***7TH***

***INTERNATIONAL DRUG ABUSE RESEARCH SOCIETY***

***PROGRAM AND ABSTRACTS***

***Recent Advances in Drug Addiction***

***September 2 – 6, 2019***

***Hyatt Regency Hotel***

***Casablanca, Morocco***

**Conference Organizers:**

**Syed Ali, Nick Gilpin, Barbara Mason, Sulie Chang, Emmanuel Onaivi, Michael Kuhar; Abdelouahhab Tazi, Antonio Noronha and George Koob**

**Local Organizing Committee:**

 **Pr. Abdelouahhab TAZI (Chairman), Pr. Driss MOUSSAOUI, Pr. Meriem El YAZAJI, Sara LAKEHAYLI, Dr. Nadia SAID, Soumaya EL GANOUNI**

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

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**Ministry of Health Morocco**

**The Addiction Research Center of Casablanca, Morocco**

**Faculty of Medicine, University Hospital**

**Monday, September 2 - 6, 2019**

**REGISTRATION AND WELCOME**

 **TBA – Check for sign at Lobby**

 **3:00 – 6:00 PM**

**Tuesday, September 3, 2019**

**8:00AM - 2:00PM Registration: Forum 2/3 Conference Area**

**8:00AM - 9:00AM Breakfast: Forum 2/3 Foyer**

**Conference: Forum 2/3 - Meeting Hall**

**9:00 - 9:30AM Opening of the Meeting**

 **Welcome/Travel Award Presentations**

 **George Koob/Syed Ali**

**9:30 – 10:30AM Plenary Lecture I**

 **Alcohol and Drug Addiction: The Gain in the Brain is in the Pain**

 **George Koob,** *NIAAA, Baltimore, Maryland, USA*

**Moderator: Abdelouahhab Tazi, Casablanca, Morocco**

**10:30 -10:50AM COFFEE/TEA BREAK**

**SESSION I: CORTICAL REGULATION OF REWARD, COMPULSIVITY AND ALCOHOL DRINKING**

**Moderators:** **George Koob (USA) and Nick Gilpin (USA)**

**10:50 - 11:10AM Cortical-Amygdala circuitry in reward learning and pursuit**

 ***Kate M. Wassum*** *Department of Psychology, University of California Los Angeles, Los Angeles, California USA*

11:10 - 11:30AM Prefrontal regulation of punished ethanol self-administration.

 ***Andrew Holmes,*** *Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, Maryland, USA*

**11:30 - 11:50AM Context-induced relapse of nicotine seeking after punishment-imposed abstinence: role of anterior insular cortex.**

 ***Nathan Marchant,****,* *Department of Anatomy & Neurosciences, Amsterdam University Medical Center, Amsterdam, The Netherlands, National Institute on Drug Abuse, Baltimore, Maryland, USA*

**11:50 - 12:10PM Anterior insular cortex regulates renewal of alcohol-seeking after punishment imposed abstinence**

 ***Andrew Lawrence,***  *The Florey Institute of Neuroscience and Mental Health, Parkville, Victoria 3052, Australia*

# 12:10 - 12:30PM Exercise reduces escalated drinking in alcohol dependent mice via elevated BDNF in prefrontal cortex

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

# 12:30 - 12:50PM Adolescent rat alcohol exposure alters adult brain mimicking human AUD

#  *Fulton T. Crews*, *Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel, North Carolina, USA.*

**12:50 - 2:00 PM LUNCH**

**SESSION II: POSTER SESSION: 12:50 – 2:00 PM**

**Moderators :** **Ashraf Virmani (The Netherlands) and Syed Ali (USA)**

**(See Pages 17-19 for the List of Posters)**

**SESSION III: EXTENDED AMYGDALA REGULATION OF ADDICTION**

**Moderators:** **Antonio Noronha (USA) and Roberto Ciccocioppo (Italy)**

2:00 – 2:20 PM Reward and aversion biases in projector populations of the amygdala and insular cortex.

 *Anna Beyeler, Neurocentre Magendie, INSERM 1215, University of Bordeaux, Bordeaux, France*

 **2:20 – 2:40 PM Coactivation of MOP and NOP opioid receptor to treat substances use disorders.**

***Roberto Ciccocioppo,*** *School of Pharmacy, Pharmacology Unit, University of Camerino, Italy*

**2:40 – 3:00 PM Adolescent intermittent ethanol exposure alters fear conditioning and extinction behavior in adulthood**

 ***Judson Chandler*,** *Department of Neuroscience, Medical University of South Carolina*, *Charleston, South Carolina, USA*

**3:00 – 3:20 PM Repeated binge drinking causes plastic increases in central amygdala corticotropin releasing factor neurons in vivo.**

 ***Dennis Sparta***, *Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland, USA.*

**3:20 – 3:40 PM Epigenetic mechanisms underlying molecular changes in the amygdala following adolescent alcohol exposure in humans and rodents.**

 ***Subhash Pandey****,* *Center for Alcohol Research in Epigenetics, Department of Psychiatry, University of Illinois at Chicago, Chicago, Illinois, USA.*

**3:40 - 4:00 PM COFFEE/TEA BREAK**

 **SESSION IV: BRAIN OPIOIDS SIGNALING IN PAIN AND ADDICTION**

**Moderators:** **Eliot Gardner (USA) and Leandro Vendruscolo (USA)**

**4:00 – 4:20 PM Heroin addiction engages glucocorticoid-dependent brain stress systems.**

 ***Leandro Vendruscolo, NIDA-IRP, Baltimore, Maryland, USA*.**

**4:20 – 4:40 PM Involvement of endogenous ghrelin in oxycodone self-administration and brain stimulation reward.**

 ***Eliot Gardner****, National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland USA*

**4:40 – 5:00 PM Functional upregulation of kappa opioid receptors in chronic pain states.**

 ***Catherine Cahill,*** *University of California Los Angeles, Los Angeles, California USA*

**5:00 – 5:20 PM Opioid use disorder in humans.**

***Sandra Comer,*** *Columbia University, New York, NY, USA*

**5:20 - 5:30 PM TRANSITION**

 **SESSION V: TRAVEL AWARD WINNER PRESENTATIONS**

**Moderators:** **Barbara Mason (USA) and Eliot Gardner (USA)**

**5:30 – 5:45 PM A multi-species neurogenomics approach to identify pathways & medications for alcohol use disorder**

***Sean Farris\*,*** *Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, Texas, USA*

(\* George Koob Travel Awardee)

5:45 – 6:00 PM Manipulation of central amygdala neurotensin neurons alters the consumption of alcohol.

 *Zoe McElligott\*\*, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA*

 (\*\* Michael Kuhar Travel Awardee)

**6:00 – 6:15 PM Cocaine self-administration induces transcriptional adaptation in the ventral pallidum**

 ***Michel Engeln****\*\*\*, Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland, USA*

(\*\*\*Eliot Gardner Travel Awardee)

**6:15 – 6:30 PM Prescription opioids, synaptic alterations and neurodevelopment: a systems biology approach.**

***Gurudutt Pendyala\*\*\*\*,*** *Department of Anesthesiology, University of Nebraska Medical Center, Omaha, Nebraska, USA*

(\*\*\*\* Institute of NeuroImmune Pharmacology, Seton Hall University Travel Awardee)

**Wednesday, September 4, 2019**

**8:00AM - 11:00 AM Registration: Forum Meeting area**

**8:00AM - 9:00AM Breakfast: Forum 2/3 Foyer**

**9:00 - 10:00 AM Plenary Lecture II**

 **Ventral tegmental area neuronal diversity and motivated behavior**

***Marisela Morales***, *Integrative Neuroscience Research Branch. Neuronal Networks Section. National Institutes of Health. National Institute on Drug Abuse. Intramural Research Program. Baltimore, Maryland, USA.*

**Moderator: Michael Kuhar (USA)**

**10:00 – 10:20 AM COFFEE/TEA BREAK**

**SESSION VI: BINGE DRINKING: NEUROIMMUNE BIOMARKERS**

**Moderators:** **Sulie Chang (USA) and Dipak Sarkar (USA)**

**10:20 - 10:40 AM** **Binge exposure to ethanol-mediated neuroinflammation modulating**

 **morphine’s anti-nociception in B6 adolescent mice**

***Sulie L. Chang****, Institute of Neuroimmune Pharmacology and Department of*

 *Biological Sciences, Seton Hall University, South Orange, New Jersey, USA*

**10:40 - 11:00 AM** **Microglia control stress axis function via secreting exosomes**

***Dipak Sarkar***; *Rutgers Endocrine Research Program, Department of Animal Sciences, Rutgers University, New Brunswick, New Jersey, USA*

11:00 – 11:20 PM Alcohol and Cholesterol interact to control cerebral artery bk current via a PKC-dependent mechanism.

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

11:20 -11:40 PM Toluene constricts cerebral arteries by targeting smooth muscle BK channels

 *Alex Dopico,* *University of Tennessee Heath Science Center, Memphis, Tennessee, USA*

11:40 – 12:00 PM Female gonadal hormone modulates etanol-induced memory déficit in post-pubertal female rats.

 *Ratna Sircar, The City College of New York, New York, New York, USA.*

12:00 – 12:20 PM Involvement of specific nicotine receptors in alcohol, nicotine, Cocaine and methamphetamine intake and attentional performance.

 ***Amir Rezvani,*** *Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina, USA*

**12:20 - 2:00 PM LUNCH**

**SESSION VII: POSTER SESSION: 12:20 – 2:00 PM**

**Moderators : Ashraf Virmani (The Netherlands) and Syed Ali (USA)**

**(See Pages 17-19 for the List of Posters)**

**SESSION VIII: MEDICATIONS DEVELOPMENT: Alcohol/Cocaine/Nicotine**

**Moderators:** **Barbara Mason (USA) and Edith Sullivan (USA)**

**2:00 – 2:20 PM A novel cutaneous gene therapy for alcohol abuse and co-abuse with cocaine.**

***Ming Xu*,** *The University of Chicago, Chicago, Illinois USA*

2:20 – 2:40 PM Translational studies of alcohol use disorder.

 ***Adolf Pfefferbaum,*** *SRI International, Menlo Park, California USA*

2:40 – 3:00 PM Multisystem approaches to treating tobacco and other addictions.

 ***Ed Levin,*** *Duke University Medical Center, Durham, North Carolina, USA*

3:00 – 3:20 PM Methamphetamine-associated cognitive decline involves aberrant proliferation of neural progenitor cells and is attenuated by neutralizing IL-1 signaling.

 ***Michal Toborek,*** *Department of Biochemistry and Molecular Biology, University of Miami, School of Medicine, Miami, Florida, USA*

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

**SESSION IX: HIV-INFECTIONS AND DRUG ABUSE**

**Moderators:** **Mohan Sopori (USA) and Shilpa Buch (USA)**

**3:40 – 4:00 PM Interaction between HIV and cigarette smoke in lung comorbidities.**

 ***Mohan Sopori,*** *Lovelace Respiratory Research Institute, Albuquerque, New Mexico, USA*

4:00 – 4:20 PM Opiates potentiate HIV-associated neuroinflammation: Role of EVS in microglial activation and migration.

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

**4:20 – 5:00 PM Alcohol-SIV/HIV-ART interactions & risk for comorbidities.**

 ***Patricia Molina,*** *Louisiana State University Health Sciences Center, New Orleans, Louisiana USA*

**5:00 – 5:20 PM Activation of GPR55 induces neuroprotection of hippocampal neurogenesis during chronic systemic inflammation.**

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

**5:20 – 5:40 PM Morphine tolerance is attenuated in germ-free mice and reversed by probiotics implicating the role of gut microbiome.**

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

**SESSION X: FLASH TALKS by TRAINEES**

**Moderator: Nick Gilpin (USA)**

**5:45 - 5:50 PM Intranasal oxytocin on alcohol withdrawal, craving and relapse: a double-blind RCT.**

 ***Katrine Melby,*** *Department of Clinical Pharmacology, St. Olav University Hospital, Norway*

5:50 - 5:55 PM Sucrose bingeing modifies alcohol reward in mice.

***Gaёlle Awad,*** *Labortorie de Neurosciences Cognitive et Adaptatives UMR 7364, CNRS/Universitie de Strasboug, France*

**5:55 - 6:00 PM Involvement of NFKB in cocaine-associated reconsolidation using intravenous self-administration.**

 ***Caroline Correia,*** *Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), Faculte de Psychologie, France*

**6:00 - 6:05 PM Sex differences in cocaine craving: role of the estrous cycle.**

 ***Celine Nicolas,*** *Behavioral Neuroscience Branch, Intramural Research Program, NIDA, Baltimore, Maryland, USA*

**6:05 - 6:10 PM Initial characterization of opioid-induced respiratory depression in rats.**

***Renata Marchette,*** *Neurobiology of Addiction Section, Integrative Neuroscience Branch, NIDA-IRP, Baltimore, Maryland, USA*

**6:10 – 7:00 PM IDARS Business Meeting**

**Thursday, September 5, 2019**

**Conference Organizers plan a tour of Casablanca**

**Dinner will be Hosted by**

**Mr. Sâadeddine EL OTHMANI**

**Head of Government**

**The Kingdom of Morocco**

**No Scheduled Conference activities**

**Friday, September 6, 2019**

**8:00 - 9:00 AM Registration: Forum 2/3 Meeting Ballroom**

 **Breakfast: Forum 2/3 Foyer**

**9:00 – 9:30AM Plenary Lecture III**

 **Addictions in Morocco: A Historical Prospective**

 ***Driss Moussaoui,*** *Ibn Rushd University Psychiatric Centre*

 *Casablanca, Morocco*

**Moderator: George Koob (USA)**

**9:30 – 10:00 AM COFFEE/TEA BREAK**

**SESSION XI: MARIJUANA/CANNABINOIDS I**

**Moderators:** **Prakash Nagarkatti (USA) and Emmanuel Onaivi (USA)**

# 10:00 – 10:20 AM Alcohol preference and behavioral modifications following deletion of CB2RS dopamine neurons and *microglia*.

 ***Emmanuel Onaivi*** , *William Paterson Universtiy, Wayne, New Jersey, USA*

**10:20 – 10:40 AM Effects of cannabidiol on cocaine seeking: dose-response profiles and neurobiological substrates.**

 ***Friedbert Weiss,*** *Department of Neuroscience, The Scripps Research Institute, La Jolla, California USA*

**10:40 – 11:00 AM Epigenetic regulation of inflammation by marijuana cannabidiol.**

***Prakash Nagarkatti,*** *Universtiy of South Carolina, Columbia, South Carolina USA*

**11:00 – 11:20 AM Protective effects of Δ9-tetrahydrocannabinol (THC) against SEB-induced acute lung injury results from alterations in lung dysbiosis.**

***Mitzi Nagarkatti,*** *Universtiy of South Carolina, Columbia, South Carolina USA*

**11:20 - 1:00 PM LUNCH**

**SESSION XII: MARIJUANA/CANNABINOIDS II**

**Moderators:** **Mitzi Nagarkatti (USA) and Declan Ali (Canada)**

**1:00 – 1:20 PM Acute exposure to cannabinoids alters early neuronal development in Zebrafish.**

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

1:20 – 1:40 PM Δ9-THC and related cannabinoids suppress substance p-evoked vomiting via activation of cannabinoid CB1receptors.

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

**1:40 – 2:00 PM Neuroadaptations of the endocannabinoid system following cocaine or sucrose intake.**

***Katia Befort****, CNRS, Universite de Strasbourg, Strasbourg, France*

**2:00 – 2:20 PM Alcohol preference and depression in cnr2 knockout mice**

***Hiroki Ishiguro,*** *University of Yamanashi, Chuo, Yamanashi, Japan*

**2:20 – 2:40 PM Classic amphetamines versus new cathinones: comparative behavioral, neurochemical and glial signature.**

 *Frederico Pereira, Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

**2:40 – 3:00 PM COFFEE/TEA BREAK**

**SESSION XIV: 3:00 – 5:00 PM**

 **PANEL DISCUSSION, SUMMARY & RECOMMENDATION**

George Koob, Abdelouahhab Tazi, Sulie Chang, Shilpa Buch, Emmanuel Onaivi, Prakash Nagarkatti, Barbara Mason, Susan Schenk, Eliot Gardner, and Syed Ali

**7:00 - 10:00 PM**

**FAREWELL DINNER**

**Poolside Terrace**

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**ABSTRACTS**

1. **Cortical-amygdala circuitry in reward learning and pursuit**

**Kate M. Wassum1**,2, Melissa Malvaez1, Nina Lichtenberg1, Ashleigh Morse1, Venuz Greenfield1, Zachary Pennington1, Linnea Sepe-Forest1, Michael Murphy1, Christine Shieh1, Sandra Holley3, Carlos Cepeda3, Michael Levine2,3

*1Department of Psychology, 2 Brain Research Institute, 3Intellectual and Developmental Disabilities Research Center, Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California 90095*

To make adaptive decisions we must cast ourselves into the future and consider the outcomes of our potential choices. This prospective consideration is informed by our memories. I will discuss our lab’s recent work investigating the neural circuits responsible for encoding, updating, and retrieving reward memories for use in the considerations underlying decision making. We have taken a multifaceted approach to these investigations, combining recording, circuit dissection, and behavioral tools. Our results are indicating that the orbitofrontal cortex and basolateral amygdala work in a circuit to participate in these functions. The cognitive symptoms underlying addiction can result from a failure to appropriately learn about and/or anticipate potential future events, making these basic science data relevant to the understanding and potential treatment of addiction to drugs or alcohol.

Acknowledgements: This research was supported by NIH grant DA035443, MH106972, and NS087494 to KMW and NIH grant DA038942 and DA024635 to MM.

Conflict of interest: No conflict of interest.

1. **Prefrontal regulation of punished ethanol self-administration**

**A. Holmes**; L.R. Halladay

*Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, USA*

A clinical hallmark of alcohol use disorder (AUD) is that drinking persists despite an awareness of the potential adverse consequences. The ventral (vmPFC) and dorsal (dmPFC) medial prefrontal cortex are positioned to exert top-down control over subcortical regions, such as the nucleus accumbens shell (NAcS) and basolateral amygdala (BLA), encoding the positive and negative valence of EtOH-related stimuli. Prior studies in rodents have implicated these prefrontal regions in the regulation of punished EtOH self-administration (EtOH-SA). Here, we conducted *in vivo* electrophysiological recordings in the vmPFC and dmPFC to obtain neuronal correlates of footshock-punished EtOH-SA in mice. *Ex vivo* recordings were performed in NAcS D1-positive MSNs receiving vmPFC input to examine punishment-related changes in this pathway. To assess the functional contribution of the vmPFC and dmPFC neurons, and vmPFC projections to the NAcS or BLA, these regions/pathways were optogenetically-silenced during testing. In response to punishment, mice exhibited approaches to the EtOH-lever but aborted making an actual lever-press, leading to a reduction in the rate of EtOH-lever pressing. Neurons in both vmPFC and dmPFC exhibited phasic firing related to both aborts and EtOH-lever pressing, but population-level coding of aborts was only evident in the vmPFC. Photosilencing the vmPFC, but not dmPFC, reversed punished-suppression of EtOH-SA. Punished-suppression was associated with plasticity at vmPFC inputs to D1-MSNs in the NAcS, and photosilencing vmPFC→NAcS projections, but not vmPFC→BLA, partially reversed punished-suppression. These data identify a corticostriatal circuit regulating EtOH-SA after punishment, with implications for understanding the neural basis of compulsive drinking in AUDs.

Work supported by the NIAAA Intramural Research Program.

No conflict of interest

1. **Context-induced relapse of nicotine seeking after punishment-imposed abstinence, role of anterior insular cortex.**

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In humans, cues and environments associated with nicotine use cause relapse during abstinence. Previous rodent models of nicotine relapse have used extinction to suppress nicotine seeking. However, human abstinence is typically initiated out of a desire to avoid the negative consequences of nicotine use. Here, I will present recent studies aimed to better model this motivation for abstinence by using punishment-imposed abstinence. Rats are first trained to self-administer intravenous nicotine in one context. Subsequently nicotine self-administration is punished in another context. After punishment-imposed abstinence, we observed increased nicotine seeking on a test in the nicotine context, compared to a test in the punishment context. To identify the neural correlates of this relapse, we measured fos expression in rats tested for context-induced relapse of nicotine seeking. We made comparisons of fos expression in rats tested for context-induced relapse after either punishment or extinction. We found that context-induced relapse of nicotine seeking after either extinction or punishment is associated with increased activity in the anterior insular cortex. In contrast we found that fos expression in BLA was increased in rats tested for context-induced relapse after punishment, but not extinction. Finally, we found that chemogenetic inhibition of the anterior insular cortex during test for context-induced relapse after punishment caused a significant decrease in nicotine seeking in the nicotine context. These studies show that punishment of nicotine self-administration is encoded as a new, context-dependent, memory. Furthermore, the neural substrates of relapse may differ depending on the motivation for abstinence.

1. **Anterior insular cortex regulates renewal of alcohol-seeking after punishment imposed abstinence**

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Negative consequences associated with excessive drinking, such as health, legal and/or relationship issues can motivate individuals with alcohol use disorder typically to voluntarily abstain. Exposure to contexts previously associated with alcohol use can trigger craving and subsequent relapse. There is variability in relapse propensity following extended periods of abstinence, and this impacts successful treatment. We employed a rat model that features voluntary abstinence from alcohol use due to negative consequences. In rodents (male alcohol preferring iP rats) we model this using random but contingent punishment upon delivery of alcohol in a distinct context, a variation of the ABA protocol. We assessed relapse following acute (1 day) or prolonged (30 days) abstinence. Relapse propensity was high in Context A but low in Context B when tested at day 1. After 30 days abstinence, there was no change in relapse propensity in Context A, but increased relapse propensity in Context B. Therefore, 30 days abstinence increases relapse propensity in the punishment context. Neither alcohol intake history nor the motivational strength of alcohol predicted the propensity to relapse. Fos expression in the anterior insular cortex (AI) correlated with alcohol seeking behavior (lever pressing) in Context B after prolonged (30 days) abstinence. Therefore, we tested a causal role of the AI in context-induced relapse in the punishment context after prolonged abstinence. Local pharmacological inactivation of the AI reduced relapse propensity in Context B after 30 days abstinence. Our results show individual variability in relapse propensity in the punishment-associated context after prolonged abstinence, mediated by activity in the AI.

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No conflict of interest.

**5. Exercise reduces escalated drinking in alcohol dependent mice via elevated bdnf in prefrontal cortex**

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Repeated cycles of chronic intermittent ethanol (CIE) exposure results in increased ethanol consumption along with reduced brain-derived neurotrophic factor (BDNF) mRNA and protein expression in medial prefrontal cortex (mPFC) of mice. We previously demonstrated BDNF infusion directly into the mPFC of ethanol-dependent mice reversed CIE-induced escalation of ethanol intake, while viral-mediated over-expression of BDNF prevented this CIE-induced escalated drinking. Since exercise is known to increase BDNF expression in brain, the present study investigated the effects of wheel running on ethanol consumption and Bdnf mRNA expression in mPFC of mice in the CIE-Drinking model. Adult male C57BL/6J mice were given 2-hr daily access to activity wheels in the home-cage. After 6 weeks, Bdnf mRNA levels were increased in the mPFC compared to sedentary mice (no-wheel access). Separate groups of mice that continued wheel running showed an attenuation in CIE-induced escalated ethanol intake compared to CIE-exposed mice with no access to wheel running (CIE: ~145% increase vs. CIE+WH: ~50% increase over baseline ethanol consumption at end of study; p< 0.05). In another study, systemic administration of the TrkB receptor antagonist ANA-12 (1 mg/kg) attenuated the ability of wheel-running to reduce CIE-related escalated drinking. Mice that received placebo (DMSO vehicle) showed the expected effect of exercise on drinking. Collectively, these data suggest that exercise can attenuate dependence (CIE) induced escalation of ethanol drinking and this effect may be mediated by exercise-induced elevation of Bdnf mRNA expression as well as BDNF-TrkB receptor signaling in the mPFC.

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Conflict of Interest: None.

1. **Adolescent rat alcohol exposure alters adult brain mimicking human AUD**

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Adolescent age of alcohol drinking onset is related to risks of developing lifetime AUD. To determine if adolescent binge drinking caused persistent effects, rats exposed to adolescent intermittent ethanol (AIE) across puberty were assessed in adulthood for changes in synaptic proteins, immediate early genes, cognition and fcMRI. AIE-induced changes in rat brain are compared to post-mortem human control or AUD brain. AIE increased adult brain expression of innate immune genes HMGB1, CCL2, Toll-like receptors, and RAGE. AIE also increased activation of the transcription factor NFkB and increased epigenetic silencing marker H3K9me2. AIE also increased adult risky decisions and blunted behavioral flexibility in parallel with reduced adult hippocampal neurogenesis, reduced forebrain cholinergic and midbrain serotonergic neurons as well as increases in markers of neurodegeneration. MRI found altered cortical thickness and myelin and a decrease in fcMRI cortical-striatal connectivity in adults after AIE. Post-mortem AUD human brain assessments find increased HMGB1, TLR, CCL2 and pNFkB that mimic those found following AIE. Further, increases in human brain gene expression correlated with age of drinking onset. Exercise, indomethacin, donepezil and galantamine were found to prevent and/or reverse AIE induced loss of adult neurogenesis, loss of cholinergic neurons, increased RAGE, pNFkB and proinflammatory gene induction. Rat studies finding adolescent alcohol exposure alters brain gene expression, synapses and neuronal phenotypes linked to altered adult PFC connectivity, responses to alcohol and risk-taking behaviors are consistent with increasing risks for AUD. Prevention and reversal of these pathologies links them mechanistically, providing new therapeutic targets for AUD.

Funding from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) through the Neurobiology of Adolescent Drinking in Adulthood (NADIA) consortium.

No conflict of interest.

1. **Reward and aversion biases in projector populations of the Amygdala and insular cortex**

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Inappropriate assignment of emotional valence and dysfunctions of the circuits that govern valence processing are thought to underlie many psychiatric diseases including anxiety, depression and addiction. The basolateral amygdala (BLA) and the insular cortex (insula) are known to play a critical role in the formation of associative memories of both positive and negative valence, are reciprocally connected and contain populations of projection neurons targeting the central nucleus of the amygdala (CeA), the ventral hippocampus (vHPC) and the nucleus accumbens (NAc).

To define the critical, yet unknown, role of projector populations in the amygdala and insula we used a combination of cutting edge technologies in mice models. Using optogenetics to activate or inhibit these populations in vivo, we found that BLA-vHPC projection regulates anxiety-related behaviors, while BLA-NAc population support reward seeking and BLA-CeA population induce avoidance. In addition, projection neurons in the anterior insula showed an increase calcium signal in anxiogenic spaces and optogenetic activation of posterior insula-CeA populations induces real time place aversion.

By combining large-scale single-unit recordings and optogenetic photo-identification in vivo, we identified that despite the heterogeneity of single-cell coding, BLA-NAc and BLA-CeA populations differentially encode the retrieval of positive and negative associations. Consistently, we provided evidence that experiences of opposite valence differentially regulate the synaptic inputs onto BLANAc and BLA-CeA populations. Finally, using retrograde tracing we identified a topographical organization of the three populations within the amygdala and mapped their local connectivity using *ex vivo* whole-cell patch-clamp recordings.

Together, our findings reveal that encoding of emotional valence in the insula and the amygdala is at least partially accomplished through the divergent activity of anatomically defined neural populations.

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1. **No conflict of interest Coactivation of mop and nop opioid receptor to treat substances use disorders.**

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Buprenorphine has been used for the treatment of opioid addiction for several years. However, preclinical evidence indicates that it is also efficacious in reducing the motivation for psychostimulants and alcohol. The potential efficacy of buprenorphine on cocaine and on alcohol consumption has merged also in few clinical studies. Classical studies demonstrated that buprenorphine activates MOP and antagonize DOP and KOP opioid receptors. However, more recent experiments showed that at higher doses it also acts as a partial agonist at nociceptin NOP receptor, a fourth member of the opioid family. Engagement of this latter receptor seems to play an important role in mediating the effect of this drug on alcohol and on cocaine. Based on these evidence, we sought interesting to explore the effects of cebranopadol, a potent MOP/NOP dual agonist, that at higher concentration stimulates also DOP and KOP. Using operant self-administration paradigms in the rat we found that cebranopadol attenuates the motivation for cocaine, heroin and alcohol. Furthermore, in drug self-administration training extinction/reinstatement paradigms, pretreatment with cebranopadol significantly reduced yohimbine stress-induced reinstatement of drug seeking. Finally, in rats trained to intravenous (i.v.) heroin self-administration, we observed a progressive decrease in operant responding when animals were switched to i.v. cebranopadol. Together, these data indicate that cebranopadol has limited abuse liability compared to heroin while it is highly efficacious in reducing opioids, psychostimulants and alcohol self-administration. These results suggest that drugs co-activating MOP and NOP receptors have low abuse liability and may represent a suitable strategy to treatment substance use disorders (*Grant: NIH- AA014351*). *No conflict of interest are reported*.

1. **Adolescent intermittent ethanol exposure alters fear conditioning and extinction behavior in adulthood.**

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Post-Traumatic Stress Disorder (PTSD) and Alcohol Use Disorder (AUD) are two of the most prevalent psychiatric conditions and are highly comorbid. The social and financial burden that results from a lack of effective treatments for these disorders is enormous. Therefore, there is a clear and urgent need to gain a greater understanding of the pathophysiological basis of PTSD and AUD comorbidity. Current evidence suggests the neurocircuitry of PTSD and AUD significantly overlap, which includes dysfunctional control of behavior by the prefrontal cortex (PFC) and components of the extended amygdala. There is a strong correlation between the age of onset of alcohol use during adolescence and the likelihood of developing an AUD later in life. In addition, adolescence is a critical period of continued development, and accumulating evidence indicates that the abuse of alcohol during adolescence can have long-term consequences on brain and behavior. In the current study, we used a rat binge ethanol model consisting of repeated cycles of Adolescent Intermittent Ethanol (AIE) exposure by vapor inhalation and a Single Prolonged Stress (SPS) procedure that is thought to model a traumatic stress event in humans. In the first set of studies, we observed that AIE-exposed male rats exhibited a greater level of fear conditioning compared to controls. This conditioning was acquired at a faster rate as reflected by a fewer average number of tone/shock pairings required. AIE rats also exhibited a slower rate of extinction of the fear memory that was also reflected by fewer tone/shock pairing required to reach criteria (tone/shock pairing data not shown). AIE-exposed rats also exhibited increased freezing behavior during testing for both extinction recall and spontaneous recovery. In a separate set of follow-up studies, we examined the effect of a positive allosteric modulator of mGlu5 (CDPPB) on AIE-induced deficits in extinction learning. The results replicated the deficits observed in the first study, and further demonstrated that CDPPB administered 20 min prior to each daily extinction session completely reversed the AIE-induced deficits in extinction recall, renewal and spontaneous recovery. In a final set of studies, a we subjected control and AIE-exposed rats to the SPS procedure. Following a 1-week stress consolidation period, rats were then tested in the fear conditioning procedure. The results revealed that both AIE and SPS exposed rats exhibited reduced extinction learning compared to controls. An especially interesting observation is that while extinction recall was not altered by SPS alone, it had a synergistic effect with AIE (e.g., deficits in the ability to retain the extinction memory). Taken together, these observations provide preclinical support for the hypothesis that individuals who experience a traumatic event and also abused alcohol during adolescence, may be at increased risk for developing PTSD.

1. **Repeated binge drinking causes plastic increases in central amygdala corticotropin releasing factor neurons in vivo**

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Binge ethanol drinking is an increasingly problematic component of alcohol use disorder costing the United States approximately over $150 billion every year. Binge drinking likely causes progressive neuroplasticity alterations in numerous brain regions. However, the precise nature or mechanisms by which they alter binge drinking have not yet been determined. Corticotropin releasing factor (CRF) neurons in the central amygdala (CeA) are thought to modulate binge drinking, but the precise circuit mechanisms remain poorly understood. Here, for the first time we combined optogenetics with *in vivo* electrophysiology to identify and record from CeA CRF neurons in mice during a repeated binge drinking task. First, we found that CeA CRF neurons were more excitable than CeA non CRF neurons in our binge drinking. We also observed that CeA CRF neurons displayed a heterogeneous spike profile in response to a lick of ethanol including lick predictive, lick excited, lick inhibited, and no response. Lick predictive CeA CRF neurons could be further grouped into 2 classes based on their activity in response to a binge alcohol session, with the majority showing increases in firing and bursting. Furthermore, lick predictive CeA CRF neurons increased their activity over repeated binge drinking sessions, indicating possible synaptic plasticity. These data indicate that microcircuits within the CeA CRF system as well as their projections may modulate specific components of binge drinking.

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No conflict of interest

1. **Epigenetic mechanisms underlying molecular changes in the amygdala following adolescent alcohol exposure in humans and rodents**

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Alcohol consumption during adolescence is a primary risk factor for developing alcohol use disorder (AUD) and comorbid anxiety disorder later in life. This study investigated how adolescent drinking effects epigenetic mechanisms mediated by EZH2 (H3K27me3 specific histone methyltransferase) in humans and rodents. EZH2 is the catalytic component of the PRC2 complex and plays a vital role in development by controlling developmental transcription programs. Human postmortem amygdala from individuals with AUD who began drinking before or after the age of 21 and control subjects was used for mRNA and epigenetic analysis. Additionally, Sprague-Dawley male adolescent rats were exposed to intermittent ethanol (2g/kg) (AIE) or saline (AIS). Adult rats were infused with EZH2 siRNA into the central nucleus of amygdala (CeA) and then evaluated for anxiety-like and alcohol drinking behaviors. qPCR and ChIP assay were used to examine mRNA levels and epigenetic modifications in the rat amygdala after EZH2 siRNA infusion. We also performed transcriptomic profiling in the amygdala of AIE and AIS adult rats using RNA-sequencing (RNA-seq). Individuals with early onset drinking had significantly decreased Arc mRNA and BDNF mRNA and protein expression in the amygdala; a finding which was not observed in the late onset drinking group. ChIP analysis revealed that this decrease was likely caused by increased repressive H3K27me3 deposited by increased EZH2 at the BDNF-IX and Arc promoters in early onset AUD. Our results also indicate that AIE exposure in rats caused decreased Bdnf and Arc expression and increased EZH2 and H3K27me3 associated with the BDNF-IX and Arc promoter. These alterations were prevented by knocking down EZH2 in the CeA of rats in adulthood. Interestingly, EZH2 siRNA infusion into CeA also attenuated AIE-induced anxiety and alcohol-drinking behaviors in adulthood. RNA-seq data indicates that AIE produced deficits in several axon-guidance and synaptic plasticity-associated genes in adult amygdala. Furthermore, these transcriptomic changes are mediated by the increased occupancy of EZH2 in the promoters of these genes in adult amygdala due to adolescent alcohol exposure. Together, these data suggest that EZH2 is an important epigenetic regulator of AIE-induced molecular changes in the amygdala and could be a potential target for treatment of adolescent alcohol exposure-induced adult psychopathology [Supported by NIH-NIAAA P50AA022538, UO1AA-019971, U24AA-024605 and by the VA Senior Research Career Scientist award to SCP].

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1. **Heroin addiction engages glucocorticoid-dependent brain stress systems**

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Repeated opioid withdrawal episodes dysregulate brain stress systems. We hypothesized that this dysregulation drives addiction-like behaviors and depends on glucocorticoid receptors (GRs). Rats that were allowed long access (LgA; 12 h sessions) to heroin exhibited an increase in heroin addiction-like behaviors compared with rats that were allowed short access (ShA; 1 h sessions). Chronic GR antagonism with mifepristone blocked the increase in addiction-like behavior in LgA rats, with no behavioral changes in ShA rats. Electrophysiological experiments indicated that corticotropin-releasing factor (CRF) neurons in the central nucleus of the amygdala, a stress-related brain region, were hyperactive in LgA rats compared with ShA rats, an effect that was blocked by chronic mifepristone treatment. To test the functional role of CRF in the central nucleus of the amygdala in addiction-like behavior, we used an antisense oligonucleotide (ASO) approach that was designed to favor transcription of the steroid receptor co-regulator-1a (SRC-1a) isoform and thus inhibit the transcription of CRF. The bilateral infusion of SRC-1 ASO in the central nucleus of the amygdala decreased addition-like behaviors in LgA rats. These results suggest that GRs and GR co-regulators may be potential non-opioid targets for the treatment of opioid use disorder.

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No conflict of interest.

1. **Involvement of endogenous ghrelin in oxycodone self-administration and brain stimulation reward**

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Prescription opioid abuse is a serious worldwide health problem. Identifying novel mechanisms underlying opioid addiction and developing innovative medications for treatment of opioid addiction are high priority objectives. Ghrelin, an orexigenic hormone secreted primarily from stomach and gut, modulates mesolimbic dopamine transmission and food reward, and regulates the rewarding effects of abused drugs as assessed using conditioned place preference. However, the role of endogenous ghrelin signaling in drug self-administration remains unexplored. We assessed responses of ghrelin in bloodstream and of ghrelin brain receptors to oxycodone self-administration behaviors, and the effects of the ghrelin receptor antagonist JMV2959 on such behaviors. We found that acquisition of oxycodone self-administration (0.1 mg/kg/infusion) in rats is associated with significant elevations in plasma ghrelin. Acquisition of oxycodone self-administration also dramatically increased mRNA expression of ghrelin receptors in the ventral tegmental area (VTA), a brain region critical for drug reward. Elevation of ghrelin receptor mRNA levels in the VTA was localized predominantly in dopamine neurons. Pretreatment of rats with JMV2959 (0-5 mg/kg, i.p.) dose-dependently reduced oxycodone (0.05 or 0.0125 mg/kg/infusion) self-administration. JMV2959 pretreatment also dose-dependently decreased breakpoint for oxycodone self-administration under progressive ratio reinforcement. JMV2959 pretreatment (5 mg/kg, i.p.) in mice significantly inhibited intracranial self-stimulation maintained by optogenetic activation of VTA dopamine neurons. Thus, oxycodone experience is associated with development of endogenous ghrelin hyperactivity in rats, ghrelin signaling contributes to maintenance of oxycodone self-administration, and contributes to motivation for oxycodone-taking behavior. Thus, manipulations of ghrelin systems may represent a feasible approach for treatment of prescription opioid addiction.

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No conflict of interest.

1. **Functional upregulation of kappa opioid receptors in chronic pain states**

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Pain is a multidimensional experience and negative affect, or how much the pain is “bothersome”, significantly impacts the sufferers’ quality of life. It is well established that the kappa opioid system contributes to depressive and dysphoric states, but whether this system contributes to the negative affect precipitated by the occurrence of chronic pain remains tenuous. Using a model of persistent pain, we show by quantitative RT-PCR, florescence *in situ* hybridization, western blotting and GTPgS autoradiography an upregulation of expression and the function of kappa opioid receptors (KORs) and its endogenous ligand dynorphin in the mesolimbic circuitry in animals with chronic pain compared to surgical controls. Using *in vivo* microdialysis and microinjection of drugs into the mesolimbic dopamine system, we demonstrate that inhibiting KORs reinstates evoked dopamine release and reward related behaviors in chronic pain animals. Chronic pain enhanced KOR agonist-induced place aversion in a sex-dependent manner. Using various place preference paradigms, we show that activation of KORs drives pain aversive states in male but not female mice. However, KOR antagonist treatment was effective in alleviating anxiogenic and depressive affective-like behaviors in both sexes. Finally, ablation of KORs from dopamine neurons using AAV-TH-cre in KORloxP mice prevented pain-induced aversive states as measured by place aversion assays. Our results strongly support the use of KOR antagonists as therapeutic adjuvants to alleviate the emotional, tonic-aversive component of chronic pain, which is argued to be the most significant component of the pain experience that impacts patients’ quality of life.

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1. **Opioid Use Disorder in Humans: The Potential Role of Serotonin Pathways**

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Serotonin modulates mood, appetite, and sleep. Of particular interest is the growing evidence suggesting that serotonin receptors, specifically the 5HT2c subtype, are also involved in modulating impulsive behaviors, reactivity to drug-related cues, and the reinforcing effects of various drugs. For example, preclinical studies show that 5HT2c agonists decrease cocaine, alcohol, nicotine, and opioid self-administration. Lorcaserin, a selective, high-affinity agonist at 5HT2c receptors, reduced the reinforcing effects of nicotine in rats, as well as nicotine- and cue-induced reinstatement of responding for nicotine. Lorcaserin also dose-dependently decreased responding for oxycodone-associated cues and oxycodone self-administration by rats. Another selective 5HT2c agonist, MK-212, inhibited morphine-induced increases in extracellular dopamine levels in the nucleus accumbens. Interestingly, the 5HT2c/2b antagonist SB206553 produced the opposite effect in that it enhanced morphine-induced increases in striatal and accumbal dopamine output, and morphine-induced increases in neuronal firing rate in the ventral tegmental area and the substantia nigra pars compacta. Thus, the ability of 5HT2c agonists to inhibit opioid-induced increases in dopamine release, and their ability to reduce opioid self-administration provided the rationale for evaluating the ability of lorcaserin to reduce opioid self-administration in humans with opioid use disorder (OUD). At a maintenance dose used clinically for the treatment of obesity (10 mg twice daily), lorcaserin was well tolerated but it failed to alter oxycodone self-administration and it produced small but statistically significant increases in ratings of Drug Liking, Good Drug Effect, and Want Heroin. Future studies should evaluate higher doses of lorcaserin as a potential treatment for OUD.

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No conflict of interest.

1. **A multi-species neurogenomics approach to identify pathways & medications for alcohol use disorder**

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Addiction to alcohol is a heritable mental health disorder adversely affecting millions of individuals worldwide. The cellular and molecular composition of the human brain is influenced by genetic predisposition for neuropsychiatric disease and environmental conditions. To determine the CNS cell-types, brain regions, and time-frames impacted by genetic risk factors for alcohol use disorder (AUD) and alcohol consumption we evaluated the genomic profile of sixteen distinct brain regions from humans and rhesus macaques across multiple stages of development spanning fetal development, infancy, childhood, adolescence, and adulthood (N=607). Across multiple brain regions (*e.g.* amygdala and prefrontal cortex) AUD and alcohol drinking behavior demonstrated overlapping and non-overlapping profiles for transcriptional regulation; which become significantly stronger with increased developmental maturation.

RNA-Seq analysis of the amygdala and prefrontal cortex for alcohol consumption, on an separate cohort of human and non-human primate adult brain tissue (N=28/brain/species), showed conserved gene expression dysregulation associated with circulating HPA-axis neurosteroids (p < 0.01), suggesting potential gene-environmental interactions for alcohol drinking behavior and stress-responses. To identify biochemical mechanisms capable of counteracting the polygenic nature involved in excessive alcohol consumption we conducted a bioinformatics screen of > 2 million chemical and genetic perturbations. Contrasting these perturbations with the neurogenomic landscape of AUD revealed a number of interrelated systems, including glucocorticoid receptor modulators (p < 0.01), subsequently verified in C57BL/6J alcohol drinking studies. Collectively, our results provide evidence for novel molecular signatures involved in alcohol consumption with the potential for gene/pathway- based diagnosis and targeted treatment of AUD.

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*No conflict of interest.*

1. **Manipulation of central amygdala neurotensin neurons alters the consumption of alcohol**

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The central nucleus of the amygdala (CeA) is a heterogeneous structure that plays an important role in the regulation of appetitive, aversive, and ethanol-mediated behaviors. While some data have shed light on the neuronal subpopulations influencing fear- and feeding-related behaviors, it remains less clear which CeA efferents and neuronal subpopulations influence alcohol consumption. To investigate this we used mice expressing bacterial cre-recombinase under the gene encoding the 13-amino acid neuropeptide neurotensin (NTS-cre mice). We then injected a cre-dependent virus expressing caspase 3 to genetically lesion the NTS neurons in the CeA, and then explored how this affected consumption of alcohol. We found that the lesion of CeA-NTS neurons reduced both moderate and extended access alcohol consumption. To probe which projection may alter the consumption of alcohol, we expressed channelrhodopsin (ChR2) in the CeA-NTS neurons of the NTS-cre mice. We found that optical stimulation of the axon terminals from the CeA projecting to the parabrachial nucleus (PBN) promoted the consumption of alcohol. Furthermore stimulating in the PBN projection promoted positive valence behavior (real-time place preference), and was reinforcing (optical intracranial self-stimulation), suggesting that the NTS projection from the CeA🡪PBN may be important for ethanol reward. Interestingly, neither manipulation altered anxiety-like behaviors. These data suggest that NTS-expressing CeA neurons, and especially their projection to the PBN, play a significant role in modulation of ethanol consumption and may alter incentive salience.

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The authors have no conflicts of interest

1. **Cocaine self-administration induces transcriptional adaptations in the ventral pallidum**

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Growing evidence suggests the ventral pallidum (VP) is critical for drug intake and seeking behavior. While repeated exposure to cocaine alters VP neuronal activity, there is limited information on the molecular adaptations occurring in VP neurons following cocaine intake. To provide insight into cocaine-induced transcriptional alterations we performed RNA-sequencing on VP of mice that underwent 10 days of cocaine self-administration followed by twenty-four hours of abstinence. Cocaine intake was associated with important alterations in gene expression levels and subsequent Gene Ontology analysis pointed toward alterations in dendrite- and spine-related genes. Searching for a common regulator for these sets of genes, the expression of the transcription factor Nr4a1 showed a robust increase following cocaine self-administration. Further, we observed an increase in the Nr4a1 transcriptional target Plk2, a molecule important for synaptic and structural plasticity. Analysis of Plk2 molecular targets showed alterations in Actin and Rap2 dynamics after cocaine exposure, supporting impaired dendritic and spine functions. Overexpression of Nr4a1 in the VP reduced cocaine seeking confirming its role in drug-related behavior. Using fluorescent *in situ* hybridization, we are now determining which VP projection neuron population displays increased Nr4a1 and Plk2 levels after cocaine self-administration. This includes VP-ventral tegmental area, VP-lateral habenula, VP-mediodorsal thalamus, or VP- nucleus accumbens projection neuron populations. Additionally, we are bidirectionally manipulating Nr4a1 and Plk2 expression in these circuits to confirm the role of these molecules in cocaine-induced behaviors. Altogether, our work can provide crucial information into the molecular adaptations occurring in VP neuron supporting cocaine self- administration and relapse-like behavior.

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1. **Prescription opioids, synaptic alterations and neurodevelopment: a systems biology approach**

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Oxycodone (Oxy) is a strong semi-synthetic prescription opioid to treat multiple types of pain. Here, we present novel evidence on the impact of oxy on neurodevelopment in offspring exposed in utero (IU) and post-partum (PP) including synaptic alterations. IU offspring showed significant reduction in physical attributes such as body weight, body length and head circumference during the first four weeks of development compared to the PP and baseline saline offspring. The decrease in head circumference correlated with reduction in the number of dendritic spines and total number of synapses at postnatal day14 (P14), a stage denoting peak synaptogenesis in the IU group. This decrease in synapses corroborated with alterations in exosomal microRNA cargo that regulated key functional pathways associated with synaptic transmission, neurodevelopment, mood disorders, addiction etc as determined by RNA-Seq. Furthermore, radio imaging by 1H- MRS revealed alterations in key brain metabolites in the IU offspring including changes in synaptic currents using electrophysiology corroborated with behavioral alterations in these offspring. To summarize, our current study using a systems biology approach integrating data at different tiers- molecular, biochemical and behavior presents a comprehensive analysis on the differential impact of prescription opioid abuse at the synapse and subsequent neurodevelopment in the exposed offspring.

1. **Ventral tegmental area neuronal diversity and motivated behavior**

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The ventral tegmental area (VTA) has been implicated in different aspects of motivated behavior, and shown to participate in the rewarding effects of drugs of abuse. Our research has been conducted towards testing the hypothesis that the different roles ascribed to VTA are mediated by distinct subsets of neurons that through specific circuitry integrate information from specific neurons from different brain areas. At the cellular level, studies on VTA information processing have been focused for over 50 years on resident *dopamine* neurons. However, we had discovered that the VTA has *glutamate* neurons that innervate some of the same brain areas targeted by *dopamine* neurons. By combination of classical and emerging anatomical techniques, we have found that VTA *glutamate* neurons establish both local and long-range connections. By optogenetic and pharmacological approaches, we have determined that VTA glutamate neurons play a role in reward by establishing synapses on neighboring dopamine neurons, and participate in aversion by establishing synapses on nucleus accumbens or medial prefrontal cortex. We had also found that the VTA has neurons with the capability of co-releasing glutamate and GABA, and concluded that these dual glutamate-GABA neurons signal rewarding or aversive stimuli. Moreover, we observed that depending on their distribution within the VTA, the neurons that release glutamate alone or in combination with GABA have mu opioid receptors. We are currently investigating the extent to which VTA glutamate releasing neurons play a role in the neurobiology of drugs of abuse.

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No conflict of interest

1. **Binge exposure to ethanol-mediated neuroinflammation modulating morphine’s anti-nociception in B6 adolescent mice**

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Correlation of alcohol abuse and increase of opioid intake have been reported. Using Ingenuity Pathway Analysis (IPA), a bioinformatics tool, we have comprehensively analyzed the studies related to alcohol use disorders (AUD) including binge drinking and opioid use disorders (OUD) collected in the IPA knowledgebase. Neuroinflammation signaling pathway was identified to be one of potential mechanisms underlying AUD being connected to OUD. Elevation of the blood ethanol (ETOH) concentration (BEC) to > 80 mg/dL (17.4 mM) after binge drinking enhances inflammation in brain and neuroimmune signaling pathways. Morphine abuse is frequently linked to excessive drinking. Morphine exerts its actions mainly via the seven transmembrane G-protein-coupled mu opioid receptors (MORs). OUD include combination of opioids with alcohol, leading to opioid overdose-related deaths. We hypothesized that binge drinking potentiates onset and progression of OUD. Using C57BL/6J (B6) adolescent mice, we first demonstrated binge exposure to EtOH elevated expression of inflammatory cytokines and MOR in the brain areas of the mice given 3 d of binge exposure to EtOH (5 g/kg/d, 42% v/v, i.g. 3 d). Since elevation of MOR could enhance morphine’s anti-nociception, we examined the impact of binge exposure to EtOH on morphine’s anti-nociception. The mice were treated with or without 3-d binge exposure to EtOH, and the anti-nociceptive changes were evaluated using the hot-plate test 24 h after the final (3rd) EtOH injection with or without a cumulative subcutaneous dose (0, 0.1, 0.3, 1.0, and 3.0 mg/kg) of morphine at intervals of 30 min. The response curve of the mice given EtOH was shifted to the left, showing enhanced latency to response to morphine up to 3 mg/kg. Co-treatment with the MOR antagonist naltrexone blocked morphine’s anti-nociception in animals given either EtOH or saline. This confirms that MOR is involved in binge exposure to EtOH-induced changes in morphine’s anti-nociception. Low dose of mentamine was reported to exert anti-inflammatory effects. Using the hot-plate test, treatment with mentamine (1 mg/kg/d, s.c.) 30 min prior to binge exposure to EtOH (5 g/kg/d, 42% v/v, i.g.) was shown to block enhancement of morphine’s anti-nociception in animals given EtOH. These studies suggested that EtOH enhanced latency to analgesic response to morphine, possibly via binge exposure to EtOH-mediated elevation of inflammation in the brain, and such effect might initiate the onset and progression of OUDs (partially supported by NIH grants DA07058, DA016149 and AA023178.

 **22. Microglia control stress axis function via secreting exosomes**

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Fetal alcohol exposure has many detrimental effects on the developing brain and can cause fetal alcohol spectrum disorders (FASDs). Many FASDs patients show lifelong stress response abnormalities, demonstrated by an augmented response to stress hormones such as adrenocorticotropin and corticosterone, which are likely driven by alterations the hypothalamic-pituitary-adrenal axis function. Using a rat animal model, we have shown that postnatal ethanol exposure reduces the number and function of stress regulatory β-endorphin producing neurons in the hypothalamus, inducing a hyper-stress response. Microglia are one of the innate immune cells in the CNS and can be categorized as activated or ramified. Activated microglia are associated with an increase in proinflammatory responses and phagocytosis while ramified microglia are associated with maintaining homeostasis through dynamic communication, remodeling of neuronal synapses, and surveying the environment. How β-endorphin neurons communicate with microglia to maintain normal homeostasis has yet to be addressed. β-endorphin can also bind to both mu- and delta-opioid receptors and may serve as a form of communication between β-endorphin neurons and microglia. Exosomes are small vesicles (30-150 nm) that play an important role in local and distant communication between cells. They carry unique cargo (proteins, mRNA, miRNA, and other non-coding RNAs) from the cells they originate from that can affect the recipient cell’s homeostasis and induce apoptosis. Additionally, complement proteins, generally known for their role to opsonize foreign pathogens and support phagocytosis of dying cells may also play a role in ethanol-induced β-endorphin neuronal cell death. We have obtained evidence to show that ethanol-induced apoptosis of β-endorphin neurons is caused by activation of microglia to release proinflammatory cytokines, pro-apoptotic exosomes, and C1q. Furthermore, mu-opioid receptors activation is critical to ethanol-induced activation of microglia to induce apoptosis of β-endorphin neurons and antagonism of mu-opioid receptors attenuated the ethanol effect. Delta-opioid receptors antagonism did not have an effect on ethanol-induced β-endorphin neuronal cell death. (Supported by a NIH Grant R37 AA08757).

1. **Alcohol and cholesterol interact to control cerebral artery bk current via a pkc-dependent mechanism**

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Alcohol at concentrations reached in blood during moderate-to-heavy episodic drinking constricts cerebral arteries via inhibition of voltage/calcium-gated, large conductance potassium (BK) channels in vascular smooth muscle (SM). Dietary cholesterol (CLR) protects against alcohol-induced constriction of cerebral arteries *in vivo*. This action is mimicked by CLR-enrichment of cerebral arteries *in vitro* and results from a competitive interaction between CLR and alcohol. Conversely, atorvastatin therapy administered to rats on high-CLR diet removes protective effect of CLR against alcohol-driven vasoconstriction. Effects of elevated CLR and atorvastatin on alcohol sensitivity are only accompanied by mild modification of CLR levels within the cerebral artery. Thus, we hypothesized that CLR-alcohol interaction was mediated by an additional molecular entity. Patch-clamp recording of BK channel activity in inside-out patches from rat cerebral artery SM cells showed that high-CLR diet protected against the BK channel inhibition evoked by 50 mM alcohol. CLR-depletion of myocytes via methyl-beta-cyclodextrin prior to patch excision from the cell removed the protective effect of high-CLR diet against alcohol. However, while *in vitro* CLR-enrichment of myocytes prior to patch excision rendered protection, application of CLR-enriching media to cell-free membrane patch did not protect against alcohol-induced inhibition of BK current. Alcohol-induced BK channel inhibition and its modulation by CLR vanished in presence of the protein kinase C (PKC) inhibitor chelerythrine. Moreover, incubation of myocytes in PKC activator diacylglycerol enabled protection against alcohol even in the absence of elevated CLR. Collectively, our findings document that alcohol and CLR interact to control cerebral artery SM BK current via an intracellular PKC-dependent mechanism(s).

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Conflict of interest: no conflict of interest.

1. **Toluene constricts cerebral arteries by targeting smooth muscle BK channels**

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Signs and symptoms of toluene acute intoxication greatly overlap with those of cerebral ischemia. Moreover, computed tomography in humans shows that toluene acute intoxication evokes brain hypoperfusion (Ryu et al., 1998). The biological targets underlying toluene-induced cerebrovascular ischemia are unknown. Using a cranial window in adult rats, we demonstrate that acute exposure to clinically relevant concentrations of toluene (<10 mM, i.e., <1,000 ppm) constricts middle cerebral arteries (MCA). This toluene action is replicated in isolated, in vitro-pressurized MCA segments, underscoring that circulating factors are not involved. Similar outcomes in de-endothelialized MCA indicate that toluene-induced constriction is endothelium-independent and likely involves targets present in the smooth muscle. In this tissue, inhibition of Ca2+- and voltage-gated K+ channels of large conductance (BK) leads to vasoconstriction (Brayden & Nelson, 1992), raising the hypothesis that cerebrovascular BK channels mediate toluene-induced cerebrovascular constriction. Indeed, pharmacological block of BK channels blunts toluene-induced constriction of isolated MCA. Smooth muscle BK channels primarily result from association of channel-forming α and regulatory β1 subunits (Dopico et al., 2018). Acute toluene inhibits smooth muscle BK channels, which is more robust in MCA from *KCNMB1-/-* vs. *wt* mice. Consistently, toluene-induced inhibition of activity is more robust in α vs. α+β1 channels reconstituted into artificial bilayers. While toluene-induced inhibition does not require Ca2+ic, it is modulated by this ion, reaching a maximum at ~10 μM Ca2+ic. Thus, toluene at concentrations reached in blood during acute intoxication inhibits cerebrovascular smooth muscle BK channel in absence of Ca2+ic and β1 subunits, yet both regulators fine-tune toluene action. BK α subunits and their immediate lipid microenvironment suffice for toluene to inhibit BK channels, which likely determines drug-induced cerebral artery constriction.

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1. Female gonadal hormone modulates ethanol-induced memory deficit in post-pubertal female rats

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Underage drinking is a global public health concern, and ethanol (EtOH) exposure during adolescence has tremendous societal and personal consequences. EtOH interferes with the ability to form memories, and "blackouts" are relatively common among young drinkers. We and others have reported that EtOH in adolescent animals disrupts the acquisition of hippocampal-based memory. Most studies in literature have reported ethanol-induced memory deficit in pre-pubertal adolescent rats. Here we report the effect of ethanol on fear conditioning in postpubertal male and female adolescent rats. Male and female rats were administered with a single injection of ethanol (2 g/kg) or equivalent volumes of vehicle. Groups of female rats were ovariectomized and given hormonal supplementation (estrogen and progesterone). Additional controls included sham-operated and oil-treated animals. Rats were trained in the fear conditioning paradigm, and 24h later they were tested for contextual fear conditioning in the same training chamber. Freezing during fear conditioning paradigm was recorded, and freezing scores were computed for each animal. Acute ethanol-treatment in intact post-pubertal female rats showed significant disruptions in hippocampus-related contextual memory but not in post- pubertal male rats did. Ovariectomized female rats did not show any deficit in fear conditioning following EtOH treatment. Ovariectomized female rats exogenously administered with estrogen and progesterone showed ethanol-induced memory impairment. Together, the data suggest that female gonadal hormones can modulate ethanol-induced memory impairment in post-pubertal female animals. (This research project was supported by grant from NIAAA).

1. **Involvement of specific nicotinic receptors in alcohol, nicotine, cocaine and methamphetamine intake and attentional performance**

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Manipulations of nicotinic cholinergic receptors have been shown to influence both alcohol and nicotine intake. In addition, nicotine and other nicotinic agonists have been shown in a variety of studies to improve attentional function. Given that nicotinic receptors are easily desensitized and all direct nicotinic agonists are also desensitizing agents, it is not clear whether the activation or desensitization of these receptors is of critical importance for attentional improvement and reducing drug intake.

Sazetidine-A [6-(5(((S)-azetidine-2-yl)methoxy)pyridine-3-yl)hex-5-yn-1-ol] is a relatively novel compound that potently and selectively desensitizes α4β2 nicotinic receptors with only modest receptor activation. We have examined the effects of sazetidine-A on alcohol intake and iv self-administration of nicotine, cocaine and methamphetamine in rats. We have also tested the effects of sazetidine-A on sustained attention in rats. Our findings showed that both acute and chronic administration of sazetidine-A caused a dose-dependent reduction in alcohol intake in alcohol preferring P rats. In the post-deprivation study, sazetidine-A also significantly reduced both alcohol intake and preference. Sazetidine-A also significantly reduced iv self-administration of nicotine, cocaine and methamphetamine in rats. We also demonstrated that similar to its acute effects, chronic infusions of sazetidine-A improve attentional performance in rats. Sazetidine-A may be exerting its improving effect on attention by selective desensitization of α4β2 nicotinic receptors. Sazetidine-A may hold promise for the treatment of drug addiction as well as improving attentional performance.

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1. A novel cutaneous gene therapy for alcohol abuse and co-abuse with cocaine

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Alcohol and cocaine are commonly abused and frequently co-abused drugs. Available medications do not meet the needs for treating ongoing alcohol and cocaine abuse, relapse and co-abuse. 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 GLP1and hBChE have very short half-lives in vivo, however, limiting their potential in treating alcohol abuse and co-abuse with cocaine. We have tested the ability to use skin grafts derived from engineered skin progenitor cells as a novel approach for long-term and efficient delivery of therapeutic agents in vivo. We first developed a novel mouse-to-mouse skin transplantation method that allows the stable introduction of engineered epidermal progenitor cells into immunocompetent host mice. We then used the CRISPR technology to target either a GLP1 gene or an hBChE gene into epidermal progenitor cells. This approach is effective in preventing mice from ethanol- or cocaine-seeking behaviors, reducing ongoing ethanol drinking and protecting mice from cocaine overdose, respectively. Co-grafting GLP1 and hBChE cells attenuated drug-seeking and lethality induced by ethanol and cocaine co-administration. The cutaneous gene therapy is long-lasting and efficient with little individual variation. This work will lay key groundwork for the development of a highly personalized and long-lasting approach for combating drug abuse and co-abuse. Supported by NIH R21AA027172.

1. **Translational studies of alcohol use disorder**

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Alcoholism follows a dynamic course, involving development, maintenance, recovery, and relapse. In vivo brain imaging studies have enabled the tracking of this course and have revealed evidence for disruption of selective neurocircuity in Alcohol Use Disorder (AUD) with evidence for improvement with sustained sobriety. Prospective studies of adolescents reveal disruption of normal maturational neurotrajectories in non-dependent youth who initiated heavy drinking. Affected brain regions include frontal, cingulate, anterior-superior cerebellum, and vermis, regions commonly affected in adult alcohol dependent individuals. Features of brain dysmorphology have been modeled in rodents under controlled conditions exposed to high levels of alcoholwith reversal after a week free of alcohol. In addition to use of within-subject designs, in vivo animal imaging enables surveying the entire brain rather than restricted examination of selective regions common in histological studies. Recently, we employed a voxel-based quantification method using MRI data to survey the entire brain of wild-type Wistar rats--13 alcohol-exposed and 12 water controls--which underwent a 4-day binge protocol. Rats were scanned at baseline, early post-binge, and one-week post binge. Our analysis identified thalamic shrinkage and ventricular enlargement early post-binge followed by significant volume recovery. By contrast, volumes of the pretectal nuclei and superior and inferior colliculi showed persistent volume loss. These brain stem structures support functional circuitry known to be relevant in human AUD yet are under-reported in animal models. Studies like these demonstrate the strength of longitudinal study using noninvasive MR imaging.

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No conflicts of interest

1. **Multisystem Approaches to Treating Tobacco and Other Addictions**

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Considerable research has highlighted the importance of dopaminergic (DA) innervation from the ventral tegmental area (VTA) to the nucleus accumbens (NACC) for the effects of addictive drugs. However, he brain is an organ of communication, with a variety of neural and glial systems interacting to provide the network bases for behavioral function and dysfunction. The VTA to NACC DA innervation is central to the reinforcing actions of drugs of abuse, but there are a variety of other neural systems that interact with and influence this pathway. In addition, drug addiction involves much more than just the reinforcing value of drugs. It also is critically dependent on circuits for cognition, emotion and habit. Another instance of limited consideration regarding biological bases of drug addiction is that the incipient mechanism of action of an abused drug is primarily important for its addiction, for example nicotinic receptors for tobacco addiction and opiate receptors for opiate addiction. Certainly, those receptors are key for the initiation of the action of the drug on brain function, but there are numerous other interacting systems that also play important roles in the biological bases of addiction to these drugs. More comprehensive understanding of how these interacting systems function and malfunction in addiction is key for both better basic understanding of drug addiction as well as the development of more effective treatments to combat addiction. In a series of studies in rats, we have found that not only dopaminergic (D1 antagonist, SCH-23390) and nicotinic (alpha3beta4 antagonists, 18-MC and dextromethorphan) treatments significantly reduce nicotine self-administration, but that a variety of other treatments are effective as well. Serotonin (5HT2c agonist), histamine (H1 antagonist, pyrilamine), glutamate (NMDA antagonists, ketamine, D-cycloserine and memantine) and broad monoaminergic (triple reuptake inhibitor, amitifadine) significantly reduce nicotine self-administration. Recently, we have also found that amitifadine also significantly reduces self-administration of the opiate remifentanil. In previous research, we found that the nicotinic antagonist mecamylamine significantly reduces cocaine self-administration. So, not only are a variety of neural systems involved in drug self-administration, a variety of treatments can be used to reduce it. This broader approach of embracing the complex interactions underlying drug addiction could provide benefits for developing a broader array of therapeutic treatments to combat it.

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1. **Methamphetamine-associated cognitive decline involves aberrant proliferation of neural progenitor cells and is attenuated by neutralizing IL-1 signaling**

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Methamphetamine (METH) abusers are prone to develop a variety of comorbidities, including cognitive disabilities, and the immunological responses have been recognized as an important component involved in the toxicity of this drug. Cytokines are among the key mediators between systemic inflammatory status and tissue responses. One of these, interleukin 1 (IL-1), has been hypothesized to be involved in cognitive functions and also appears to play a pivotal role among inflammatory molecules. In the present study, we demonstrate that exposure of mice to METH markedly increased the protein level of IL-1β in hippocampal tissue. Additionally, METH administration induced a decline in spatial learning and memory as determined by the Morris water maze test. We next evaluated the hypothesis that blocking IL-1β signaling can protect against METH-induced loss of cognitive functioning. The results indicated that METH-induced impaired spatial learning abilities were attenuated by co-administration of mouse IL-1 Trap, a dimeric fusion protein that incorporates the extracellular domains of both of the IL-1 receptor components required for IL-1 signaling (IL-1 receptor type 1 and IL-1 receptor accessory protein), linked to the Fc portion of human IgG1. This effect was associated with a decrease in hippocampal IL-1β level. In addition, exposure to METH induces aberrant proliferation of neuronal progenitor cells by affecting the CXCL12-CXCR4 axis. The current study indicates for the first time that the loss of METH-related cognitive decline can be attenuated by neutralizing IL-1 signaling. Our findings suggest a potential new therapeutic pathway for treatment of altered cognitive abilities that occur in METH abusing individuals.

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1. **Interaction between HIV and cigarette smoke in lung comorbidities**

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In the era of antiretroviral therapy (ART), noninfectious pulmonary disorders such as chronic bronchitis (CB), asthma, and chronic obstructive pulmonary disease (COPD)/emphysema are common comorbidities in people living with HIV (PLWH). These diseases are also associated or exacerbated by cigarette smoke (CS) and smoking is very common in PLWH; however, some epidemiologic data suggest that HIV is an independent risk factor for COPD/emphysema. We have shown that normal human lung bronchial epithelial (NHBE) cells express the HIV coreceptors CD4 and CXCR4 and HIVgp120 stimulates mucus formation in NHBE cells, and HIV- or SIV-infected lungs exhibit large amounts of airway mucus. To investigate the interaction between CS and HIV, cynomolgus macaques were exposed to CS and/or simian-adapted human immunodeficiency virus (SHIV) and treated with cART. The development of CB and changes in lung function were evaluated following CS±SHIV treatment. The results showed that in the lung, SHIV was a strong independent risk factor for mucus formation, loss of epithelial tight junction barrier, and proasthmatic molecular imprints. In addition, SHIV and CS synergistically reduced lung function and promoted early pro-COPD/emphysema-like changes in the lung. Moreover, SHIV-infection induced significant numbers of HIV-gp120+ epithelial cells in the small airways and the alveoli and these numbers nearly doubled in CS+SHIV-infected lungs. Furthermore, HIV infected bronchial epithelial cells in vitro and in vivo and was integrated in the host genome. Thus, even in the presence of successful cART, HIV proviral genes are present in lung epithelial cells and HIV is an independent risk factor for CB and asthma, and interacts synergistically with CS to induce pro-COPD/emphysema–like changes in the lung.

These studies were supported by the NIH grant R01 HL125000.

1. **Opiates potentiate HIV-associated neuroinflammation: Role of EVS in microglial activation and migration**

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Substance abuse and HIV-1 have been described as two linked global health crises and are a major public health concern and economic burden. Both Despite the advent of anti-retroviral therapy, HIV infection and abuse of opiates has been shown to result in increased neurocognitive deficits and neuroinflammation. Extracellular vesicles (EVs), such as exosomes are well-recognized as cell-cell communication conduits that deliver the cargo containing miRNA, mRNA and proteins to neighboring/distant cells. Herein we demonstrate that exposure of astrocytes to HIV-1 Tat protein & morphine results in increased expression and release of miR-9 and miR-138 respectively, in astrocyte-derived EVs (ADEVs). Intriguingly, uptake of miR-138 released in ADEVs by microglia resulted in their activation via the TLR7-dependent pathway. Mutation of the GUUGUGU motif in miR-138 that is homologous to a TLR7 binding domain ablated this activation. On the other hand, uptake of ADEV-miR-9 by microglia, resulted in increased microglial migration. Furthermore, we also demonstrate that EV miR-9 targets PTEN, via its binding to the 3’UTR seed sequence of the PTEN mRNA, leading to its downregulated expression that underlies microglial migration. In summary, our findings demonstrate that morphine and HIV Tat cooperate to exacerbate neuropathogenesis via two complementary mechanisms: **a)** Morphine exposed astrocytes upregulate expression & release of miR-138 in the ADEVs, which, upon uptake by the microglia, results in their activation via the TLR7-dependent pathway and, **b)** HIV Tat exposed astrocytes upregulate expression & release of miR-9 in the ADEVs, that is taken up by the microglia, leading in turn, to their migration.

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**Conflict of interest**: No conflict of interest.

1. **Alcohol‒SIV/HIV‒ART interactions & risk for comorbidities**

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Reduced mortality rates with antiretroviral therapy (ART) and increased survival of persons living with HIV (PLWH) transformed HIV infection into a chronic disease that frequently coexists with at-risk alcohol use. Increased longevity of PLWH is complicated with comorbidities that may be exacerbated by at-risk alcohol consumption, unhealthy dietary behaviors, and ART adverse effects. Complex interactions of HIV, ART, and lifestyle behaviors like at-risk alcohol use in the increasingly older PLWH population can increase the risk for geriatric comorbidities, particularly through compromised metabolic health. Our bidirectional translational studies have shown that chronic binge alcohol (CBA) administration markedly impairs metabolic homeostasis in simian immunodeficiency virus (SIV)-infected ART-treated non-human primates (NHP). Furthermore, we observe marked brain upregulation of inflammatory gene expression and suppressed growth factor signaling that is only partially improved by ART. This presentation will discuss the clinical relevance of the NHP findings. Our data show high exposure to stressors at the community and interpersonal levels that impact an individuals’ behavior. Together with disadvantaged demographics of PLWH enrolled in our New Orleans Alcohol Use in HIV (NOAH) longitudinal study we find these chronic stressors affect several biological processes. The immunopathological effects of alcohol at the gut mucosa decrease mucosal barrier function leading to gut leak, resulting in systemic immune activation, inflammation, and subsequent immune senescence. The resulting inflammatory/oxidative stress environment produces tissue injury and dysregulation of homeostatic mechanisms that we hypothesize promotes cellular energy metabolism dyshomeostasis and underlies the increased risk for comorbidities in PLWH.

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No conflict of interest.

1. **Activation of gpr55 induces neuroprotection of hippocampal neurogenesis during chronic systemic inflammation**

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The cannabinoid system exerts functional regulation of neural stem cell (NSC) proliferation and adult neurogenesis. The recently de-orphaned GPR55 has been shown to be activated by numerous cannabinoid ligands suggesting that GPR55 is a third cannabinoid receptor. We examined the role of GPR55 activation in NSC proliferation and early adult neurogenesis.

The effects of GPR55 agonists (LPI, O-1602, ML184) on human NSC proliferation *in vitro* were assessed by flow cytometry and expression of cytokine receptors was assessed by RT-PCR. hNSC differentiation was determined by flow cytometry, qPCR, and immunohistochemistry. Early adult neurogenesis in the hippocampus of C57BL/6 and GPR55-/- mice was evaluated by immunohistochemistry. We exposed NSCs to IL-1β *in vitro* to assess inflammation-caused effects on NSC differentiation and the ability of GPR55 agonists to attenuate NSC injury. GPR55 agonist treatment protected against IL-1β induced reductions in neurogenesis rates. Inflammatory cytokine receptor mRNA expression was down regulated by GPR55 activation in a neuroprotective manner. To determine inflammatory responses *in vivo,* we treated C57BL/6 and GPR55-/- mice with LPS continuously for 14 days via osmotic mini-pump. Reduced NSC survival, immature neurons, and neuroblast formation due to LPS were attenuated by concurrent direct intrahippocampal administration of the GPR55 agonist. Molecular analysis of the hippocampal region showed a suppressed ability to regulate immune responses by GPR55-/- animals manifesting in prolonged inflammatory responses after chronic, systemic inflammation as compared to C57BL/6 animals. These results suggest a neuroprotective role of GPR55 activation on NSCs *in vitro* and *in vivo* and that GPR55 poses a novel therapeutic target against negative regulation of hippocampal neurogenesis by inflammatory insults.

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Conflicts of interests: None

1. **Morphine tolerance is attenuated in germ-free mice and reversed by probiotics implicating the role of gut microbiome**

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Prolonged exposure to opioids results in analgesic tolerance, drug overdose, and death. The mechanism underlying morphine analgesic tolerance still remains unresolved. We show that morphine analgesic tolerance was significantly attenuated in germ-free (GF) and in pan-antibiotic-treated mice. Reconstitution of GF mice with naïve fecal microbiota reinstated morphine analgesic tolerance. We further demonstrated that tolerance was associated with microbial dysbiosis with selective depletion in Bifidobacteria and Lactobacillaeae. Probiotics, enriched with these bacteria, attenuated analgesic tolerance in morphine-treated mice. These results suggest that probiotic therapy during morphine administration may be a promising, safe, and inexpensive treatment to prolong morphine’s efficacy and attenuate analgesic tolerance. We hypothesized a vicious cycle of chronic morphine tolerance: morphine-induced gut dysbiosis leads to gut barrier disruption and bacterial translocation, initiating local gut inflammation through TLR2/4 activation, resulting in the activation of pro-inflammatory cytokines, which drives morphine tolerance.

1. **Intranasal oxytocin on alcohol withdrawal, craving and relapse: a double-blind RCT**

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Background and aims

Intranasal oxytocin (OT) has been proposed as treatment in addiction (1-3). Our aim was to compare the effect of self-administered OT and placebo on prolonged alcohol withdrawal and relapse during a four-week post-detoxification period.

Methods

A double-blind RCT on patients admitted at the Blue Cross Lade Addiction Treatment Center, Trondheim, Norway. In total, 38 patients (19 oxytocin, 19 placebo) completed inpatient detoxification, and were included in this follow-up trial. Patients received either OT or placebo to use as needed, maximum daily dose of 2 insufflations x 3 (12 IU). Main outcomes: Number of days to relapse and total number of alcohol units consumed. Other outcomes: HSCL-10 score, ACQ-SF-R score, the concentration of PEth in blood, and self-reported nervousness.

Results

No differences between the two treatment groups (16 OT, 17 placebo) in days until the relapse by Kaplan-Meier analysis (p=0.91), or alcohol units consumed (6.9±4.2 vs. 8.1±7,0; p=0.72; 95% confidence interval (Cl) -5.9 to +8.2). Neither were there any differences between the treatment groups in HSCL-10 scores (p= 0.39;; Cl -0.81 to 0.33) or in ACQ-SF-R scores (p=0.67; Cl -1.25 to 0.82), nor PEth concentrations (0.46 µmol/I in the OT group and 0.55 µmol/I in the placebo group (p = 0.78; Cl -0.75 to 0.57)). The OT group reported a significant lower level of nervousness compared to the placebo group (p=0.005).

Conclusion

Oxytocin had no significant effects on time to relapse, nor the number of alcohol units consumed. It did, however, have a significant effect on self-reported nervousness Source of funding

This trial is granted by Department of Research and Development -AFFU, St. Olav University Hospital, Trondheim, Norway, and funded by the Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology- NTNU, Trondheim, Norway, the Research Department, St. Olav University Hospital, Trondheim, Norway and the Joint Research Council between St. Olav University Hospital, Trondheim, Norway and the Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology- NTNU, Trondheim, Norway (FFU). No conflict of interest

1. Sucrose bingeing modifies alcohol reward in mice

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Eating disorders and substance abuse are highly comorbid (Conason A.H. et al., 2006) although the relationship between the expression of these two maladaptive behaviours is unclear. Obese and subjects with alcohol use disorder show similar characteristics: continuous use despite adverse consequences, persistent craving, and repeated unsuccessful attempts to quit (Leigh et al, 2018), suggesting that they are linked by a common etiology. To understand the nature of this relationship, we induced sucrose bingeing in C57Bl6/J male and female mice using 14-day limited access protocol (17.1% w/v). Binge intake was measured as increased solution consumption during the first hour (Yasoshima and Shimura, 2015). All mice given limited access to sucrose developed binge-like intake. We then examined the impact of sucrose bingeing on alcohol reward using a conditioned place preference paradigm with ethanol (i.p.;3%). Interestingly, all mice developed a conditioned place preference to 3% ethanol with the exception of female and male mice given limited access to sucrose. Our findings suggest that binge-like sucrose intake affects reward processing, at least to alcohol- related cues. We will examine this further by assessing ethanol consumption and preference across a range of concentrations (3%, 6%, 9%, 12%, 15%). Our study will provide insights into the relationship between eating disorders and vulnerability to alcohol consumption.

Fundings: FRA Fondation pour la Recherche en Alcoologie ; PICS : Projet international de coopération scientifique (CNRS)

Authors declare no conflict of interest

1. Involvement of NFΚB in cocaine-associated reconsolidation using intravenous self- administration

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Persistent and intrusive memories play a key role in maintaining certain psychiatric disorders, such as posttraumatic stress disorder and substance abuse. In the latter, memory for drug-paired cues are potent triggers for relapse, playing a critical role in sustaining drug use. Modulation of these memories may alter the propensity to relapse, suggesting that reconsolidation of drug-cue associations may be a potential target to reduce addictive behaviours. Experimental data in animals confirms that retrieved memories become transiently labile for a few hours post-retrieval: altering a drug-cue memory reduces drug- seeking behavior. Previous work from our lab showed that chronic cocaine intake alters DNA methylation in the rat prefrontal cortex; pathway analysis clustered some of the target genes around the nuclear factor κ B (NFκB) system. Although implicated primarily in inflammation, this transcription factor also plays important roles in learning and memory. Specifically, NFκB is involved in consolidation and reconsolidation of memories in different conditioning paradigms (e.g., conditioned fear, place preference). Our study assessed the effect of NFκB pharmacological inhibition on reconsolidation of cocaine-associated memories using an intravenous self-administration paradigm. Briefly, male rats were allowed to self-administer cocaine over 12 daily sessions under a FR1 schedule of reinforcement; a light stimulus (conditioned stimulus, CS) was associated with each infusion. After instrumental extinction with no CS presentation, rats underwent one CS reactivation session and were immediately injected with NFκB inhibitors. CS-induced reinstatement was assessed 24h later. Our study shows that two different NFκB inhibitors reduced cocaine-seeking behavior, suggesting NFκB involvement in the reconsolidation process.

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1. Sex differences in cocaine craving: role of the estrous cycle

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Studies using continuous-access [drug self-administration](https://www.sciencedirect.com/topics/neuroscience/drug-self-administration) showed that cocaine seeking increases during [abstinence](https://www.sciencedirect.com/topics/medicine-and-dentistry/abstinence) (incubation of cocaine craving). Recently, studies using intermittent-access self- administration showed increased motivation to self-administer and seek cocaine. Additionally, studies report sex differences and a role of ovarian hormones in cocaine relapse. Here, we studied whether intermittent cocaine self-administration would increase incubation of craving in male and female rats, and we investigated the role of the estrous cycle in this effect. We trained male and female rats to self-administer cocaine continuously or intermittently for 12 days (8-h/day). We found, in both sexes and under both training conditions a higher cocaine seeking in the relapse test after 29 days than 2 days. Moreover, in both sexes and independent of the abstinence day, intermittent drug access increased cocaine seeking. Importantly, in both training conditions, female rats showed an increase of drug seeking on both day 2 and 29 compared to males. Finally, by monitoring the estrous cycle in females, we found a potentiation of incubation of craving after either intermittent or continuous drug access in females in estrus.

Our results demonstrate that intermittent cocaine access caused a time-dependent increases in drug seeking during abstinence. In female rats, incubation of craving is critically dependent on the estrous cycle phases. Thus, to the degree that results from animal models generalize to humans, our findings implicate the phase of the [menstrual cycle](https://www.sciencedirect.com/topics/medicine-and-dentistry/menstrual-cycle) as a risk factor for relapse in women and, therefore, should be taken into consideration in the development of [relapse prevention](https://www.sciencedirect.com/topics/medicine-and-dentistry/relapse-prevention) treatments.

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1. **Initial characterization of opioid-induced respiratory depression in rats**

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Respiratory depression is the main cause of deaths by opioid overdose. Therefore, we sought to characterize the respiratory depression induced by heroin and fentanyl in rats. Long-Evans rats had catheters implanted in the right jugular vein. The rats were tested using whole-body plethysmography, which is a non-invasive technique that allows the measurement of several respiration parameters in a breath-by-breath basis in conscious, freely moving animals. The rats were habituated to the plethysmograph chamber for three consecutive days. On the fourth day, they were allowed to habituate to the chamber for 30 min followed by a 20-min baseline recording. Then, they received a single intravenous bolus infusion of heroin 600 µg/kg or fentanyl 25 µg/kg. Respiration was recorded for 90 min. This test was repeated two more times, 2 weeks apart, to test for tolerance development. Heroin produced a long-lasting (60 min) respiratory depression characterized by an increased inspiratory time, decreased tidal volume (volume of air inhaled in each breath), minute ventilation (volume of air inhaled per minute) and inspiratory drive (tidal volume/inspiration time) compared to baseline values. Fentanyl produced the same changes in respiration, but with a significantly shorter duration (30 min). The repeated testing indicated the development of tolerance to the respiratory depression produced by heroin, but not fentanyl. In conclusion, this preliminary experiment with only one dose of each drug suggests that heroin and fentanyl produced respiratory depression with distinct duration and tolerance development.

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The authors declare no conflict of interest.

1. **Addictions in Morocco: a historical perspective**

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As it is the case all over the world, addictions always existed in Morocco. Cannabis was introduced from the Middle East during the Arab conquest of the Maghreb in the 8th Century. Its use was however forbidden by the religious zealots, such as the Almohade dynasty (12-13th Century) and users were severely punished publicly for such offense. Despite this, cannabis has been used for centuries both in rural and urban areas by craftsmen and peasants. Alcohol was also widely used, for centuries, and not only by Moroccan jews who produced aquavit in their neighbourhoods. Hundreds of thousands of hectolitres of wine were imported on a regular basis from France and Germany at the end of the 19th Century, 20 years before French protectorate was set up, for a total population of about 4 million inhabitants only.

Other addictions were introduced in the 19th Century to Morocco, especially tea that became the national beverage, but with a new illness ‘théisme’ described by French doctors when consumption was excessive and detrimental to the health of the subject. Some psychotropic medications became ‘street drugs’ and led to epidemics (such as Optalidon\* in the 1970s), and cocaine, heroin, among others became more and more available to Moroccans, and not only for the highest socio-economic strata. Addiction to Internet, video games and other new technologies is the latest of this list and will probably not be the last ones.

1. **Alcohol preference and behavioral modifications following deletion of cb2rs in dopamine neurons and microglia**

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Our previous report demonstrated that selective deletion of type 2 cannabinoid receptors (CB2Rs) in dopamine (DA) neurons; DAT-Cnr2 conditional knockout (cKO) mice exhibit a hyperactive phenotype. Neuroinflammation is emerging as a key component in the effects of CB2Rs expressed in neurons and glial cells that are key regulators of immune response. Using Cre-LoxP technology, we generated Cx3Cr1-Cnr2 cKO mice with selective deletion of CB2Rs in microglia. We utilized immunoblotting, immunohistochemistry, behavioral tests, and alcohol behavioral effects to determine the neuro-immuno-modulatory effects of CB2Rs in the DAT-Cnr2, Cx3Cr1-Cnr2 cKO and wild type (WT) C57BL/6J mice. Here we report 1). That CB2Rs are involved in the tetrad assay induced by cannabinoids in the WT and the CB2R cKO mice contrary to the long-standing notion that the characteristic tetrad tests were induced mainly by CB1R agonism. 2). In the hippocampus, there was enhanced IBA1 immunoreactivity in both CB2R cKO mice, and microglia activation was detected by CD11b in the dentate gyrus in WT, DAT-Cnr2 and Cx3cr1-Cnr2 mice with clear morphological difference in the Cx3cr1-Cnr2 mice after stress. 3). Neuroinflammation signaling pathways of PI3K/AKT/mTOR, MAP/ERK and NF-ĸB were differentially affected by the cell-type specific deletion of CB2R in cerebral cortexes of CB2R cKO and WT mice. 4). Alcohol preference ratio was significantly higher in Cx3cr1-Cnr2 cKO and WT, than DAT-Cnr2 cKO mice that consumed less alcohol. WT mice and Cx3cr1-Cnr2, but not DAT-Cnr2 cKO mice showed robust conditioning to alcohol in the CPP paradigm. We conclude that microglia and DA neuron specific deletion of CB2Rs reveals a neuro-immune basis for the behavioral alterations, and modulation of alcohol behavioral effects by type 2 cannabinoid receptors.

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1. **Effects of cannabidiol on cocaine seeking: dose-response profiles and neurobiological substrates**

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Cannabidiol (CBD) has received attention for potential in drug relapse prevention. In animal models of relapse CBD attenuates reinstatement of cocaine, alcohol, and heroin seeking as well as morphine- and cocaine-conditioned place preference (CPP). In our previous studies, CBD attenuated context-induced cocaine and alcohol seeking across a 7-day treatment period. However, maximal attenuation of drug seeking by the single CBD dose in these studies occurred well before CBD reached maximal levels in brain. Here, we follow up on these findings by establishing the dose-response (D-R) profile and neurobiological substrates for suppression of drug seeking by CBD using a cocaine CPP model. Following acquisition of CPP, rats received one of six CBD doses daily and were tested on day four for expression of CPP. CBD produced linear increases in CBD brain/plasma concentrations across doses, but suppressed CPP with a distinct U-shaped D-R profile characterized by efficacy at intermediate doses and lack of effects, but not aggravation of CPP, at the highest dose. Neural activation (Fos expression) in brain of rats treated with the most effective CPP suppressant dose was reduced in the prelimbic but not infralimbic mPFC or nucleus accumbens. RNAscope® revealed reduced glutamatergic neural activation as the predominant effect of CBD. These findings confirm anti-relapse potential for CBD. However, CBD’s D-R profile may have important implications for clinical treatment regimens and the understanding of CBD effects (or lack thereof) in the literature. Lastly, the findings identify suppression of prelimbic glutamatergic neural activation as a potential mechanism for CBD’s “anti-relapse” actions.

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No conflict of interests.

1. **Epigenetic regulation of inflammation by marijuana cannabidiol**

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Cannabidiol (CBD) is a non-psychotropic cannabinoid derived from marijuana. Studies from our lab have shown that CBD exhibits potent anti-inflammatory properties. In fact, our patent on use of CBD to treat autoimmune hepatitis has been approved recently by FDA as an orphan drug. We have observed that CBD is highly effective in attenuating a variety of autoimmune and inflammatory diseases including Experimental Autoimmune Encephalomyelitis (EAE). We have identified multiple pathways through which CBD exerts anti-inflammatory properties. Importantly, CBD triggers robust induction of CD11b(+)Gr-1(+) myeloid-derived suppressor cells (MDSC) which expressed functional arginase 1, and potently suppressed T cell proliferation both in vitro and in vivo. Induction of MDSC by CBD was markedly attenuated in Kit-mutant (Kit(W/W-v)) mast cell-deficient mice. MDSC response was reconstituted upon transfer of wild-type bone marrow-derived mast cells in Kit(W/W-v) mice, suggesting the key role of cKit (CD117) as well as mast cells. Histone methylation signal was differentially enriched in the binding sites of certain transcription factors. Using microarray, we found that the expression of many protein coding transcripts as well as non-coding RNA was significantly altered in EAE mice which was reversed by CBD. Expression of many miRNAs and lncRNAs was dramatically affected by CBD. In summary, the current study demonstrates that CBD is a potent anti-inflammatory agent and that it mediates these effects through induction of MDSCs and through epigenetic mechanisms, from histone methylation and miRNA to lncRNA (Supported by NIH grants R01AI123947, R01AI129788, P01AT003961, P20GM103641, and R01AT006888). Authors report no conflicts of interest.

1. **Protective effects of δ9-tetrahydrocannabinol (thc) against seb-induced acute lung injury results from alterations in lung dysbiosis**

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Inhalation of Staphylococcal Enterotoxin B (SEB) is known to induce acute lung injury (ALI) and studies from our laboratory have shown that THC, the psychoactive ingredient in *Cannabis sativa,* can attenuate the ALI. In the current study, we investigated the role played by lung microbiota in ALI with or without THC treatment. Lung microbiota was collected and 16S rRNA sequencing was performed. The data were analyzed to determine the alpha and beta diversity. THC treatment led to elevated Firmicutesphylum due to significant increase in the beneficial genus, *Lactobacillus* in the lungs of SEB-exposed group. In contrast, vehicle-treated SEB-exposed mice expressed the Phylum Proteobacteria and class Alphaproteobacteria. Lipopolysaccharide (LPS), bacterial endotoxin was found at significantly higher concentration in the BALF of vehicle-treated group in comparison with THC-treated mice. Beneficial metabolome levels were significantly higher in the colonic flush of THC-treated mice when compared to vehicle-treated SEB group, specifically focusing on butyric, propionic and acetic acids. The role of increased butyrate was further studied by administration of Na butyrate which was found to prevent SEB-induced ALI. In addition, NaB was found to induce immunosuppressive Tregs and MDSC while decreasing Th1 activity as well as beneficial microbiota. Transciptome analysis of lungs of SEB-administered mice following butyrate-treatment showed a decrease in chemokines/receptors CCL12, CCL15, CCL19, CCL22 and CXCR2 and an increase in claudin2, claudin34, alfa and beta-defensins. Together, our data suggest that THC attenuates SEB-induced mortality and ALI by altering the microbiota and other molecules in the lungs (Supported in part by NIH P01AT003961, R01AI123947, R01AI129788 and P20GM103641 to PN and MN). Authors report no conflicts of interest.

1. **Acute exposure to cannabinoids alters early neuronal development in Zebrafish**

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Marijuana is one of the most commonly used illicit recreational drugs. It is reported that up to 14% of pregnant females aged between 12-44 have used cannabis during their first trimester. In this study we wanted to determine the effect of brief exposure of cannabinoids ((+/-)THC, CBD and CBN) during early development. To do this, we exposed zebrafish embryos to different cannabinoids during early stages of development. Cannabinoid-treated fish were less responsive to touch whereas controls exhibited robust escape responses upon touching the head or tail with forceps. Because the movement of treated fish was severely limited, we examined Mauthner cell (M-cell) and motor neuron (MN) development, muscle fiber morphology and activity at the neuromuscular junction (NMJ). Fluorescent labelling of primary and secondary MNs indicated a change in branching patterns and a reduction in the number of axonal branches in the trunk musculature. To examine the structural details of muscle fiber morphology, we performed TEM (transmission electron microscopy) imaging of longitudinal sections of trunk muscle and observed the presence of large-sized mitochondria in several muscle layers in treated embryos but not in controls. To determine if cannabinoid treatment altered the ability of fish to respond to sound at 5 dpf, we examined the C-start escape response in larvae. We found that larvae treated with cannabinoids exhibited a drastic reduction in the number of C-start escape responses to sound stimuli. To examine how cannabinoids mediate their activity, we carried out co-exposure of cannabinoids with the CB1R blockers AM251, CP9455 or the CB2R blockers AM630 and JTE907. Additionally, we examined the effects of knocking down CB1 and CB2 receptor expression using morpholino technology. Our results suggest that the effects of δ9THC occur largely (but not exclusively) through CB1R whereas the effects of CBD and CBN occur mainly (but not exclusively) through activation of CB2Rs. These findings suggest that cannabinoids alter neuronal development via acting through either CB1R or CB2R.

1. **Δ9-THC and related cannabinoids suppress substance** **p-evoked vomiting via activation of cannabinoid CB1 receptors**

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Δ9-THC and related cannabinoids suppress cisplatin-induced vomiting via active

ation of central and peripheral cannabinoid CB1 receptors in the least shrew model of vomiting. Cisplatin-evoked emesis is mainly due to release of serotonin and substance P (SP) in both the gut and the brainstem emetic loci which subsequently stimulate their corresponding 5-HT3- and neurokinin NK1-receptors to induce vomiting. Δ9-THC inhibits vomiting caused by the serotonin precursor 5-HTP, or the 5-HT3 receptor selective agonist, 2-methyserotonin.

In this study we explored whether Δ9-THC and related cannabinoids (WIN55,212-2 and CP55,940) can suppress vomiting caused by SP (50 mg/kg, i.p.) or the NK1 receptor selective agonist GR73632 (5 mg/kg, i.p.). Administration of either Δ9-THC (0, 0.5, 1.25, 2.5 and 5 mg/kg, i.p. or 0, 2.5, 5, 10 and 20 mg/kg, s.c.), WIN55,212-2 (0, 1, 2.5 and 5 mg/kg, i.p.) or CP55,940 (0, 0.025, 0.05 and 0.1 mg/kg, i.p.) caused significant suppression of SP-evoked vomiting in a dose-dependent manner. Δ9-THC (0, 5, 10 and 20 mg/kg, i.p.) pretreatment also significantly suppressed vomiting caused by GR73632. The antiemetic effect of Δ9-THC (20 mg/kg, s.c.) against SP (50 mg/kg, i.p.)-induced vomiting was reversed by the CB1 receptor inverse agonist/antagonist SR141716A (0, 5 and 10 mg/kg, s.c.) in a dose-dependent manner. We have also previously demonstrated that 10 -20 mg/kg (i.p.) doses of SR141716A cause vomiting in shrews. Here we tested the antiemetic efficacy of the NK1 receptor selective antagonist netupitant (0, 1, 2.5, 5 and 10 mg/kg), i.p.) against SR141716A-induced vomiting. Netupitant caused significant and dose-dependent reductions in both the frequency and number of animals vomiting in response to SR141716A (20 mg/kg, i.p.). The discussed data strongly supports the notion that Δ9-THC and related cannabinoid agonists suppress vomiting evoked by nonselective (SP) as well as the selective (GR73632) neurokinin NK1 receptor agonists.

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No conflict of interest.

1. **Neuroadaptations of the endocannabinoid system following cocaine or sucrose intake**

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The endocannabinoid system (ECS) plays a key role in a number of cognitive and motivational processes, including modulation of pain responses, emotional regulation, and memory. The ECS may impact these functions through an interaction with the reward system, particularly as cannabinoid compounds alter responses to a variety of abused drugs and also disrupt feeding behaviours. Both drug abuse and maladaptive eating progress with continued use suggesting that the ECS may be involved in the progression to maladaptive feeding that characterizes obesity and eating disorders. Our project examined this connection by assessing molecular adaptations of the ECS in brain reward circuits following uncontrolled drug or food intake. Separate groups of rats were trained to self-administer intravenous cocaine or were given intermittent access to sucrose using a protocol that induces binge intake. We investigated ECS regulation following these behaviours by assessing CB1 and CB2 receptors, endocannabinoids, and enzymes for endocannabinoid synthesis and degradation. Interestingly, we found a marked increase in CB1 gene expression induced by cocaine in the hippocampus. CB1 transcript was also increased in the NAc following both cocaine and sucrose intake. The 2AG level was oppositely modulated in the hippocampus. We are currently examining whether associated epigenetic processes participate in these transcriptional modulations. Our main objective is to uncover the impact of epigenetic processes on plasticity mechanisms that lead to addictive behaviors. In particular, our approach will bring new insights into maladaptive changes in the brain leading to uncontrolled intake of both drugs of abuse and palatable food and clarify the role of the ECS in these processes.

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Authors declare no conflict of interest

1. **Alcohol preference and depression in cnr2 knockout mice**

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Our previous study demonstrated the genetic association between Cannabinoid type 2 receptor (CB2R) gene (CNR2) and two psychiatric disorders, major depressive disorder and alcoholism. Although a comorbidity of alcoholism and depression is well-known in epidemiological studies, the biological mechanism underlying the comorbidity has been unexplained. The aim of the study is, first to evaluate how CB2R is involved in depression. Reduction of Phosphoethanolamine (PEA), which is one of components of endocannabinoid system, in cerebrospinal fluid and blood have been reported in patients with major depressive disorder. CB2R expressed in neurons and glial cells may be a key regulators of immune and HPA responses. To seek these biological mechanism of Cb2 receptor function underlying alcoholism and depression, we develop model mice using Poly I:C and ACTH administration as depression model. Second aim of our study is to examine how depressiveness/anxiety develop alcohol preference using Cnr2 knockout mice as model of stress-induced depression.

The study indicated that 1) The Cnr2 knockout mice treated with Poly I:C increased anxiety and reduced locomotion. 2) Neuroinflammation related signaling, such as interleukins, Bdnf and Fkbp5, were differentially expressed in brains of Cnr2 Het and WT mice. 3) Pre-administration of PEA reduced anxiety in depression model mice. 4) The Cnr2 Het mice treated with Poly I:C reduced alcohol consumption in comparison to WT, while they drink water equally between them.

Taken together, we conclude that cannabinoid type2 receptor has one of important roles in modulation of stress-induced enhancement of alcohol drinking.

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No conflict of interest.

1. **Classic amphetamines versus new cathinones: comparative behavioral and glial signature**

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Aims: Methylenedioxypyrovalerone (MDPV) is the most common synthetic cathinone found in the blood and urine of patients admitted to emergency departments after taking "bath salts". In spite of MDPV posing growing neuropsychiatric complications, its neuropharmacological profile is dramatically less explored, comparing to classic amphetamines such as methamphetamine (METH). This study determined the early behavioral (locomotor and emotional phenotype) and glial signature (WB and IHC analysis) of this new psychoactive substance in comparison with METH. Methods: Adult male C57BL/6 mice were injected with MDPV or METH binge (4x10 mg/Kg, two hours-apart, i.p.) and their striata were analyzed after probing their locomotor and emotional phenotypes at 18-24 h post-injection, time-window known to encompass the onset of classical amphetamines neurotoxicity. Differences between experimental groups (SAL; MDPV; METH, n=8-9) were compared using one-way ANOVA followed by Tukey multiple comparisons or Kruskal-Wallis followed by Dunn multiple comparisons. Results: MDPV mice showed normal vertical and horizontal locomotor activity in the open field. This was paralleled by unchanged emotional parameters as assessed by elevated plus maze (anxiety-like behavior), splash test (index of well-being) and tail suspension (despair-like behavior). Molecular and cellular analysis showed that MDPV did not change glia as gauged by normal densities in Iba1 (microglia) and GFAP (astrocytes). Finally, striatal receptor for advanced glycation end-products (RAGE; early responder to dopaminergic toxins) density in MDPV mice was not statistically different from control animals. In sheer contrast with MDPV, METH decreased general motor activity in mice. This was paralleled by a significant striatal TH depletion which was accompanied by changes in microglia arborization (Scholl analysis) and astrocytic hypertrophy (increased GFAP labeling). In spite of glial changes, RAGE levels were not significantly different from control animals Conclusion: This comparative study newly highlights that binge MDPV exposure comes without evident behavioral and striatal glial changes at an early time-point, when METH-induced behavioral and striatal glial changes are already evident in mice. However, neuropharmacological MDPV signature needs further profiling.

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1. **Protective effects of oleamide on quinolinated-induced neurotoxicity in rat-brain**

 **synaptosomes and cortical slices**

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Physiological responses in the Central Nervous System (CNS) are regulated by the endocannabinoid system (ECS). Inhibition of neuronal excitability through cannabinoid receptors (CBr) or by independent mechanisms, constitute a potential neuroprotective response. Oleamide (ODA) is a fatty acid amide and endocannabinoid exerting several effects in the CNS, though its neuroprotective properties remain unknown. Quinolinic acid (QUIN) induces toxic effects in the CNS via overactivation of N-methyl-D-aspartate receptors (NMDAr). We investigated the possible protective properties of ODA against the excitotoxic damage induced by Quin in vitro in rat brain synaptosomes and cortical slices, and if these effects are related with the activation of the endocannabinoid system via CB1 and/or CB2 receptors. ODA(1-50 μM) reduced the QUIN (100 μM)-induced loss of mitochondrial reductive capacity in synaptosomes in a mechanism partially mediated by CB1 receptor, as evidenced by the recovery of mitochondrial function induced by the CB1 and CB2 receptor-dependent manners. These findings demonstrate the Neuroprotective and modulatory properties of ODA in biological preparations exposed to excitotoxic insults, and the partial role that the CB1 and CB2 receptors activation exerts in these effects.

1. **Nicotine-afforded neuroprotection of dopaminergic neurons is mediated by the α4 subunit of nicotinic acetylcholine receptors**

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The cause and mechanisms underlying the loss of dopaminergic (DA) neurons in Parkinson’s disease (PD) are poorly understood. A major barrier to the development of new and effective therapies for PD is the current limitation in our understanding of the molecular and cellular events that lead to degeneration of the nigrostriatal DA system. Currently, there are two clinical trials underway to determine whether nicotine might serve as a useful therapeutic for the treatment of PD. Here, we evaluated the effect of nicotine pretreatment (0.5 or 1.0 μM 2 h prior to MPP+ exposure) in 1-[methyl](http://en.wikipedia.org/wiki/Methyl)-4-[phenyl](http://en.wikipedia.org/wiki/Phenyl)[pyridinium](http://en.wikipedia.org/wiki/Pyridine) (MPP+)-treated (1.0 μM) SHSY-5Y neuroblastoma cells, as an in vitro model of PD-related neuronal damage. Assessments were made 24 h after MPP+ exposure. Treatment with MPP+ significantly reduced the number of active mitochondria in neuronal cells and , pretreatment with nicotine prevented this effect. Additionally, nicotine significantly prevented MPP+ induced loss of cellular proliferation. A significant, concentration-dependent neuroprotection afforded by nicotine against MPP+ induced loss of DA was also observed. In a separate set of experiments, in which neuronal cells were transfected with 27-mer primer specific siRNA towards nicotinic acetylcholine receptor (nAChR) α4 subunits prior to MPP+ treatment, nicotine was no longer able to protect cells from the loss of DA cause by treatment with MPP+ suggesting that the neuroprotection afforded by nicotine is likely mediated via nAChRα4 subunits. These data suggest a role for nicotine in protecting mitochondria and facilitating neuronal proliferation via nAChRα4 subunits. Further dissection of this pathway might lead to critical insight into therapeutic approaches means for neuroprotection in PD.

1. **Binge ethanol drinking enhances activity of central amygdala corticotropin releasing factor neurons**

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Binge alcohol drinking has been linked to long-term alteration in brain stress and reward circuitry and is considered a critical step in the development of alcoholism. Corticotropin-releasing factor (CRF) neurons in the central amygdala (CeA) seem to be involved in binge drinking as pharmacological blockades reduce drinking. However, their neural activity in acute and repeated binge drinking is still unclear.

Here we combined optogenetics and *in vivo* electrophysiology to record CeA CRF neurons and define their role in a rodent model of binge ethanol drinking. We found that CeA CRF units had higher firing rate and a more regular pattern of discharge when compared to non-CRF units. Furthermore, we identified four types of CRF units by their ethanol lick-responses: lick-excited (CRF-E), lick-inhibited (CRF-I), lick-predictive (CRF-P), and no response (CRF-NR). We focused our analysis on CRF-NR and CRF-P units, due to their higher prevalence. We observed that CRF-P units had a higher firing rate, as well as the percentage of spikes in bursts compared to CRF-NR units, during ethanol sessions, making them uniquely responsive to alcohol consumption. We then further investigated if the distinct firing activity of CRF units changes over repeated sessions of ethanol consumption, and we found out that CRF-P units firing rates increase significantly in later ethanol sessions compared to early sessions, suggesting alcohol-induced plasticity.

Altogether, our data show an important role for a subpopulation of CeA CRF neurons in binge drinking and could lead to novel, specific circuit strategies for therapeutic intervention for alcohol use disorder.

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No conflict of interest.

1. Sucrose bingeing modifies alcohol reward in mice

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Eating disorders and substance abuse are highly comorbid (Conason A.H. et al., 2006) although the relationship between the expression of these two maladaptive behaviours is unclear. Obese and subjects with alcohol use disorder show similar characteristics: continuous use despite adverse consequences, persistent craving, and repeated unsuccessful attempts to quit (Leigh et al, 2018), suggesting that they are linked by a common etiology. To understand the nature of this relationship, we induced sucrose bingeing in C57Bl6/J male and female mice using 14-day limited access protocol (17.1% w/v). Binge intake was measured as increased solution consumption during the first hour (Yasoshima and Shimura, 2015). All mice given limited access to sucrose developed binge-like intake. We then examined the impact of sucrose bingeing on alcohol reward using a conditioned place preference paradigm with ethanol (i.p.;3%). Interestingly, all mice developed a conditioned place preference to 3% ethanol with the exception of female and male mice given limited access to sucrose. Our findings suggest that binge-like sucrose intake affects reward processing, at least to alcohol- related cues. We will examine this further by assessing ethanol consumption and preference across a range of concentrations (3%, 6%, 9%, 12%, 15%). Our study will provide insights into the relationship between eating disorders and vulnerability to alcohol consumption.

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Authors declare no conflict of interest

1. **Clonidine effects on rat oxycodone self-administration, extinction, and conditioned place preference**

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Clonidine, an alpha-2 noradrenergic receptor agonist, attenuates stress-induced relapse to opioids in laboratory animals. Clinically, clonidine is used to alleviate withdrawal signs during drug detoxification and for drug relapse prevention in opioid addicts. Concerns about such clinical use are raised by case reports that clonidine itself may produce reinforcing effects. Here, in rats, we investigated: 1) clonidine’s rewarding effects, using conditioned place preference (CPP); 2) clonidine’s effects on oxycodone self-administration and oxycodone-seeking behaviors, and 3) clonidine’s effects on oxycodone’s antinociceptive efficacy, using a tail flick test in oxycodone-naïve rats. We found that clonidine (0, 5, 25, 50 µg/kg, i.p.) did not alter CPP in either oxycodone-naïve rats or in oxycodone self-administration rats subjected to CPP conditioning following the completion of self-administration. Thus, animals were in gradual opioid withdrawal during CPP conditioning and testing (2 CPP pairings per day; 5 days, including CPP pre-conditioning and testing). Other animals were subjected to extinction of oxycodone self-administration (10 days) with clonidine (0, 5, 25 µg/kg) given each day to assess clonidine’s effects on extinction. Clonidine pretreatment dose-dependently reduced oxycodone self-administration tested under 0.025 or 0.1 mg/kg/infusion. Clonidine pretreatment also significantly decreased the breakpoint for oxycodone self-administration tested under progressive ratio reinforcement. A 1:1 mixture of clonidine (50 μg/kg i.p.) and oxycodone (1 mg/kg, i.p.) significantly potentiated antinociceptive effects induced by oxycodone (1 mg/kg, i.p.). Our findings indicate that clonidine can affect the reinforcing, motivational, and antinociceptive effects of oxycodone, but does not appear – by itself – to be reinforcing.

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No conflict of interest.

1. **Effects of prenatal maternal ethanol exposure on offspring neurodevelopment in dat-*cnr2* and cx3cr1- *cnr2* conditional knock out mice**

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Several lines of evidence have demonstrated that endocannabinoid system (eCB) plays a significant modulatory role in specific brain and immune functions and neurodevelopment. The eCB system consists of endocannabinoids and cannabinoid receptors (CB1Rs and CB2Rs). For a long time, CB2R was considered to be a periphery receptor. However, accumulating evidence now shows the presence and neuronal functional role of CB2Rs as well as in the microglia. With the use of new genetic strategies such as conditional knockout (cKO) animals, new possibilities are open in studying the role of the CB2Rs in specific behaviors. We use cKO mice that do not express CB2 receptors in dopaminergic neurons (DAT-*Cnr2*) and microglia (Cx3cr1- *Cnr2*). Prenatal ethanol exposure results in neurobehavioral abnormalities. In the present study, we investigated the neurodevelopmental and behavioral alterations of DAT-*Cnr2* and Cx3cr1-*Cnr2* cko and wild type (WT) mice. We also evaluated the effect of perinatal exposure to ethanol on the neurodevelopmental features of these animals (16% alcohol intraperitoneal from gestational days 7 through 17). Ultrasonic vocalizations (USVs), physical development, and reflex activities of the DAT-*Cnr2* and CX3cr1-*Cnr2* cKO and WT pups were analyzed. Results showed that USV rates at 60-80 hertz were higher in the cKO mice than in the WT mice. Ethanol perinatal-exposure induced neurodevelopmental and behavioral alterations. The DAT-*Cnr2* cKO pups started crawling and walking sooner than the WT mice. Locomotor ambulation was affected in the pups that were prenatally exposed to alcohol. No major differences in physical features and reflexes were found in the cKO mice, but ethanol had a differential impact on them.

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No conflict of interest

1. **Involvement of NFΚB in cocaine-associated reconsolidation using intravenous self-administration**

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Persistent and intrusive memories play a key role in maintaining certain psychiatric disorders, such as posttraumatic stress disorder and substance abuse. In the latter, memory for drug-paired cues are potent triggers for relapse, playing a critical role in sustaining drug use. Modulation of these memories may alter the propensity to relapse, suggesting that reconsolidation of drug-cue associations may be a potential target to reduce addictive behaviours. Experimental data in animals confirms that retrieved memories become transiently labile for a few hours post-retrieval: altering a drug-cue memory reduces drug- seeking behavior. Previous work from our lab showed that chronic cocaine intake alters DNA methylation in the rat prefrontal cortex; pathway analysis clustered some of the target genes around the nuclear factor κ B (NFκB) system. Although implicated primarily in inflammation, this transcription factor also plays important roles in learning and memory. Specifically, NFκB is involved in consolidation and reconsolidation of memories in different conditioning paradigms (e.g., conditioned fear, place preference). Our study assessed the effect of NFκB pharmacological inhibition on reconsolidation of cocaine-associated memories using an intravenous self-administration paradigm. Briefly, male rats were allowed to self-administer cocaine over 12 daily sessions under a FR1 schedule of reinforcement; a light stimulus (conditioned stimulus, CS) was associated with each infusion. After instrumental extinction with no CS presentation, rats underwent one CS reactivation session and were immediately injected with NFκB inhibitors. CS-induced reinstatement was assessed 24h later. Our study shows that two different NFκB inhibitors reduced cocaine-seeking behavior, suggesting NFκB involvement in the reconsolidation process.

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1. **Fentanyl abstinence causes structural and molecular changes specific to nucleus accumbens d1 neurons**

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Opioid abuse has risen dramatically over the last decade. Potent, synthetic opioids like fentanyl are responsible for nearly half of opioid-related deaths, yet synthetic opioid abuse remains broadly understudied. Opioids, like other drugs of abuse, engage and alter dopaminergic circuitry to promote continued use and eventual relapse. Neurons in the Nucleus Accumbens (NAc) play a key role in drug abuse and receive dopaminergic input from the midbrain. NAc medium spiny neurons (MSNs) express either dopamine D1 or D2 receptors, and manipulation of their activity can oppositely regulate drug-related behaviors. While it is known that morphine exposure causes a loss of dendritic spines on NAc MSNs, it is unknown if this is true for synthetic opioids like fentanyl, and if the changes are specific to D1- or D2- MSNs. Here we show that after homecage fentanyl exposure and abstinence, both male and female mice show increased social-withdrawal and reduced dendritic complexity specific to D1-MSNs. To identify the molecular mechanisms behind cell-type specific dendritic remodeling, we used D1- or A2A-Cre mice crossed with RiboTag mice to isolate ribosome-associated mRNA in specific cell types after fentanyl. We performed RNA sequencing of the D1- and D2-MSN translatome and found >1,000 differentially expressed genes. Preliminary weighted correlation network analysis (WCGNA) indicates the most strongly down-regulated genes (n=115) are associated with Wnt signaling, ion channels, and neurogenesis. Ongoing experiments aim to delineate the precise molecular mechanisms underlying the social behavior deficits and neuron subtype specific atrophy, as well as to characterize changes to NAc function following operant fentanyl self-administration.

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No conflict of interest

1. **Understanding the effects of giving/receiving naloxone on future drug use behaviors**

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There is limited research that includes the perspective of opioid abusers and their experiences using and/or receiving Naloxone. There is also a limited understanding of the role their drug using behaviors play in their likelihood to administer/be administered Naloxone, nor any understanding from the users’ perspectives of behavioral changes (if any) that result from being administered Naloxone. Understanding the perspective of drug users during this high-risk overdose event can lead to the development of targeted interventions at such key points to prevent future overdoses. This study (n=803) examines the demographics and circumstances of Naloxone usage by drug users in Philadelphia. Additional interviews were conducted with 20 active drug users who either saved someone with Naloxone or were themselves saved to explore the effects of Naloxone use on future drug use behaviors. Participants also share their experiences with Fentanyl testing strips and their intention regarding future use of the soon to open safe injection facility. Data on the location of drug user use of Naloxone is geocoded and compared to data from police and ambulance use for 2018 and the intersection of these systems is explored. Although immediately effective, approximately 35% of people who are administered Naloxone to reverse an overdose are dead within a year of another overdose, and of those, half died within one month. We use this data to propose overdose reduction policies that take into account the perceptions and behaviors of the users themselves to confront this overdose crisis that caused over 70,000 US deaths in 2018.

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No conflict of interest.

1. **Excitatory input from the insula to the vbnst governs the acquisition of cues that predict reward**

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Individuals suffering from substance use disorder often experience relapse events that are attributed to drug craving. Insular cortex (IC) function has been implicated in processing drug-predictive cues and is thought to be a critical substrate for drug craving. Here, we uncover the functional connectivity of a novel projection from the IC to the ventral bed nucleus of the stria terminalis (vBNST), a portion of the extended amygdala that modulates dopaminergic activity within the ventral tegmental area, and investigate the role of this pathway in establishing reward-predictive cues. We reasoned that these cues activate IC projections that synapse onto projection neurons within the vBNST, which then activate the mesolimbic dopamine pathway resulting in the acquisition of associations between exteroceptive stimuli and rewards. To examine this hypothesis we utilized in vivo optogenetics to bidirectionally control the IC-vBNST projection in various behavioral paradigms. We found that the IC is connected to the vBNST via excitatory projections, that when activated, result in a real-time place preference (RTPP) and sustain intracranial self-stimulation (ICSS). We also determined that dopamine neurotransmission is required for these effects. We then silenced the IC-vBNST projection and found that it is necessary for the acquisition of cue-reward pairs, but not for the maintenance of established reward-predictive cues. Thus, the IC-vBNST projection is sufficient to drive reward-related behavior and necessary for establishing reward-predictive cues, making this pathway a previously undescribed target for addictive substances to usurp the brain’s natural reward systems.

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No conflict of interest

1. **Microglial CX3CR1/NLRP3 axis regulates cocaine responses *in vivo***

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Microglia, the resident CNS macrophages account for about 10 -15% of all brain cells, constitute the first line of defense and quickly transit into activation in response to various stimuli. Microglial activation is coordinately and tightly regulated by multiple molecules including CX3CR1. CX3CR1 is exclusively expressed in adult microglia and restrains them in quiescence by binding with neuronal CX3CL1. CX3CR1 deficiency primes microglia leading to exaggerated immune responses upon a second insult. Microglia are sensitive to cocaine exposure which leads to their activation both *in vitro* and *in vivo* and subsequent neuroinflammation has been suggested as integral in the development of cocaine addiction. Whether CX3CR1 deficiency could potentiate cocaine-mediated microglial activation and behavioral changes has not been explored till now. Here we demonstrated that cocaine induced more significant behavioral changes and neuroinflammation in CX3CR1-/- mice than in wild type counterparts. While the TLR4 signaling and autophagy pathways did not show differences between these two strains of mice, cocaine significantly increased NLRP3 inflammasome activity resulting in enhanced production of mature (m) IL1β in the striatum of CX3CR1-/- mice. Further mechanistic studies revealed that CX3CR1 deficiency primed the NLRP3 inflammasome as shown by increased NLRP3 protein and IL1β mRNA expression. Cocaine also served as signal 2 with increased binding of NLRP3 and lysosomal cathepsin D resulting in the full activation of NLRP3 signaling. The contributing role of NLRP3 activity in cocaine mediated behavioral effects was corroborated by the evidence that MCC950 (NLRP3 inhibitor) and IL1β KO blunted cocaine-mediated hyper-locomotion. In summary, we discovered that the CX3CR1/NLRP3 axis could regulate the intensity of cocaine-mediated microglial activation and behavioral changes implying a positive association between neuroinflammation and cocaine addiction. Our findings indicated that engendering microglial quiescence by targeting NLPR3 inflammasome may provide another therapeutic approach to reverse/ameliorate cocaine addiction.

1. **Characterising the variability in behavioural flexibility to predict addiction susceptibility**

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Self-administration procedures conducted in laboratory animals have suggested that only some develop a profile consistent with addiction; only some increase their drug-taking over successive sessions, only some show extensive drug-seeking, and only some are resistant to the inhibitory effect of punishment on drug-taking. It has been hypothesised that behavioural flexibility, a cognitive skill required to alter behaviour in response to changes in environmental circumstances, may be a determinant of the tendency to develop and maintain compulsive drug-taking. Specifically, behavioural inflexibility prevents the shift from maladaptive drug-taking to more adaptive behaviours required to maintain abstinence. Accordingly, behavioural flexibility should be a relatively stable trait and exhibit marked variability across subjects.

I have characterised the variability in behavioural flexibility for a large cohort of drug-naïve rats. The procedure is a visual discrimination reversal learning task. Initially, the rat learns to depress a lever located under an illuminated light. Following acquisition of this discrimination, the reinforced task is reversed so that depression of the lever under the unilluminated light is now reinforced. The number of trials required to learn the reversal rule is measured. This procedure was repeated five times. There was substantial between-subject variability, and the number of trials required to reach criterion for each reversal approximated a normal distribution; some rats were very slow to learn the reversals whereas some were very fast. This procedure provides a means of testing the hypothesis that behavioural flexibility is a determinant of susceptibility to drug addiction.

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No conflicts of interest.

1. **The consumption of psychoactive substances among medicine students in casablanca**

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**Introduction**

Our Study aims at assessing the prevalence of the PAS in take among medicine students of Casablanca during the academic year 2016/2017.

**Materiel and methods**

We carried out an inquiry in 1054 students of the Faculty of Medicine and Pharmacy of Casablanca through an online questionnaire, made of 50 questions that allowed us to study the use of several PAS, namely tobacco, cannabis, alcohol, psychotropic medicines and other substances. The mean age is 21.5 years old.

The use of tobacco was assessed by using the FAGERSTROM test. The use of cannabis, was assessed by the cannabis screening test (CAST), whereas the questionnaire ASSIST (Alcohol, smoking, and Substance Involvement Screening Test) was used to assess the other PAS.

**Findings**

The consumption prevalence of PAS is 16.1% for tobacco, 15.2% for cannabis, 14.8% for alcohol, 14.4% for the other kind of tobacco using, 5.8% for tranquilizers, 3.3% for the opioids, 3% for the stimulants, 2.5% for cocaine, 1.9% for the hallucinogens and 0.7% for the volatile substances while 0.4% of students use drug injections.

We detected a statistically significant association with the male gender except for opioids and volatile substances, the repeating , those who live away from the parent’s home, those of broken families due to divorce or death and those who have sleep disorders.

Psychiatric comorbidity is found in a great number of abusers especially schizophrenia, bipolar disorder, suicidal ideas.

**Conclusion**

Our survey reinforces the findings of the other surveys carried out among students or general population.

1. **Fluoxetine history decreases cocaine and sucrose preference in female mice**

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**Introduction**. Preclinical literature indicates that exposure to antidepressant medications, during early stages of development, results in long-term altered behavioral responses to drugs of abuse (Iñiguez et al., 2015, Sci Rep, 5:15009). However, to date, these studies have been conducted in male subjects primarily. This is surprising, given that females, when compared to males, are more likely to be diagnosed with mood-related disorders, and thus, be prescribed with antidepressants. Therefore, the objective of this study is to assess whether exposure to the selective serotonin reuptake inhibitor fluoxetine (FLX) results in long-lasting alterations in sensitivity to the rewarding properties of cocaine and sucrose, using female mice as a model system.

**Methods**. Adolescent (postnatal day [PD]-35) and adult (PD70) female C57BL/6 mice were exposed to FLX (in their drinking water, 250 mg/l) for 15 consecutive days. Twenty-one days later (PD70+ and PD105+, respectively), mice were assessed on behavioral responsivity to cocaine (0, 2.5, 5, 7.5 mg/kg) using the conditioned place preference paradigm, or their sensitivity to a 1% sucrose solution using the 2-bottle choice test.

**Results**. Data was analyzed with ANOVA techniques, with antidepressant pre-exposure and cocaine post-exposure as sources of variance, followed with Tukey post hoc tests. Our results indicate that female mice pre-exposed to FLX during adolescence or adulthood displayed reliable conditioning to the cocaine-paired compartment, in a dose-dependent manner. However, when compared to respective age-matched controls, antidepressant pre-exposure decreased the magnitude of conditioning at the 5 (p<0.05, R2= 0.21) and 7.5 mg/kg (p<0.05, R2= 0.51) cocaine doses. Similarly, independent of age of antidepressant pretreatment, FLX-pretreated mice also displayed a decrease in sucrose preference (p<0.05, R2= 0.62), without altering total liquid intake (p>0.05).

**Conclusions**. Collectively, our results suggest that exposure to FLX, in adolescent and adult female C57BL/6 mice, leads to prolonged decreases in sensitivity to the rewarding properties of both drug- and natural-rewards. This data further highlight the need for investigations assessing the potential enduring neurobiological side effects that may arise later in life, as a result of antidepressant exposure, in a sex dependent manner.

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1. **Anxiolytic-like effect of nicotine and other minor tobacco alkaloids in zebrafish**

**K. Koshibu**, S. Frentzel, O. Alijevic, D. McHugh, M. Van der Toorn, J. Hoeng, M.C. Peitsch

*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*

Smoking has been repeatedly demonstrated to have anxiolytic and antidepressant effects in smokers, which is thought to contribute to the reinforcing effect of smoking addiction. There are more than 8,000 constituents in cigarette smoke. To understand which of the tobacco components can have these psychoactive properties, we investigated the anxiolytic effects of selected tobacco alkaloids using the zebrafish novel tank test. This behavioral paradigm is higher throughput compared to rodent tests, taking advantage of the fact that zebrafish have the tendency to dive and dwell at the bottom of a body of water to avoid danger or stress. Many anxiolytic drugs, such as diazepam and buspiron, have been shown to reduce this anxiety-driven behavior. Using this model, we found that nicotine, cotinine, anatabine, and harmane induce an anxiolytic-like effect in a dose-dependent manner. To understand the possible mechanisms underlying this effect, we extensively investigated the molecular targets of these alkaloids in vitro. The results indicated that nicotine, cotinine, and anatabine showed binding selectivity to subtypes of nicotinic acetylcholine receptors (nAChR) among more than 170 targets, and functional activity of receptor binding was confirmed electrophysiologically. Harmane, however, bound to multiple potential binding targets, in addition to its well-documented effect on monoamine oxidase (MAO). Taken together, these results indicate that in addition to nicotine, there are other tobacco alkaloids that may possess anxiolytic-like properties, the mechanisms of which are mostly likely mediated by nAChRs, MAO, and potentially other molecules. Further investigations are necessary to elucidate the exact mechanisms.

All authors are employees of Philip Morris International. Philip Morris International is the sole source of funding and sponsor of this research.

1. **Anxiolytic- and antidepressant-like effects of nicotine**

**K. Koshibu**, W. Xia, S. Frentzel, O. Alijevic, D. McHugh, J. Hoeng, M.C. Peitsch

*PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*

Cigarette smoking has been repeatedly demonstrated to have anxiolytic and antidepressant effects in smokers, which are thought to contribute to the reinforcing effect of smoking addiction. Among more than 8,000 compounds in cigarette smoke, nicotine is the major psychoactive and most well-documented. We investigated the acute and subchronic neurological effects of nicotine using SmartCube® system, focusing on psychiatric phenotypes. SmartCube® is a phenotypic behavioral investigation tool that can provide classification of compounds by comparing their effects on behavioral features in mice against a database compiled from the behavioral features obtained from known reference compounds of various drug classes. Using this technology, we observed that a single administration of nicotine induced similar behavioral features as anxiolytics. In contrast, subchronic nicotine administration over seven days induced antidepressant-like behavioral features, the effect of which was corroborated by the classic behavioral test in mice. To understand the possible mechanisms underlying these effects, we extensively investigated the molecular targets of nicotine in vitro, including receptors, channels, and enzymes, that have been predicted largely by in silico models to have binding affinity to nicotine. As a result, nicotine bound selectively to subtypes of nicotinic acetylcholine receptors (nAChR) among more than 170 other targets, and the precise affinities (Ki) were determined for nAChRs. Furthermore, functional activity (EC50) was subsequently confirmed electrophysiologically in mammalian cells overexpressing individual nAChR subtypes. Taken together, these results confirm and augment the previous findings that nicotine has substantial anxiolytic- and antidepressant-like effects, the mechanism of which is mediated by specific subtypes of nAChRs.

All authors are employees of Philip Morris International. Philip Morris International is the sole source of funding and sponsor of this research.

1. **The αlpha 7 nicotinic acetycholine receptors in nucleus accumbens play a role in cue-induced nicotine seeking behavior**

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Exposure to smoking-associated environmental cues triggeres relapse to tobacco use in abstinent subjects. Our previous work has demonstrated that the nicotinic acetylcholine receptors (nAChRs) are required for cue-induced relapse of nicotine-seeking behavior. The present study examined neuroanatomic substrates for the nAChR mediation of nicotine seeking. Male SD rats were trained to intravenously self-administer nicotine on an FR5 schedule. A nicotine-conditioned cue was established via associating a sensory stimulus with each nicotine delivery. After extinction, the relapse tests were performed with response-contingent re-presentation of the cue without nicotine availability. Prior to the test session, rats were subjected to microinjection of antagonists selective for the α4β2 and α7-subtypes of the nAChRs into the nucleus accumbens core (NAc) and ventral tegmental area (VTA). In different set of rats, after the test session, rat brains were prepared for western blot analysis of expression of the α4, α7, and β2 subunits of the nAChRs. Injection of the α7-selective antagonist methyllycaconitine (MLA) and α-conotoxin ArIB (ArIB) in the NAc but not VTA effectively reduced cue-reinstated nicotine-seeking responses. However, microinjection of dihydro-β-erythroidine (DHβE), a α4β2-selective antagonist in both brain regions produced no effect. Increased level of the α7 subunit was observed in the NAc but not VTA in the relapse rats. These data indicate that activation of the α7-nAChRs in the NAc region mediates expression of conditioned reinforcement by nicotine cues. The findings would shed a light on our understanding of the neurobiological mechanisms for smoking relapse triggered by exposure to environmental cues.

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No conflict of interest.

1. **Initial characterization of opioid-induced respiratory depression in rats**

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Respiratory depression is the main cause of deaths by opioid overdose. Therefore, we sought to characterize the respiratory depression induced by heroin and fentanyl in rats. Long-Evans rats had catheters implanted in the right jugular vein. The rats were tested using whole-body plethysmography, which is a non-invasive technique that allows the measurement of several respiration parameters in a breath-by-breath basis in conscious, freely moving animals. The rats were habituated to the plethysmograph chamber for three consecutive days. On the fourth day, they were allowed to habituate to the chamber for 30 min followed by a 20-min baseline recording. Then, they received a single intravenous bolus infusion of heroin 600 µg/kg or fentanyl 25 µg/kg. Respiration was recorded for 90 min. This test was repeated two more times, 2 weeks apart, to test for tolerance development. Heroin produced a long-lasting (60 min) respiratory depression characterized by an increased inspiratory time, decreased tidal volume (volume of air inhaled in each breath), minute ventilation (volume of air inhaled per minute) and inspiratory drive (tidal volume/inspiration time) compared to baseline values. Fentanyl produced the same changes in respiration, but with a significantly shorter duration (30 min). The repeated testing indicated the development of tolerance to the respiratory depression produced by heroin, but not fentanyl. In conclusion, this preliminary experiment with only one dose of each drug suggests that heroin and fentanyl produced respiratory depression with distinct duration and tolerance development.

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The authors declare no conflict of interest.

1. **Striatal fast-spiking interneurons contribute to habitual alcohol consumption**

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Habit formation is an adaptive learning process that allows for efficient reward acquisition. If the reward is a drug of abuse, such as alcohol, habit learning accelerates, and habitual use is promoted. The dorsolateral striatum is necessary for habit formation and the function of its resident parvalbumin expressing fast-spiking interneuron (FSI) population is significantly modulated by alcohol. To determine whether FSIs are necessary for the development of habitual alcohol drinking, we selectively ablated dorsolateral FSIs in mice undergoing a voluntary chronic intermittent ethanol consumption paradigm (drinking-in-the-dark). Adult male and female C67B6J mice voluntarily consumed alcohol for 4-weeks before being challenged on a habitual ethanol drinking assay. Selectively ablating FSIs in the dorsolateral striatum curtailed the development of organized lick sequence behavior without affecting overall ethanol consumption on the drinking-in-the-dark paradigm. On the habitual ethanol consumption assay, FSI ablation significantly decreased drinking of ethanol with the added bitterant quinine.  Collectively these results demonstrate causal involvement of FSIs in habitual ethanol consumption and highlight this neuron population as a key target for therapeutic intervention in compulsive alcohol consumption.

1. **Intranasal oxytocin on alcohol withdrawal, craving and relapse: a double-blind RCT**

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# Background and aims

Intranasal oxytocin (OT) has been proposed as treatment in addiction (1-3). Our aim was to compare the effect of self-administered OT and placebo on prolonged alcohol withdrawal and relapse during a four-week post-detoxification period.

# Methods

A double-blind RCT on patients admitted at the Blue Cross Lade Addiction Treatment Center, Trondheim, Norway. In total, 38 patients (19 oxytocin, 19 placebo) completed inpatient detoxification, and were included in this follow-up trial. Patients received either OT or placebo to use as needed, maximum daily dose of 2 insufflations x 3 (12 ICJ). Main outcomes: Number of days to relapse and total number of alcohol units consumed. Other outcomes: HSCL-IO score, ACQ-SF-R score, the concentration of PEth in blood, and self-reported nervousness.

# Results

No differences between the two treatment groups (16 OT, 17 placebo) in days until the relapse by Kaplan-Meier analysis (p=O.91), or alcohol units consumed (6.9±4.2 vs. 8.1±7,0; p=O.72; 95% confidence interval (Cl) -5.9 to +8.2). Neither were there any differences between the treatment groups in HSCL-IO scores (p= 0.39;; CI -0.81 to 0.33) or in ACQ-SF-R scores (p=O.67; Cl -1.25 to 0.82), nor PEth concentrations (0.46 gmol/l in the OT group and 0.55 gmol/l in the placebo group (p = 0.78; Cl -0.75 to 0.57)). The OT group reported a significant lower level of nervousness compared to the placebo group (p=O.005).

# Conclusion

Oxytocin had no significant effects on time to relapse, nor the number of alcohol units consumed. It did, however, have a significant effect on self-reported nervousness.

# Source of funding

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# No conflict of interest

1. **Roots of decision-making in addiction: the insular cortex, nucleus accumbens, striatum**

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Methamphetamine is a widely abused psychostimulant. Repeated intake of methamphetamine leads to drug addiction, a chronically relapsing disorder characterized by compulsive drug taking, inability to limit intake, and intense drug cravings. Accumulating evidence also indicates that patients addicted to methamphetamine exhibit impaired cognitive functions such as executive function, attention, social cognition, flexibility, and working memory. Furthermore, decision-making is altered in patients with drug addiction, including methamphetamine abusers. Altered decision-making in methamphetamine abusers may contribute to the high rate of relapse even after long-term withdrawal with psychosocial support. Thus, a better understanding of the mechanisms underlying impaired decision-making would provide insights into novel and successful treatments for drug addiction. Recently, we found that insular cortex is a critical region related to risky decision-making in methamphetamine-treated rats. Moreover, we found that both direct and indirect pathway in the nucleus accumbens were involved in reward-based decision-making under the uncertainty condition. Because the insular cortex is connected to the amygdala, nucleus accumbens and striatum, it constructs the frontostriatal and limbic loops related to decision-making. Thus, addicts may have dysfunction in these regions, which is associated with increased risk-taking.

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The authors have no conflict of interest associated with this research.

1. **Ghrelin mRNA in ventral tegmental dopamine neurons is altered by addictive drug self-administration**

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Ghrelin, a stomach/gut hormone, modulates dopamine (DA) transmission within the brain’s reward circuitry, and modulates rewarding effects of abused drugs. Here, we explored localization of ghrelin receptor mRNA within the reward-regulating ventral tegmental area (VTA), and also examined changes induced by addictive drug self-administration. Three rat cohorts were studied: 1) rats given saline, 2) rats self-administering oxycodone, and 3) rats self-administering cocaine. Self-administration rats were allowed to self-administer oxycodone (0.1 mg/kg/infusion i.v.) or cocaine (0.5 mg/kg/infusion i.v.) in 3-hour daily sessions. They were then euthanized by acute decapitation following last self-administration session. Rats given saline were euthanized at the same time post-saline. Brains were removed, placed into 2-methylbutane, and then onto dry ice. Sections through the ventral mesencephalon were mounted on glass slides. Staining for ghrelin and DA transporter (DAT) mRNA was by standard protocol. Sections were then visualized for ghrelin and DAT using RNAscope, an in situ hybridization assay for quantifying mRNA within cells at single molecule sensitivity. Software containing a hybrid cell count function was used to count and analyze (for area and shape) fluorescent particles. In saline-treated animals, ~50% of VTA DA neurons contained ghrelin. In oxycodone self-administration animals, the amount of ghrelin within VTA DA neurons was increased. In cocaine self-administration animals, the amount of ghrelin was decreased (at least within medial VTA DA neurons). We conclude that ghrelin is contained within VTA DA neurons, and that addictive drug self-administration alters the amount of DA-ghrelin co-localization within VTA.

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No conflict of interest.

1. **Cognitive Impairments Induced by Adolescent *binge-like* Ethanol Intoxication in Rats : Neuroprotective Role of Argan Oil**

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Adolescent alcohol *binge drinking* constitutes a major vulnerability factor to develop cognitive disorders. However, the pathways of treatment or prevention against this susceptibility remain less explored. Argan oil (AO), commonly used in traditional Moroccan medicines, is rich in oleic and linoleic acids, polyphenols, sterols, and tocopherols. This composition gives it numerous beneficial pharmacological effects of mental health. In the current study, we evaluated the short-term and long-term AO effects on (i) memory and learning deficits induced by adolescent *binge-like* ethanol intoxication (ii) the oxidative status of the hippocampus and the prefrontal cortex (PFC) in Wistar rats. To model *binge-like* ethanol intoxication, every 2 days, rats received an ethanol injection (3.0 g/kg) for 2 consecutive days across 14 days either from postnatal day 30 (PND30) to 43 (early adolescence). Two weeks before the onset of ethanol intoxication (21PND), rats were daily administered by oral gavage with AO (1 ml/100 g/day), for 5 (PND 53) or 20 (PND 160) weeks. The Y-Maze, Object Recognition and Morris water maze tests were used to assess the working memory, recognition memory, spatial learning and memory performance in adolescent (PND53) or adult (PND160) animals. Also, the catalase and superoxide dismutase (SOD) activities, the lipid peroxidation and nitrite concentrations were measured using spectrophotometric methods. AO pretreatment increased the performance of working memory, recognition memory and spatial memory in rats previously intoxicated by ethanol, regardless of the age and sex of the animals. These behavioral improvements were accompanied by stress oxidative marked changes in hippocampus and CPF. AO pretreatment produces significant decrease of the lipid peroxidation and nitrite levels. On the contrary, AO increased the catalase and SOD activities in adolescent and adult animals. For the first time, our results suggest that AO pretreatment is capable of attenuating cognitive impairments and oxidative stress in the hippocampus and CPF of Wistar rats. This indicates that AO may exhibit a neuroprotection against the toxicity of ethanol in brain adolescent rats. Further investigations are in progress to confirm this pharmacological property.

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1. Sex differences in cocaine craving: role of the estrous cycle

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Studies using continuous-access [drug self-administration](https://www.sciencedirect.com/topics/neuroscience/drug-self-administration) showed that cocaine seeking increases during [abstinence](https://www.sciencedirect.com/topics/medicine-and-dentistry/abstinence) (incubation of cocaine craving). Recently, studies using intermittent-access self- administration showed increased motivation to self-administer and seek cocaine. Additionally, studies report sex differences and a role of ovarian hormones in cocaine relapse. Here, we studied whether intermittent cocaine self-administration would increase incubation of craving in male and female rats, and we investigated the role of the estrous cycle in this effect. We trained male and female rats to self-administer cocaine continuously or intermittently for 12 days (8-h/day). We found, in both sexes and under both training conditions a higher cocaine seeking in the relapse test after 29 days than 2 days. Moreover, in both sexes and independent of the abstinence day, intermittent drug access increased cocaine seeking. Importantly, in both training conditions, female rats showed an increase of drug seeking on both day 2 and 29 compared to males. Finally, by monitoring the estrous cycle in females, we found a potentiation of incubation of craving after either intermittent or continuous drug access in females in estrus.

Our results demonstrate that intermittent cocaine access caused a time-dependent increases in drug seeking during abstinence. In female rats, incubation of craving is critically dependent on the estrous cycle phases. Thus, to the degree that results from animal models generalize to humans, our findings implicate the phase of the [menstrual cycle](https://www.sciencedirect.com/topics/medicine-and-dentistry/menstrual-cycle) as a risk factor for relapse in women and, therefore, should be taken into consideration in the development of [relapse prevention](https://www.sciencedirect.com/topics/medicine-and-dentistry/relapse-prevention) treatments.

This work was supported by NIDA/NIH. No conflict of interest

1. **Regional changes in ∆FosB expression in rat brain following MDMA self-administration predict increased sensitivity to effects of locally-infused MDMA**

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Background. Repeated exposure to drugs produces a plethora of persistent brain changes, some of which underlie the development of drug addiction. An important objective of addiction research is to identify and map the brain changes that might mediate the transition from drug use to drug misuse. The persistent accumulation of the transcription factor, ∆FosB, following drug self-administration provides a means of achieving this objective

Methods. All experiments were conducted on sexually mature male Sprague-Dawley rats. The effects of extensive MDMA self-administration on immunohistochemical measurements of ∆FosB accumulation in 12 brain regions was compared with a matched, drug-naive, control group. Other groups were pretreated with MDMA (0.0 or 10.0 mg/kg, IP, once daily for 5 days) and the locomotor activating effect of MDMA microinjected into brain regions selected on the basis of the ∆FosB results was subsequently determined.

Results. MDMA self-administration significantly increased ∆FosB expression in the nucleus accumbens core, ventromedial and dorsomedial caudate-putamen, anterior cingulate, prelimbic, infralimbic, and orbitofrontal cortex, central and basolateral amygdala, but not in the nucleus accumbens shell or the ventrolateral or dorsolateral caudate-putamen. MDMA pre-treatment enhanced MDMA-produced hyperactivity only when administered into the nucleus accumbens or the medial, but not the lateral, caudate-putamen, mirroring the ∆FosB results.

Conclusions. These data show selectivity in terms of ∆FosB accumulation and provide indices of selective neuroplastic changes relevant to addiction.

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The authors declare no conflict of interest.

1. Development of a competitive c[olloidal gold-based](https://link.springer.com/article/10.1007/s00216-007-1642-z) lateral flow immunoassay strip for the rapid salivary benzodiazepines and melatonin detection

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In Morocco, road accidents are responsible for the deaths of more than 4,000 person each year. The majority of these accidents are mainly due to oversight, excessive speed but also to the state of the driver, due to the consumption of some drugs. In order to better control the drug-impaired drivers and to decrease the level of crash risk, the available tests are either in the blood or in urine. These tests require specialized equipment and staff training, which are unavailable in resource-limited and roadside environments where the need is greatest. The main objective of this project is to develop fast, noninvasive Lateral flow immunoassay test (quick test) that allows the detection of drugs in the saliva of drivers.

The target molecules for our test are benzodiazepines and melatonin (sleep hormone in form of dietary supplement). An assay based on gold nanoparticles has been developed to detect the presence of these molecules in saliva samples. This qualitative test is based on a competitive technique in which the benzodiazepine-BSA and melatonin-BSA conjugates, immobilized in the test zone, compete with the targeted molecules in the saliva of the drivers. A positive test is represented by the absence of a colored line whereas a negative test is associated with any trace of line in the test zone. Then, the results obtained will be validated by a quantitative technique, proton nuclear magnetic resonance (1H-NMR) and liquid or gas phase chromatography techniques.

This test could be used as a monitoring device, in contexts with limited resources, as an inexpensive, accurate and fast solution.

# Student’s use of illicit drugs in the faculty of medicine and pharmacy of Casablanca

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Introduction.

The prevalence of drug use among medical and pharmaceutical student’s remains poorly studied, and particularly poorly understood.

Aim of the study.

Our work aims to assess the prevalence of illicit drugs use among medical and pharmacy students at the faculty of Casablanca.

Method.

A cross-sectional observational descriptive survey will be conducted among medical and pharmacy students enrolled in the Casablanca Faculty of Medicine and Pharmacy during the 2018/2019 academic year. The data collection will be based on an anonymous self-completed questionnaire. The consent and free choice of students will be respected.

Results.

The analysis of the results will allow us to identify the following characteristics:

- The prevalence of illicit drugs use in faculty settings;

- The different types of illicit drugs used in faculty settings;

- Factors triggering the use of illicit drugs in faculty settings;

- Students' knowledge of the consequences of illicit drugs in faculty settings;

Conclusion.
This survey is part of an effort to combat drug addiction among our students in its preventive component, in order to limit this phenomenon and minimize its impact on an increasingly younger population.

1. **Do carnitines play a neuroprotective role against beta-amyloid peptides toxicity?**

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The amyloid beta (Aβ-peptides have been implicated in the excitotoxic mechanism of neuronal injury in the pathogenesis of Alzheimer's disease. The mechanism of Aβneurotoxicity is still not clear but is thought to involve mitochondrial inhibition, production of free radicals, changes in membrane characteristics leading to loss of Ca2+ homeostasis and apoptosis. Our previous studies have shown that exposure of neurons to Aβ25-35 or Aβ1-40 at concentrations as low as 1 microgram/ml significantly inhibited the MTT response. Furthermore, 10-min pretreatment of neurons with acetyl-L-carnitine (ALC) at concentrations of 0.1, 1, 5, and 10 mM reversed the inhibition of the MTT response caused by Aβ25-35 (50 micrograms/ml). Similar protection was also exhibited by vitamin E (100 microM) and catalase (4 mg/ml) (Ref 1). The mechanism for the protective actions of these compounds against Aβ25-35 toxicity was not clear, but thought to involve free radical scavenger action and preservation of energy production, since the Aβ25-35 toxicity is believed to disrupt mitochondrial function, increasing reactive oxygen species (ROS) generation. Therefore, further experiments were carried out with ALC, as well as with L-carnitine (L-C) and propionyl-L-carnitine (PLC) against Aβ1 effects in hippocampal neurons and effects on various mitochondrial function parameters, namely mitochondrial membrane potential (mmp), mitochondrial oxygen consumption rates, basal and maximal respiration and ATP production, were analyzed using the Seahorse apparatus. Preliminary results suggest that all three forms of carnitines, LC, ALC and PLC, are effective against prevention of the Aβ evoked neurotoxicity (Ref 2). Part of this effect may be linked to the preservation of the mmp. However, the exact mechanism underlying the protective action is not clear and may involve multiple cellular processes. It is possible that the carnitines prevent cell damage by stabilizing the membrane against Aβ action and thereby prevent free radical damage and mitochondrial injury, thus preserving energy production and membrane functions. Although other mechanisms, especially for ALC, such as a direct effect on Aβ interaction with charged anionic phospholipids and/or stabilizing action on membranes, are also possible (Ref 1).

Further studies are necessary to understand the neuroprotective potential of these compounds in neurodegeneration models.

1. **Opioid addiction case study for the last 3 years**

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Opioid analgesics (Tramadol, Myantalgique, Fentanyl.) are prescribed and used inappropriately, they cause side effects (nausea, drowsiness, and constipation, also slow breathing which can lead to overdose deaths), they also induce dependence and addiction.

Analgesics accounted for 8.1% of all cases of drug poisoning in Morocco on 2016.

In this study we have detailed the different cases seen at the center with severe analgesics opioid addiction and we have examined the characteristics of two groups: the first includes 17 Tramadol (cp 50 mg) users, the second with 5 patients Fentanyl users.

The average age of the two groups is 36.24 years old.

In the first group: the male sex is predominant with 71% versus 29% of the female sex, Tramadol initiation was essentially made following a drug prescription at 53%, the doses consumed vary between 2 cp and 75 cp per day.

The second group includes only men, aged between 27 and 55, working in the medical field with 2 nurse anesthetists and two doctors in training, the doses consumed vary between half a light bulb and 5 bulbs a day. Initiation is done mainly for experimentation.
Our findings help identify factors associated with problematic prescriptions and underscore the importance of targeted public health interventions.

With this study we hope to highlight an emerging problem that can become a public health problem in Morocco.

**80. Glucocorticoid receptor antagonism reduces opiod addiction-like behaviors in rats**

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Opioid use disorder and opioid overdose deaths are a major public health crisis in the United States. New therapeutic targets are urgently needed. Chronic cycles of opioid intoxication and withdrawal lead to dysregulation of brain stress systems. We hypothesized that this dysregulation depends on glucocorticoid receptor (GR) signaling and contributes to addiction-like behaviors. To test this hypothesis, we used a preclinical model of heroin addiction, where rats given long access (LgA; 12-h/d) to heroin exhibit increased drug taking and seeking compared with rats given short access (ShA; 1-h/d) to heroin. Rats were first trained to self-administer heroin (60 µg/kg/intravenous infusion) in 1-h sessions. Rats were then subcutaneously implanted with a pellet for chronic delivery of mifepristone, a GR antagonist (200 mg/21-day release) or vehicle. In LgA rats, chronic GR antagonism blocked the escalation of heroin self-administration in a fixed-ratio 1 schedule of reinforcement, as well as the enhanced motivation to work for heroin in a progressive ratio test. Additionally, chronic GR antagonism blocked naloxone-induced increases in heroin self-administration and mechanical hypersensitivity observed during spontaneous heroin withdrawal. In contrast, chronic mifepristone treatment did not change the behavior of ShA rats. These findings indicate that GR dysregulation is functionally involved in the addiction-like behavior observed in rats given extended access to heroin. We propose that the GR signaling system is a potential therapeutic target in opioid use disorder.

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No conflict of interest.

**81. Can bioactive foods modulate epigenetics and play a role in neuroprotection and healing drug abuse-evoked epigenetic ‘scars’.**

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The food apart from providing nutrition also actively interacts with the body at the signalling level as well as the genetic (epigenetic) level. Everyday nutrition provides metabolically active compounds that play a role in the maintenance and upkeep of the living organism and is able to modulate the epigenetic information on genes and influence cellular function. Changes in the cellular availability of the food bioactives and their metabolites can alter the status of DNA associated proteins such as the histones, thereby affecting gene activity and intracellular signaling molecules including miRNA expression patterns. Important genes for enzymatic and mitochondrial function can be affected, leading to changes in metabolism in the body such as in the nervous system.

These biochemical changes at the molecular and cellular level will impact the organism and its overall susceptibility to disease. Epigenetic dysregulation in terms of histone-mediated acetylation/deacetylation imbalance may underline neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD). Evidence suggests that targeting histone deacetylase (HDAC) and histone acetyltransferases (HATs) may be beneficial in the treatment and prevention of neurodegenerative disorders. Certain deacetylases mediate neurotoxicity, whereas others provide neuroprotection. Rogue epigenetic patterns formed by drug addiction or epigenetic ‘scars’, found in drug users, can affect pathways in the striatum and the reward system. These may be targeted by compounds restoring epigenetic balance.

Food bioactives can affect HDAC, HAT as well as affect genome stability, mRNA (miRNA) and protein expression. Dietary bioactive compounds such as genistein, phenylisothiocyanate, carnitine, curcumin, resveratrol, indole-3-carbinol, and epigallocatechin-3-gallate can regulate HDAC and HAT activities and acetylation of histones as well as non-histone chromatin proteins. Therefore, their potential health benefits which could be related to epigenetic mechanisms in addition to their usual metabolic and antioxidant properties. The protection by food bioactives, therefore, extend from the molecular, to cellular to organism level and influence genomic, metabolic and proteomic pathways. This influence extends throughout the lifetime of the organism from conception to adulthood and even to a few generations beyond. The challenge for research is to identify the right foodstuffs and to understand the underlying protective mechanism for each type and to extend its efficacy in a preventative and also treatment role.

**82. An in vivo imaging study for detecting rewarding effects of drugs in mice**

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Many drugs of abuse directly or indirectly act on dopamine receptors to activate their downstream signaling pathways. It has been reported that transcription factors and their target genes are activated in response to the pharmacological action of addictive drugs. Therefore, monitoring the activities of transcription factors in vivo might enables us to predict the rewarding effects of drugs. In this study, we attempted to develop an in vivo screening method for detecting rewarding effects of addictive drugs by using cyclic AMP response element (CRE) promoter. As an in vitro assay, HEK293 cells were transfected AAV plasmid which encodes a sequence of CRE promoter and luciferase gene (AAV-CRE). Forskolin treatment significantly increased the expression level of luciferase 3 and 6 h after the treatment in HEK293 cells. In vivo experiment, we injected the AAV particle (AAV-CRE) into the striatum of C57BL/6 mice (AAV-CRE mice). Three weeks later, AAV-CRE mice were administrated saline or cocaine and then placed in the chamber for in vivo imaging system. Cocaine treatment significantly increased luciferase activity compared to saline-treated control group 6 h after the treatment. Morphine or methamphetamine treatment also increased luciferase activity compared to saline-treated control group. These results suggest that the in vivo imaging system using AAV-CRE mice is useful as an in vivo screening system of rewarding effects of drugs. Because we have previously demonstrated that activation of medium spiny neurons (MSNs) expressing dopamine D1 but not D2 receptors, in the nucleus accumbens plays a crucial role in rewarding effect of methamphetamine, it might be possible to enhance the sensitivity of AAV-CRE mice to detect rewarding effects of drugs in vivo, by expressing CRE- luciferase only in the dopamine D1 receptor-expressing MSNs.

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The authors have no conflict of interest associated with this research.

**83. Neuronal ensemble-specific projections mediate cocaine seeking and extinction**

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**Aims:** We previously found that functionally distinct neuronal ensembles in the ventral medial prefrontal cortex (vmPFC) mediate self-administration and extinction of cocaine-seeking. In the present study, we examined whether neuronal ensembles associated with cocaine self-administration or extinction project to different subregions of the nucleus accumbens (NAc). We then examined whether these activated projections differentially mediate the cocaine self-administration and extinction behaviors.

**Methods:** We trained rats to self-administer cocaine for 14 days, then extinguished the behavior for either 0 or 7 days. We used a non-reinforced test to reactivate either the self-administration memory (0 extinction session group) or the extinction memory (7 extinction session group). We retrogradely labeled projections from the vmPFC to NAc and combined this with double-labeling for Fos in the vmPFC. Next, we performed a disconnection experiment combining Daun02 inactivation of vmPFC neuronal ensembles associated with self-administration or extinction of cocaine seeking followed by ipsilateral or contralateral inactivation of the NAc core or shell prior to a probe test.

**Results:** The tracing experiment indicated that self-administration ensembles project preferentially to NAc core, while extinction ensembles project preferentially to shell. Contralateral inactivation of the extinction-related ensemble and NAc shell increased cocaine seeking when compared to ipsilateral inactivation. Conversely, contralateral inactivation of the self-administration-related ensemble and NAc core increased cocaine seeking when compared to ipsilateral inactivation.

**Conclusions:** Different neuronal ensembles within the vmPFC mediate cocaine self-administration and extinction of cocaine seeking. These ensembles are functionally distinct, and project to different anatomical subregions of the nucleus accumbens to exert their effects.

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No conflict of interest.

**84. Optogenetic interrogation of the rewarding vs. Aversive effects of cannabinoids**

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Cannabinoids can exert both rewarding and aversive effects in humans and animal models. However, the neural mechanisms underlying cannabinoids’ paradoxical effects are poorly understood. Cannabis reward is canonically attributed to activation of the inhibitory cannabinoid receptor type 1 (CB1R) on GABAergic inputs to the ventral tegmental area (VTA). Our lab recently reported that Δ9-tetrahydrocannabinol (Δ9-THC), the major psychoactive component of cannabis, also produces aversive effects in mice that are mediated by CB1R on glutamatergic inputs in the VTA. In addition, the discovery of inhibitory cannabinoid receptor type 2 (CB2R) expression on VTA dopamine (DA) neurons suggests a further mechanism of action by which cannabinoids may induce aversive effects. Here, we hypothesized that the net outcome of cannabis reward vs. aversion depends upon the relative activity of a cannabinoid ligand at CB1R vs. CB2R, respectively. To test this hypothesis, we used optogenetic and transgenic approaches to express light-sensitive channelrhodopsin (ChR2) in VTA DA neurons using DAT-cre mice. Optical stimulation of VTA DA neurons induced frequency- dependent intracranial self-stimulation behaviors. Responding was significantly enhanced by systemic administration of a highly selective CB1R agonist (XLR-11), but suppressed by highly selective CB2R agonists (Xie2-64 and Xie2-49) in a dose-dependent manner. Cannabinoids with less selective CB1R/CB2R activity progressively reduced (Δ9-THC and Win55,212-2) or had no effect (JWH-133) on optical self-stimulation responding. Taken together, these findings support our hypothesis that CB1R activation may account for the rewarding effects of cannabinoids, while CB2R activation may account the aversive effects of cannabinoids in different subjects.

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**85. Behavioral and epigenetic effects of paternal exposure to cannabinoids during adolescence on offspring vulnerability to stress**

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 Chronic cannabinoid exposure during adolescence in male rats induces chronic cognitive and emotional impairments. However, the impact of this form of exposure on offspring vulnerability to stress is unknown. The aim of this study was to evaluate the behavioral and epigenetic effects of stress in the offspring of male rats whose fathers were exposed to cannabinoids during adolescence.

 Male adolescent offspring of Win55,212-2 (1.2 mg/kg) treated rats were exposed during one week to variable stressors and subjected to behavioral tests of anxiety and episodic-like memory, followed by an assessment of global DNA methylation and expression of DNA methyltransferases enzymes DNMT1 and DNMT3a mRNA in the prefrontal cortex.

 Stress exposure induced a significant anxiogenic-like effect but did not affect the episodic-like memory in the offspring of Win55,212-2 exposed fathers in comparison to the offspring of non-exposed fathers. These behavioral changes were subsequent to a significant increase in global DNA methylation and DNMT1 and DNMTa3 transcription in the prefrontal cortex.

 These data suggest that the deleterious effect of chronic exposure to cannabinoids during adolescence are not limited to the exposed individuals but may increase the vulnerability to stress-induced anxiety in the offspring and alter their epigenetic programming.

**Keywords** : Adolescence; Anxiety; Cannabinoids; DNA methylation; Offspring; Stress

**86.** **Prenatal stress alters D2 and 5HT1A receptors expression in adult rats**

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Early-life events have long-term effects on brain structures and cause behavioral alterations that persist into adulthood. Our results show high levels of Drd2 expression in the nucleus accumbens of prenatally stressed rats compared to control subjects, while repeated diazepam administration in adulthood down-regulated Drd2 expression and prevented the effect of prenatal stress. Interestingly, the results of this study provide more evidence that prenatal stress has a profound impact on the descendant’s behavior and dopaminergic and serotoninergic systems. Our findings reveals that maternal exposure to chronic footshock stress increases the intensity of diazepam withdrawal symptoms and significantly reduce levels of 5HT1A receptors in the raphe nuclei of adult offspring, suggesting that prenatal stress may increase vulnerability to drugs.

**87. Accumbal dopamine release tracks the expectation of dopamine neuron-mediated reinforcement**

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The efficient pursuit of rewards is crucial for survival and relies on environmental cues directing and energizing goal-oriented behavior. Dopamine (DA) transmission in the nucleus accumbens (NAc) facilitates cue-reward associations and invigorates appetitive action. Reward-related accumbal DA release dynamics are traditionally ascribed to ventral tegmental area (VTA) DA neurons, as VTA cell firing and accumbal DA release phasically increase following better than expected events and–as learning proceeds–the DA response transfers along with action initiation to cues predicting access to the instigating stimulus. Accordingly, the ability to phasically activate DA neurons is thought to allow rewards such as food or addictive drugs to function as goals and for cues to subsequently drive DA signaling and goal-seeking. But whether DA neuron activation sufficiently drives learning about, and dopaminergic encoding of, predictive cues is not known. Here we used optogenetics to control DA neuron firing and voltammetry to record accumbal DA release in order to quantify how reinforcer-evoked dopaminergic activity shapes conditioned DA transmission and behavior. We find that cues predicting access to DA neuron self-stimulation elicited conditioned responding and NAc DA release, but cue-evoked DA release did not reflect the cost or magnitude of DA neuron activation. Accordingly, conditioned accumbal DA release selectively tracks the expected availability of DA neuron-mediated reinforcement. This work provides insight into how mesolimbic DA transmission drives and encodes goal-directed behavior.

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No conflict of interest.

**88. Prenatal stress induces vulnerability to nicotine addiction by altering behavioral and molecular patterns in adult Wistar rats**

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Addiction is a pathological learning process overtraining and altering decision-making patterns, leading to a loss of control over common behaviors. Although systematically initiated after exposure to drugs of abuse, this process only occurs to compulsion in some vulnerable individuals. Many factors may explain a person's predisposition to addiction, such as genetics, stress, PTSD…

In this study, we chose to focus our research on the effect of prenatal stress on the vulnerability to nicotine addiction since its prevalence rate has significantly risen recently in modern societies. First, we investigated the effect of prenatal stress on the reinforcing properties of nicotine in the CPP paradigm and its withdrawal intensity in the water consumption test in both in utero stressed rats and their control counterparts. Then, we examined the mRNA expression of their D2 dopaminergic receptors in the nucleus Accumbens (NAcc) and their 5HT1A receptors in the raphe nuclei using quantitative real-time PCR (qPCR).

The results showed that prenatally stressed rats exhibited a greater place preference for the nicotine- paired compartment and a heightened anxiety symptom following nicotine withdrawal when compared to the controls. Additionally, they presented an overexpression of the DRD2 gene in the NAcc and a significant decrease in 5HT1A receptors’ levels in the raphe nuclei.

Taken together, these findings suggest that gestational stress can permanently alter the offspring’s addictive behavior throughout adulthood on a behavioral and molecular level.