons of animals that received prior injection of BD1063 before the sessions. Amplitude of mEPSC and mIPSC were not altered across the conditions. However, unexpectedly, the electrophysiological activity of the serotonin 2A receptor (5HT-2AR) in the OFC of different treatment groups did not differ. To reveal the sub-global profile of molecular changes among the group, a relatively new tag, APEX2, combined with mass spectrometry analyses, is being used to reveal the underlying molecular changes among the groups. Although more experiments are needed, for example by testing the deficit reversal by using the Sig-1R antagonist during cocaine withdrawal, our data suggest the Sig-1R as a potential therapeutic target in the treatment of cocaine addiction. (This study supported by IRP/NIDA/NIH/DHHS)

62. Sigma-1 Receptor is Involved in Cocaine-induced AMPA Receptor Synaptic Plasticity in the VTA Dopamine Neurons

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Sigma-1 receptor (Sig1R) is a single transmembrane protein residing mainly at endoplasmic reticulum and has no homology with other mammalian proteins. Sig1R interacts with many proteins and modulate their functions, therefore Sig1R regulates a variety of cellular functions. Since some psychostimulants including cocaine have affinity to Sig1R, Sig1R is implicated in drug addiction. In fact, Sig1R ligands can attenuate behavioral effects of cocaine. Cocaine causes neuroadaptation in the brain accompanied by strong and persistent plasticity in the mesocorticolimbic dopamine system essential for reward and motivational process. The system connects the ventral tegmental area (VTA) to the major projected areas, nucleus accumbens (NAc) and prefrontal cortex. Glutamate receptors play an important role in drug-induced synaptic plasticity in the system and long-lasting behavioral effects of cocaine such as craving. Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) is an ionotropic glutamate receptor that mediates most of the fast excitatory synaptic transmission in the central nervous system. AMPARs are tetramers composed of four subunits, GluR1-4, and the presence of GluR2 subunit in the tetramers prevents Ca^{2+} permeability. While the great majority of AMPARs in the adult brain are GluR2-containing AMPARs which are Ca^{2+} impermeable, the expression of GluR2-lacking AMPARs, which are Ca^{2+} permeable, is quite uncommon and strongly regulated under development, basal and stress conditions. It has been shown that single cocaine exposure in vivo induces potentiation of AMPAR-mediated currents at excitatory synapse onto the dopamine neurons in the VTA through partly insertion of GluR2-lacking AMPARs, which allows to trigger later changes in the NAc. However, molecular mechanisms underlying the cocaine-induced potentiation of AMPAR-mediated currents in the VTA are not fully understood. In this study, we hypothesize that Sig-1R may play a role in the regulation of trafficking and/or expression of the AMPAR subunits that affects cocaine-induced changes in AMPARs-mediated currents in the VTA. To investigate if Sig1R is involved in cocaine-induced potentiation of AMPAR currents, we examined miniature AMPAR-mediated EPSCs in the VTA of wild-type (wt) or Sig1R knockout (KO) mice. As previously reported, a single cocaine injection significantly increased both amplitude and frequency in wt mice. Conversely, cocaine treatment did not change amplitude in Sig1R KO mice. We further found that cocaine caused inward rectification of AMPAR excitatory postsynaptic currents in wt mice, which indicates insertion of GluR2-lacking AMPARs, but not in Sig1R KO mice. These results suggest that Sig1R is involved in cocaine-induced increase of GluR2-lacking AMPARs. We next examined the intracellular distribution of AMPAR subunits and found that expression of GluR2 and GluR3 were significantly decreased on the surface and increased in endosomes with cocaine treatment in wt but not in Sig1R KO mice. Meanwhile, cocaine did not affect total expression of GluR2 and GluR3 either in wt or Sig1R KO mice. These results indicated that Sig1R regulates cocaine-induced distributions of GluR2 and GluR3. Together, we demonstrate that Sig1R play a role in cocaine-induced changes related to AMPARs. This research was supported by the Intramural Research Program, NIDA, NIH.

SESSION XV: ALCOHOL DEPENDENCE AND TOXICITY IN ADOLESCENT AND ADULTHOOD

MODERATOR: Yousef Tizabi & Youssef Sari

SPEAKERS: Yousef Tizabi, Shaketha Hauser, Youssef Sari, & Burk Getachew

63. Overview of Behavioral and Neurobiological Effects of Alcohol

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The discovery and abuse of alcohol is a spectacular historic phenomenon that carries on to today's civilization. Its dissociative properties and easy access, can in vulnerable individuals, lead to devastating consequences not only on self but also on family and society in general. The abuse cycle usually starts during adolescence, a period of particular vulnerability and may be influenced by gender and ethnicity. In this talk, alcohol's effects on reward circuitry as well as its toxicological consequences, including some co-morbid conditions and postulated mechanisms will be briefly discussed. This introduction will set the stage for more detailed discussion of specific factors by other speakers in this symposium. The overall goal is to enhance insights into differences between adolescent and adult responses to alcohol as well as potential novel treatments for alcohol dependence and neurotoxicity.

64. Adolescent Ethanol Exposure Alters the Adult Response to Drugs of Abuse

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Adolescent alcohol consumption increases the rate of drug addiction during adulthood and introduces further challenges in treatment for such addictions. It has been postulated that alcohol consumption during adolescence may cause brain alterations, resulting in a desire to use drugs more readily during adulthood. This presentation will focus on studies that have examined the effects of alcohol exposure during adolescence on neurochemical responses to microinjections of both ethanol (EtOH) and nicotine into the posterior ventral tegmental area (VTA), a reward brain region, during adulthood. Some highlighted findings indicate that voluntary EtOH consumption during adolescence results in a hypersensitive mesolimbic dopaminergic system and enhanced responding not only to EtOH but also to nicotine during adulthood. It is concluded that EtOH consumption/exposure during adolescence produces persistent alterations within key brain regions that enhance the response to drugs of abuse during adulthood. Moreover, the potential utility of these findings in treatment modalities will be briefly discussed.

65. Neurocircuits Involved in Alcohol Dependence: Neuropharmacological Studies

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Ample evidence implicates the glutamatergic system in drugs of abuse. Chronic exposure to several abused substances, including alcohol, can lead to increase in extracellular glutamate concentration in brain reward regions, such as nucleus accumbens (NAc) and prefrontal cortex (PFC). Extracellular glutamate concentration is regulated mainly by the astrocytic glutamate transporter 1 (GLT-1) and cystine/glutamate antiporter (xCT). Our laboratory showed that chronic alcohol exposure increased extracellular glutamate concentration in the NAc and downregulated GLT-1 and xCT expression in the NAc, PFC, amygdala, and hippocampus in alcohol-preferring rats. Importantly, chronic alcohol exposure downregulated GLT-1 expression in the NAc shell but not core. Furthermore, treatment with beta-lactams upregulated GLT-1 and xCT expression in these brain regions, and attenuated alcohol dependence and relapse to alcohol drinking behavior. Recently, our laboratory identified a new beta-lactam, MC100093, that showed similar attenuating effect in alcohol intake and upregulation of GLT-1 in the NAc and PFC. These data will be discussed in this presentation to demonstrate the potential of targeting GLT-1 and xCT as major therapeutic targets for the treatment of alcohol dependence. Furthermore, the effectiveness of beta-lactams against alcohol-induced neurotoxicity will be briefly discussed.

66. Alcohol Consumption in Adolescents vs Adults: Differences in Attitudes, Consequences and Treatment

Bruk Getachew

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Adolescent alcohol abuse can, over time and in vulnerable individuals, confer liability for alcohol use disorder (AUD) during adulthood. AUD is a medical condition characterized by inability to control excessive alcohol drinking, tolerance, withdrawal syndrome, physical, and psychological dependence. AUD often entails complex interaction of alcohol with the central nervous system (CNS) resulting in neuroadaptations in the brain. The neuronal and behavioral dispositions that render adolescents more vulnerable to alcohol-induced toxicities warrant more investigation. Importantly, the extent to which these neuroadaptations can translate into behavioral and attitude changes and the mechanism(s)/neurocircuitries involved are not fully understood. Furthermore, the literature in area of adolescent alcohol treatment is limited. Here, behaviors and attitudes associated with general alcohol drinking patterns among adolescents and putative brain circuits are discussed. It is concluded that early therapeutic intervention for AUD must consider reaching out to adolescents and young adults that are not yet experiencing significant negative social, and health consequences. Moreover, treatment modalities for adolescents must include psychosocial and adjunctive treatments.

**SESSION XVI: IDENTIFICATION OF NOVEL
MECHANISMS UNDERLYING ALCOHOL, OPIOID,
AND NICOTINE ADDICTION USING SINGLE-CELL
WHOLE-BRAIN IMAGING**

MODERATOR: Olivier George

SPEAKERS: Lieselot Carrette, Alexander Smith, Samuel Centanni, & Olivier George

67. Normalization of the functional connectome in alcohol dependent mice following treatment with a CRF1 antagonist

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Andres Collazo; Olivier George*

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Using single-cell whole brain imaging of immediate early genes, our group recently showed that alcohol-dependent mice in withdrawal exhibit a widespread increase in coordinated brain activity, associated with a decrease in modularity of the whole-brain functional network, compared to naive control mice. This decreased modularity and functional hyperconnectivity has been hypothesized to represent a novel biomarker of alcohol dependence that can be used to evaluate the potential efficacy of novel medications for the treatment of alcohol use disorder, however, there is no evidence that current FDA-approved medications and experimental treatments known to reduce alcohol drinking normalize the changes in whole-brain functional connectivity. Here, we examine the normalizing effect of an FDA approved treatment for alcohol use disorder, naltrexone (opioid antagonist), and an experimental treatment, R121919 (CRF antagonist), on the functional connectome modularity of naive and alcohol dependent mice in withdrawal. Alcohol dependence was induced in mice by ethanol injections (2 g/kg or saline control; i.p., 9 days) with 4-fomepizole (9 mg/kg). On the 10th day, 24h in withdrawal, mice were treated with naltrexone (3 mg/kg), R121919 (2 mg/ml) or saline (N = 8/group, 4F + 4M). Behavior was examined 30 minutes following treatment by digging and marble burying tests. Then, 90 minutes later, mice were sacrificed, the brains immunolabeled for FOS, cleared using the iDisco+ protocol, imaged using light-sheet microscopy, and processed using the ClearMap pipeline to map and calculate the functional connectome. Alcohol dependent animals dug significantly more than naive animals, but there was no difference between the treatments within naive or dependent groups. The modularity of the withdrawal network was normalized by R121919 treatment, that mainly acted in the CRFR1 receptors in the cortical plate, causing functional disconnection between the prefrontal cortex and extended amygdala. Naltrexone on the other hand caused a further reduction of the modularity, through broad brain-wide reactivity co-repression. While naltrexone reduced alcohol intake and withdrawal-induced hyperalgesia in dependent rats in withdrawal, it was also found to cause increased anhedonia-like behaviors, which could be explained by increased activity of the lateral habenula and the decreased network modularity. These results demonstrate that whole-brain functional connectivity using immediate early genes can be used to identify the neuronal network mechanisms underlying the behavioral effects of potential medications, identify brain prints of specific compounds, and demonstrate that activation of the CRF-CRF1 system is responsible for the decrease in whole-brain modularity during alcohol withdrawal. Supported by NIAAA grants P60 AA006420 and R01 AA026081 to OG. Conflict of Interest: None.

68. Whole-Brain Mapping of Neuronal Ensembles of Oxycodone Seeking

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In the past two decades, a great deal of research using animal models drug addiction have focused on a small number of neurobiological systems, most notably the mesocorticolimbic dopamine system, the corticostriatal glutamate system, and the extended amygdala. Recent advances in tissue clearing and light-sheet microscopy technologies enable high-throughput, unbiased examination of protein expression, such as immediate early genes like c-Fos, throughout the entire brain. We trained 84 FosTRAP2-tdTomato mice in a cued reinstatement paradigm for oxycodone or sucrose (with yoked-saline controls), then fluorescently 'tagged' ensembles expressing c-Fos following either cue-induced reinstatement or a final extinction session. This revealed 33 brain regions with a significant induction of c-Fos that was specific to oxycodone compared to sucrose. We prioritized these regions based on linear modeling and correlation between regional c-Fos expression and reinstatement lever-pressing. Using chemogenetics to inhibit reinstatement-associated ensembles in FosTRAP mice, we identified the anterior pretecal nucleus (APtN) and the endopiriform nucleus (EPN) as novel regions where c-Fos expression is highly correlated with reinstatement behavior, and where activity is required for cued reinstatement of oxycodone seeking. These regions have unique molecular and cellular properties that likely contribute to their roll in opioid reward and addictive behaviors, but neither has previously been studied in the context of addiction. Future work will use single-cell transcriptomics, projection mapping, and opto/chemogenetic circuit manipulation to characterize these regions and their role in behavior, with a focus on substance use disorders. Supported by NIDA grant DA048119 (to ACWS) and DA048385 (to PJK)

69. Delineating the insula-centric negative affective circuitry engaged by stress and alcohol exposure

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Negative affective states are highly prevalent in drug addiction -especially abstinent alcoholics - and stress and negative affect can serve as potent triggers of cravings and relapse. We previously showed that a mid-insular to BNST circuit drives negative affective behavior associated with alcohol abstinence. To achieve our goal of delineating the complex abstinence network, we first need a better understanding of the fundamental circuitry governing stress on acute level. Restraint is a widely used stress model that induces a strong response in the BNST. We showed that insula-BNST pathway is recruited during active struggling behavior, and that this activity is closely paralleled by enhanced extracellular glutamatergic- and decreased GABAergic signaling. How does information regarding physical activity reach stress circuitry? Our work, in which active struggle events are tightly correlated with insula-BNST activity, suggests surveillance circuitry mechanisms exist where CNS pathways provide feedforward motor information. To delineate the larger control network governing the insula-BNST circuitry, we combined virally mediated input/output tracing with whole brain light sheet microscopy and single-cell quantification to map insula-centric stress circuitry. This revealed substantial input from motor- and premotor cortex. We isolated the cells projecting from the motor cortex to the insula and show that these cells are engaged shortly before struggle event onset. This demonstrates a unique pathway by which motor cortical regions can provide feed forward motor planning/execution information to affective circuits. This approach is now being incorporated into a chronic drinking-forced abstinence model where we are beginning to understand the larger insular circuitry governing ethanol drinking behavior and abstinence-induced negative affect. Supported by: NIAAA grants K99/R00 AA027774 (SWC) NIAAA R37 019455 (DGW), BBRF NARSAD Young Investigator Award 27172 (SWC). Conflicts of interest: None

70. Hyperconnectivity of long-range cholinergic regions contributes to the reorganization of the brain functional connectivity during nicotine withdrawal

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Withdrawal from nicotine produces a withdrawal state associated with whole-brain increases in co-activation patterns throughout the brain, with a significant decrease in whole-brain modularity, a reduction of total hub brain regions, and a shift from a cortical- to subcortical-driven network. However, it is unclear how cholinergic-rich regions contribute to the reorganization of the brain functional architecture and how this reorganization relates to the transcriptomic landscape of each brain region. To address this issue, we evaluated the contribution of long-range cholinergic regions (Ch1-Ch8) to the whole-brain networks and mapped the changes in whole-brain connectivity to genome-wide high-resolution atlas of gene expression throughout the adult mouse brain. We found that each module in the network was represented by at least one cholinergic group. The localization of each cholinergic group within each subnetwork was consistent with known anatomical and functional connections for these groups. During withdrawal, the cholinergic regions became functionally more connected in 3 clusters 1) the diagonal band nucleus, substantia innominate, and magnocellular area; 2) the lateral and medial habenula, and 3) the medial septum and pedunculopontine nucleus. Moreover, there was a significant increased correlation between the cholinergic regions and the cortical plate, subplate, striatum and pallidum regions. The cholinergic regions that clustered together in functional connectivity, relied on the same cholinergic receptor subunits. Integration of whole-brain connectivity with genome-wide high-resolution atlas of gene expression throughout the adult mouse brain showed that *Chrna3* had the highest correlation of all cholinergic receptors in the withdrawal network. Finally, we identified several gene transcripts that were highly correlated with activation of brain region throughout the brain, including glucose transferase 1 (*Slc2a1*) which is downregulated by nicotine, electron transfer flavoprotein subunit alpha (*Etfα*) which contributes to nicotine degradation, and NIMA related kinase 7 (*Nek7*) which has been associated with nicotine dependence. These results demonstrate that long-range cholinergic regions play a major role in the whole-brain network organization during nicotine withdrawal and identify key genes that are associated with nicotine withdrawal. Supported by TRDRP T32IR5384, NIAAA grants P60 AA006420 and R01 AA026081 to OG.

Conflict of Interest: None.

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