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Poster Abstract Proceedings

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P1.1: FiPhA: An Open-Source Platform for Fiber Photometry Analysis (Stevanovic)

Presenter name: Korey Stevanovic, NIEHS

Authors: Korey Stevanovic, NIEHS; Matthew Bridge, Dlhcorp; Leslie Wilson, NIEHS; Sambit Panda, Neurobiology Laboratory, NIEHS; Ayland Letsinger, NIEHS; Sandra Mcbride, Dlhcorp; Jesse Cushman, NIEHS

Fiber photometry is a widely used technique in modern behavioral neuroscience, employing genetically encoded fluorescent sensors to monitor neural activity and neurotransmitter release in awake-behaving animals. With its low cost and ease of implementation, it has become a valuable tool for the behavioral neuroscientist. However, analyzing such data can be laborious and time-consuming, especially considering that experimental setups may often vary based on the specific brain region and type of sensor being used. Consequently, many research laboratories have developed their own bespoke analysis pipelines that are not easily applicable to outside data. Here we describe a collaborative project that has been undertaken to develop a general-purpose fiber photometry analysis application in R called FiPhA (Fiber Photometry Analysis). This application utilizes the Shiny framework, providing interactive visualization, quality control, and batch processing functionalities to an otherwise tedious stage of data analysis. Simplifying a range of tasks, the program includes visualizations of event-triggered averages, event filtering, and spectral analysis capabilities. Furthermore, it is able to work with data derived from multiple photometry systems, including those that are spectrally resolved, camera-based, and involve lock-in demodulation methods. A user-friendly interface provides a highly configurable experience for researchers without necessitating knowledge of the R programming language itself.

P1.2: SeroWare: An open-source software and database for neurochemical voltammetry (Movassaghi)

Presenter name: Cameron Movassaghi, UCLA

Authors: Cameron Movassaghi, University of California, Los Angeles; Anne Andrews, University of California, Los Angeles; Cameron Movassaghi, University of California, Los Angeles

Voltammetry is widely used for fast, data-dense measurements of redox-active analytes in versatile environments, including monitoring brain neurotransmitters. Voltammetry requires minimal hardware beyond a potentiostat, a front-end amplifier, and a computer. However, researchers must either develop their own application-specific software or modify existing packages to carry out voltammetry. Of the software available, significant issues with source code that is inaccessible for modification, non-configurable data processing procedures, and hardware incompatibilities further complicate this landscape. A community-supported solution for open-source software and data, specific to neurochemical voltammetry, is becoming increasingly needed to address reproducibility and data-sharing requirements from funding agencies.We report on the development of ‘SeroWare’, an open-source, end-to-end, voltammetry acquisition, analysis, and storage software suite. The software is custom-designed for a wide variety of use cases encountered by analytical voltammetry communities. Although inspired by neurochemical analyses, the software is highly flexible, customizable, and compatible with other open-source toolkits. Our modular software architecture enables users

to generate (through customized waveforms), acquire, and analyze voltammetry data of different types ranging from pulse and sweep waveforms to fast and slow scans via easily accessible and exportable file formats. We offer user-friendly interfaces and in-depth documentation. We invite further beta testing, feature and issue requests, and contributions through GitHub. We aim to provide the voltammetry community with freely available software and tools to adopt new technologies rapidly, encourage collaboration and resource sharing, and gain new insights from the most valuable resource voltammetry has to offer – data.

P1.3: Endocannabinoids influence the dopaminergic substrates of cognitive flexibility (Zlebnik)

Presenter name: Natalie Zlebnik, University of California

Authors: Natalie Zlebnik, University of California, Riverside School of Medicine; Brandon Oliver, University of California, Riverside School of Medicine; Andrew Villa, University of California, Riverside School of Medicine; Leslie Estrada, University of California, Riverside School of Medicine

Mesolimbic dopamine is responsible for reward-based learning. Specifically, activation of dopamine neurons facilitates the association of cues with the value of related rewards and is responsible for flexibly updating cue-reward learning when conditions are altered. Endocannabinoids are key modulators of dopamine neuron activation and enable dynamic changes in reward-driven dopamine release. Therefore, endocannabinoids, such as 2-AG, may facilitate changes to cue-reward associations during reversal learning when conditions are altered. Here we employ in vivo fiber photometry to monitor dopamine release in the nucleus accumbens at distinct stages of discrimination and reversal learning following treatment with a monoacylglycerol lipase inhibitor, which prevents the degradation of 2-AG. Male and female mice (n = 40) were first trained to discriminate between two levers of differing reinforcement probabilities (80% vs. 20%); this was followed by a reversal phase in which the reinforcement probabilities were inversed across levers. During the reversal session, mice were pre-treated with a monoacylglycerol lipase (MAGL) inhibitor JZL-184 (0, 8, 18 mg/kg, ip) to increase synaptic levels of 2-AG. DA was recorded during early and late acquisition, and early, mid, and late reversal sessions. Performance was analyzed to assess mean differences across various metrics on the first day of the reversal phase. Analysis of error probabilities (win-stay and lose-shift) were also conducted. Finally, dopamine release was compared between- and within-subjects across sessions to determine differences in phasic dopamine release following different trial outcomes. Results demonstrate significant impairment in behavioral flexibility and dopamine release following elevation of 2-AG. Errors (% correct choices) were dose-dependently increased by JZL-184 administration (p < 0.05 vs. vehicle), and dopaminergic encoding of trial outcomes was attenuated. These results suggest disruption of the endocannabinoid signaling hinders reversal learning performance and dysregulates associated mesolimbic dopamine release. These findings give critical insight into the role of the endocannabinoid system in flexible reward-based learning and may have significant implications for the use of cannabinoids for recreational or therapeutic purposes.

P1.4: The transdermal patch: the first step towards obtaining a window into the neurochemistry of depression through the detection of skin inflammation (Baumberger)

Presenter name: Beatrice Baumberger, Imperial College London

Authors: Beatrice Baumberger, Imperial College London; Parastoo Hashemi, Imperial College London

Robust evidence supports the involvement of the histaminergic system in the neurochemistry that underlies depression, specifically the inhibitory effects of histamine on serotonin levels in the brain. This finding is relevant because depression is thought to stem from an underlying serotonin deficiency. Together, these highlight the importance of histamine in the pathology of depression and places this inflammation marker under the pharmacological spotlight to optimize the diagnosis and treatment of this impairing mental illness. The limited accessibility of the human brain for diagnostic purposes has driven the need to probe the peripheral system. To better understand the connections between brain and the body, with the idea of establishing a quantifiable maker, we turn to the well-known correlation between neuroinflammation and peripheral inflammation.The skin is the largest organ in the human body, is readily accessible in humans and is connected to the brain through a recently established inflammatory pathway. Through my research I aim to leverage the skin-brain axis to obtain a window into the chemistry of depression, without directly probing the human brain.The first step towards defining a skin-brain axis measurement paradigm is the development of a transdermal patch that can measure the histamine-serotonin relationship in skin, in vivo, in a minimally invasive manner.I hereby present a voltammetry-compatible transdermal probe that measures neurotransmitters in the skin, as proxy for the brain. I describe key design features, characterization and validation of the probe in skin models. This work represents important first steps to defining peripheral markers of brain inflammation, that could serve to better investigate, diagnose and treat brain diseases.

P1.5: Within-Mice Comparison of Microdialysis and Fiber Photometry- Recorded Dopamine Biosensor during Amphetamine Response (Sorensen)

Presenter name: Gunnar Sorensen, H. Lundbeck A/S

Authors: Aske Ejdrup, Department of Neuroscience, University of Copenhagen; Joel Wellbourne-Wood, H. Lundbeck A/S; Jakob Dreyer, H. Lundbeck A/S; Nina Guldhammer, H. Lundbeck A/S; Matthew Lycas, University of Copenhagen; Ulrik Gether, University of Copenhagen; Benjamin Hall, H. Lundbeck A/S; Gunnar Sorensen, H. Lundbeck A/S

Monitoring the concentration of extracellular signaling molecules is important to understand brain function and pathology. Several methods with differences in selectivity, resolution, and duration have been used to investigate the characteristics of neuronal signaling. Electrochemical methods such as fast-scan cyclic voltammetry (FSCV) can achieve subsecond temporal resolution, but chemometric data processing restricts the total duration of the measurement to a minute for most practical purposes. FCSV offers some molecular specificity but requires the molecule to be electroactive and free from interference from other molecules with similar electrochemical properties. This limits the technique to brain regions with high neurotransmitter specificity, such as dopamine (DA) in the striatum. In comparison, microdialysis (MD) provides a lower sampling rate in the range of many minutes to hours, although faster rates have been described. An advantage of MD, however, is its high specificity for molecular species and their metabolites via analysis of dialysates using high performance liquid chromatography (HPLC) or mass spectrometry (MS). Genetically encoded biosensors

offer an optical method for monitoring neurotransmission in vivo at a higher time resolution than MD and at lower analyte concentrations than FSCV. The fluorescent signal from these biosensors is typically sampled at a time resolution comparable to or higher than FSCV and offers the ability to capture changes in neurotransmitter levels over longer time scales. Biosensors for a host of molecules have been developed, making it a versatile and popular strategy for monitoring in vivo changes in neuronal signaling. A weakness of data from biosensors, however, is its relative nature. Whereas MD readily provides measures of concentration, through analysis of dialysates using HPLC or MS, and FCSV can be calibrated ex vivo to provide changes in concentration, biosensors provide relative changes in transmitter concentration. To directly compare MD and FP, we performed concurrent within-animal recordings of extracellular dopamine (DA) in the dorsal striatum (DS) before and after administration of amphetamine in awake, freely behaving mice expressing the dopamine sensor dLight1.3b. We show that despite temporal differences, MD- and FP-based readouts of DA correlate well within mice. Down-sampling of FP data showed temporal correlation to MD data, with less variance observed using FP. We also present evidence that DA fluctuations periodically reach low levels, and naiv̈e animals have rapid, predrug DA dynamics measured with FP that correlate to the subsequent pharmacodynamics of amphetamine as measured with MD and FP.

P1.6: Characterizing Fentanyl-Associated Calcium Activity of Differential Ventral Tegmental Area Projections with Fiber Photometry (Montemarano)

Presenter name: Annalisa Montemarano, Penn State College of Medicine

Authors: Annalisa Montemarano, Penn State College of Medicine; Megan Fox, Penn State College of Medicine

The ventral tegmental area (VTA) is a fundamental part of the reward neurocircuitry that shapes motivated behavior via various projection targets, such as the nucleus accumbens (NAc) and prefrontal cortex (PFC). However, these and other brain regions are often studied as independent entities rather than interconnected circuits. Understanding the circuit signatures encoding reward-related behavior is crucial to discern how these systems are dysregulated in substance use disorder, an understanding of which is especially lacking in the context of opioids as compared to other substances of abuse. Previous work indicates opioid exposure induces structural and functional adaptations in VTA dopamine neurons depending on their downstream projection target, however no work has examined how this translates to different circuit activity during behavior. Here, we use an intersectional viral strategy to monitor in vivo calcium dynamics of NAc- or PFC-projecting VTA neuronal populations with fiber photometry. We used fentanyl conditioned place preference in female mice to examine the calcium dynamics of these two different projections in drug-associated contexts. VTA-NAc projections show increased calcium activity in response to fentanyl exposure, while VTA-PFC projections show increased calcium activity in response to fentanyl-associated contexts. We also compared fentanyl-related calcium activity to that of various naturalistic reward behaviors. VTA-NAc but not VTA-PFC show increased calcium activity associated with social interaction. Both VTA-NAc and VTA-PFC calcium activity is increased upon entry into the open arms of the elevated zero maze, and VTA-PFC calcium activity is increased in response to sucrose self-administration. Future directions of this work include expanding to a fentanyl self-

administration paradigm to investigate how these and other projections relate to drug taking and seeking. Furthermore, we plan to increase the specificity of our system to address the cell-type diversity present in the neuronal populations investigated here, allowing for improved understanding of the neurocircuitry mediating drug-associated behaviors.

P1.7: Fentanyl and methamphetamine co- self-administration modifies fentanyl taking and exacerbates mesolimbic dopamine deficits (Dawes)

Presenter name: Monica Dawes, Wake Forest University School of Medicine

Authors: Monica Dawes, Wake Forest University School of Medicine; Katherine Holleran, Wake Forest University School of Medicine; Sara Jones, Wake Forest University School of Medicine

The opioid epidemic currently affecting the United States has entered a new wave of mortality, with combined use of opioid and psychomotor stimulants as a major contributing factor to the number of overdose deaths. Combined use of fentanyl and methamphetamine may be due to a number of factors, including greater rewarding effects, decreased negative side effects, and/or feelings that combined use is somehow 'safer' than use of fentanyl alone. For those reasons, there is a clear need to examine the differences in the behavioral and neurobiological alterations that occur following chronic use of fentanyl, methamphetamine, and these two substances in combination. Male and female Long Evans rats were trained to self-administer 2.5 µg/kg/inf fentanyl. Following acquisition, rats were randomly assigned to either fentanyl alone or fentanyl + methamphetamine and were tested on a short access, fixed ratio 1 schedule of reinforcement (3 hr sessions, max. 20 infusions) for ascending doses of fentanyl or combined fentanyl + methamphetamine (1.25, 2.5, 5.0 µg/kg/inf fentanyl ± 0.1 mg/kg/inf methamphetamine, 5 days per dose). A separate group of male and female Long Evans rats were trained to self-administer 0.1 mg/kg/inf methamphetamine, and following acquisition were tested on a short access, fixed ratio 1 schedule of reinforcement (3 hr sessions, max. 20 infusions, 0.1 mg/kg/inf) for 15 days to match total methamphetamine exposure in the combined fentanyl and methamphetamine group. Both male and female rats self-administering methamphetamine had greater rates of responding than those self-administering fentanyl or combined fentanyl and methamphetamine. At the highest doses tested, male rats showed greater rates of self-administration of combined fentanyl and methamphetamine than fentanyl alone, while in females, the rate of self-administration of combined fentanyl and methamphetamine was less than fentanyl alone. Additionally, in male rats, the latency to initiate responding was shorter in animals self-administering combined fentanyl and methamphetamine than those self-administering fentanyl alone, while the opposite was observed in female rats. Following self-administration, coronal brain slices containing the nucleus accumbens were prepared for ex vivo fast scan cyclic voltammetry. Combined fentanyl and methamphetamine rats had decreased evoked dopamine release and uptake rate (Vmax) compared to saline and fentanyl alone animals. Further, evoked dopamine release was decreased across stimulation amplitudes and frequencies in combined fentanyl and methamphetamine animals, compared to fentanyl alone animals. Together, these results highlight the complexities of combined opioid and stimulant use, and suggest that there may be unique sex specific neuroadaptive processes specific to combined fentanyl and methamphetamine which are not sufficiently explained by the individual changes observed following use of fentanyl or methamphetamine alone.

P1.8: Somatostatin inhibits striatal dopamine release with regional specificity that diminishes with aging. (Todd)

Presenter name: Kathryn Todd, University of Oxford

Authors: Kathryn Todd, University of Oxford; Ana Sousa, University of Oxford; Susanne Szydlowski, University of Oxford; Stephanie Cragg, University of Oxford

Nigrostriatal dopamine (DA) axons are immense structures that form extensive arbors within the striatum. Their complexity offers the potential for striatal neuromodulators to profoundly and diversely shape DA signalling and therefore striatal output. This neuromodulator landscape will also be central to circuit dysfunction during the progression of Parkinson's disease (PD), however this landscape is still far from fully understood. The striatal neuropeptide, somatostatin, is a strong candidate for a key neuromodulator of DA axons, with evidence suggesting it may be dysregulated in PD. Somatostatin is localised in striatal GABAergic interneurons and its receptors are expressed in both the dorsal and ventral striatum. However, it is poorly understood how somatostatin modulates DA release in these diverse striatal regions and if this is dysregulated in PD. We explored the regulation of DA release by somatostatin by monitoring electrically-evoked DA release using fast-scan cyclic voltammetry in acute ex vivo brain slices from mice, in wild-types and in a transgenic model of early PD. We found that somatostatin (1 μM) attenuated DA release evoked by 1 pulse and 4 pulses at 100 Hz by 40% in the dorsolateral striatum (DLS), but had no effect in the ventral striatum, an important finding given the selective vulnerability of the DLS in PD. We tested for changes in the effect of somatostatin on DA release in the human alpha-synuclein-overexpressing mouse model of PD at 18 months, and age-matched alpha-synuclein-null background controls. However, while there was no difference in the effect of somatostatin between genotypes, there was a significantly lesser inhibitory effect of somatostatin in aged (18 months old) compared to young (3 months old) mice. We also obtained preliminary data exploring the potential for the genetically-encoded fluorescent GRAB-somatostatin sensor to detect the dynamic availability of striatal somatostatin. In summary, we have shown that somatostatin differentially modulates DA release in diverse striatal regions and that while there is no significant difference in a mouse model of early PD, the effect of age, a crucial risk factor in PD, does seem to impact the somatostatin regulation of DA.

P1.9: 3D neuronal cell culture on Paper Device and Bioelectrochemical Analysis (Pelletier)

Presenter name: Juliette Pelletier, Polytechnique Montréal

Authors: Juliette Pelletier, Polytechnique Montréal; Raphaël Trouillon, Polytechnique Montréal

Context- Paper, here used as a substrate for biomedical devices, has recently opened new applications such as immunoassay, microfluidics or electrochemical sensors. The advantages of a material like paper are mainly its affordability, ease of modification and the tunability of its chemical properties. Cell culture on paper has been demonstrated to facilitate the growth of cells in a 3D environment to better describe the conditions of a real organ/ tissue. To improve the viability of cells on paper, it is possible to treat the paper with extracellular matrix molecules. Furthermore, it is possible to print electrodes on paper using techniques and materials derived from printed electronics. Paper can thus be used as an electrochemical

device to quantitatively measure molecules such as dopamine, a neurotransmitter secreted by dopaminergic neurons. These capabilities pave the way for on-paper cell analysis of neurotransmitters.Neurons on paper- By tuning the biocompatibility of paper, the viability of a cells deposit can be increased, showing the possibility of achieving neuronal cells culture on paper. The growth of theses neuronal culture can be monitored, and the neurons can survive for several days after the initial deposition. The performance of different kinds of modified paper devices were compared based on the neuron viability and the growth of axons and dendrites. Fluorescence-based imaging has been the chosen method to achieve the monitoring of theses 2 mains indicators of performance. Since paper has auto-fluorescent properties, by choosing precise fluorophores to image neurons and their projections, we have been able to produce clear fluorescent images of murine dopaminergic neurons within the paper and quantify the growth of their process. Detection of neurochemicals within the culture on paper will further prove the viability and integrity of the neurons in culture on paper. Developing a new device allowing the study of a neuronal network in precise conditions will help understand their mechanisms and find new neuropharmacological solution.Neurochemical analysis- By tuning the paper with different conductive inks, polymer poly(3,4-ethylenedioxythiophene): poly(styrene sulfonate) (PEDOT:PSS) and carbon nanotubes (CNT), paper electrodes can be used for dopamine and other neurotransmitters detection. Combining PEDOT:PSS and CNT have shown to ameliorate fouling resistance of the paper electrode, a phenomenon occurring when dopamine is oxidized, and improve electrochemical properties, lower capacitive current and higher anodic current. Optimizing spatial and temporal measures of dopamine secretion, in response to activation, can lead to a better understanding of neuronal dynamics.

P1.10: Hypocretin receptor 1 antagonism reduces motivation for cocaine and normalizes dopamine transmission in the nucleus accumbens core. (Samels)

Presenter name: Shanna Samels, Drexel University

Authors: Shanna Samels, Drexel University; Clark Phil, Drexel University; Jessica Shaw, Drexel University; Rodrigo Espana, Drexel University

Relapse to cocaine use after periods of abstinence remains one of the greatest obstacles for treating cocaine use disorder. Accumulating evidence suggests that alterations in the mesolimbic dopamine system following abstinence can lead to increases in cocaine seeking and propensity for relapse. Unfortunately, pharmacological approaches that target dopamine systems directly, are largely ineffective or intolerable and may pose abuse potential themselves. The hypocretin/orexin neuropeptide system has been shown to regulate both cocaine-associated behavior and dopamine transmission. Our previous studies demonstrate that treatment with the hypocretin receptor 1 antagonist, RTIOX-276, 30 minutes before assessment reduces motivation for cocaine and attenuates the effects of cocaine on dopamine transmission. Notably, the effects of RTIOX-276 on dopamine transmission are sustained for at least 24 hours, suggesting a long-lasting effect of hypocretin receptor 1 blockade. In the current study, we tested the hypothesis that a single treatment with RTIOX-276 reduces motivation and seeking for cocaine and that these effects are associated with normalization of dopamine transmission. Rats self-administered cocaine on an intermittent access schedule for 7 days and were then subjected to a 7-day abstinence period. On the first day of abstinence, rats were treated with RTIOX-276 and after 7 days of abstinence were assessed for either cocaine

consumption and demand using the within-session threshold schedule, or for dopamine transmission in the nucleus accumbens core using fast scan cyclic voltammetry. We found that a single dose of RTIOX-276 on the first day of abstinence reduced demand for cocaine 7 days later and normalized aspects of dopamine transmission. In a separate group of rats, we assessed the effects of RTIOX-276 on cue-induced cocaine seeking after intermittent access and abstinence. We found that RTIOX-276 reduced cue-induced seeking, which was also associated with normalization of dopamine transmission. Together these observations suggest that RTIOX-276 may be a beneficial treatment for cocaine use disorder by reducing motivation for cocaine through alterations in dopamine terminals in the nucleus accumbens.

P1.11: Bayesian optimization of rapid pulse waveforms for improved serotonin detection (Movassaghi)

Presenter name: Cameron Movassaghi, UCLA

Authors: Cameron Movassaghi, University of California, Los Angeles; Anne Andrews, University of California, Los Angeles

Pulse voltammetry is a popular electroanalytical technique for sensitive chemical detection that offers facile sensor fabrication and is less prone to electrode fouling. However, the design of pulse waveforms remains a difficult yet relatively unexplored optimization problem. Because pulse voltammograms have unique faradaic and non-faradic responses that are affected by the manner in which the pulses are layered, the design of the waveform is extremely important. A further complication is that the pulse arrangement can be analyte- and application- specific for optimal detection. Here, we present a novel optimization scheme that allows for custom-tuned analyte and interferent panels for automated waveform design, using serotonin as a case study. We find that our workflow outperforms random and human-guided waveform design and can be tuned a priori to enable implicit selectivity to interferents. We also provide interpretable analyses of the black box optimizer that allow for insight into the logic of machine-learning guided waveform design. This new method development paradigm has applications not only for neurochemical detection, but for any method requiring optimization of electroanalytical waveforms in complex matrices.

P1.12: Monitoring Serotonin dynamics from human-derived 3D organoids and spheroids by FSCV (Bohl)

Presenter name: Bettina Bohl, Imperial College London

Authors: Bettina Bohl, Imperial College London; Yuxian Lei, King's College London; Tomas Andriuskevicius, Imperial College London; Gavin Bewick, King's College London; Parastoo Hashemi, Imperial College London

Serotonin (5HT) is mainly produced by two organ systems within the body, the brain and the gut. In the brain, 5HT is produced by a specialized population of serotonergic neurons in the Raphe nucleus of the hindbrain. From there it regulates various processes in the whole central nervous system, including regulation of mood and anxiety. In the gut, 5HT is released by enterochromaffin cells of the mucosa and neurons of the enteric nervous system. While mucosal 5HT is linked to metabolic processes, e.g. secretion of pancreatic proteins, and gastric

acid, 5HT released through enteric neurons regulates gut motility. In both organs 5HT dysregulation is associated with pathophysiological conditions, most prominently in the brain with major depressive disorder (MDD) and in the gut with irritable bowel syndrome (IBS). Though the vast impact of 5HT on human health, little is known about the temporal dynamics of 5HT release and reuptake in the human context, partially due to a lag in appropriate model systems and/or suitable sensors. Here, we aimed to shed light on the 5HT dynamics in the human brain and gut using advanced cell culture models and fast-scan cyclic voltammetry (FSCV). Human stem cell-derived cell culture models provide the unique opportunity to investigate otherwise inaccessible tissues, like the brain, as well as target specific, sparse cell populations within a complex tissue by introducing reporter lines, e.g. for enterochromaffin cells of the gut. We generated 3D Raphe-type neuronal spheroids and 3D gut organoids with a fluorescent reporter for enterochromaffin cells. Using carbon-fiber microelectrodes and FSCV, we analyzed the release of 5HT from serotonergic neurons and enterochromaffin cells upon electrical and chemical stimulation, respectively. Further, we explored the effect of selective-serotonin reuptake inhibitors (SSRIs) on 5HT reuptake kinetics to better understand their pharmacological value.

P1.13: Allopregnanolone regulation of spontaneous dopamine transients and motivated behavior in freely-moving male and female rats (Mcfarland)

Presenter name: Minna Mcfarland, UNC at Chapel Hill

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Neurosteroids are compounds that are synthesized de novo in the brain and influence neuronal activity. Allopregnanolone (ALLO), a neurosteroid that is a potent, positive allosteric modulator of gamma-aminobutyric acid type A (GABA-A) receptors, has emerged as a drug with considerable potential in the treatment of mental and affective disorders, including substance use disorders and premenstrual dysphoric disorder. Moreover, ALLO is considered to have a better safety profile than other drugs that target GABA-A receptors, such as benzodiazepines. Previous work in our lab has shown that ALLO dose-dependently reduces electrically-evoked dopamine release in the nucleus accumbens (NAc) in anesthetized male and female rats, with female rats being less sensitive to ALLO than males. However, it is possible that the dopamine measurements were impacted by anesthesia effects on GABAergic neurotransmission in addition to ALLO. Thus, the present study tested the hypothesis that systemic administration of ALLO in awake rats will dose-dependently decrease the amplitude of spontaneous dopamine transients, while concurrently increasing their frequency. To test this hypothesis, we used in vivo fast scan cyclic voltammetry to measure spontaneous, phasic dopamine transients in the NAc of freely-moving male and female rats before and after systemic administration of 0.0 (vehicle), 7.5, and 15mg/kg ALLO. We found that high-dose ALLO appears to reduce the frequency of dopamine transients in female, but not male, rats. In males, the average percent change of transient frequency from the pre- to post-ALLO period in the vehicle group increased by 70.7%,

increased by 41.5% in the 7.5mg/kg group, and decreased by 32.4% in the 15mg/kg group. In females, the average percent change of dopamine transients in the pre- and post-ALLO periods increased by 161.8% in the vehicle group, decreased by 17.4% in the 7.5mg/kg, and decreased by 79.1% in the 15mg/kg ALLO group. Ongoing analyses will assess the effect of ALLO on spontaneous dopamine transient amplitude. Next, we trained animals to a self-administer sucrose on a fixed ratio schedule to assess whether ALLO would alter motivation for a natural reward. We found that ALLO (7.5mg/kg and 15mg/kg) did not alter total time to complete the session, inter-trial and inter-press interval, or rate of pressing in male or female rats, suggesting ALLO does not alter reward or motivated behaviors. Ongoing studies are currently being conducted to assess dopamine transient release concurrent with this behavior. As ALLO reduced evoked dopamine release in both anesthetized and awake (females, 15mg/kg) studies, we asked whether ALLO is aversive to rats. Using conditioned place preference, we found that 15mg/kg ALLO produced a robust place preference in both males and females, suggesting this dopamine reduction is not aversive. Overall, this study demonstrates that ALLO reduces dopamine transient frequency in females but not male rats, does not alter motivated behavior in males and females, and is not aversive to both sexes. The results from this study clarify the regulation of dopamine neurotransmission by ALLO and motivated behavior, which has clinical implications for the use of ALLO as an alternative therapeutic to benzodiazepines to treat various psychiatric disorders.

P1.14: Development of an Electrochemical Technique to Detect Ambient Brain Histamine (Batey)

Presenter name: Lauren Batey, Imperial College London

Authors: Lauren Batey, Imperial College London; Eva Borras Paredes, Imperial College London; Sergio Mena, Imperial College London; John Goodwin, Imperial College London; Marsilea A. Booth, RMIT University; Saimon M. Silva, Swineburne University of Technology; Wren Greene, Deakin University; Andriy Kozlov, Imperial College London; Parastoo Hashemi, Imperial College London

The brain is a complex structure that communicates via neurotransmission. Serotonin has long been believed to be implicated in the pathology of depression, and recently, histamine has been shown to be involved as well. Fast scan cyclic voltammetry (FSCV) using carbon fiber microelectrodes has been used to measure in vivo serotonin and histamine release and reuptake in real time, while fast scan controlled adsorption voltammetry (FSCAV) is able to measure the baseline, or ambient, levels of serotonin. Our previous work found that increased levels of histamine caused by peripherally injected lipopolysaccharide caused a decrease in the ambient levels of serotonin in the hippocampus. These lowered levels of serotonin coincided with depressive phenotypes in mice. We seek to advance this work by studying the ambient levels of histamine in the brain, during both a healthy state and a state of inflammation. To do this we must develop a new tool: FSCAV for the quantification of ambient histamine levels. I will discuss how a combination of optimizing the parameters of FSCAV and a novel polymer on the electrode facilitates the first in vivo FSCAV measurements of ambient histamine levels in the brain.

P1.15: Electroanalytical Investigation of Opioid Peptide Exocytosis Events from Single Adrenomedullary Chromaffin Cells (De Alwis)

Presenter name: Chathuri De Alwis, North Carolina State University

Authors: Chathuri De Alwis, North Carolina State University; Dylan Denison, North Carolina State University; Jenna Berger, North Carolina State University; Ruby Shah, North Carolina State University; Gregory Mccarty, North Carolina State University; Leslie Sombers, North Carolina State University; Chathuri De Alwis, North Carolina State University

Chromaffin cells have long served to inform on catecholamine exocytosis from large dense-core vesicles (LDCV), but the nature of endogenous opioid peptide release from these cells is yet to be uncovered. In a typical amperometry experiment, a carbon-fiber microelectrode is held at a constant potential of ~500 mV when detecting catecholamine release from a single cell. However, this potential is insufficient for the detection of opioid peptides, such as enkephalins (ENK), which are major peptidergic components in chromaffin cell LDCV. ENK is a 5 amino-acid neuropeptide that contains a tyrosine residue that oxidizes at ~1000 mV. Therefore, by comparing signals detected across 500 mV and 1000 mV, it is possible to reveal differences between catecholaminergic and peptidergic release profiles at the individual vesicle level. The data reveal that neuropeptides and catecholamines, which are released from a single population of LDCVs, have unique kinetic profiles. Upon repeated stimulations, the kinetics of peptide release slow, as evidenced by an increase in the event halfwidth, but does not vary for catecholamines. Degranulation of the chromogranin-dense core was found to play a crucial role in differentially modulating these neurochemical signals. In addition, solid phase extraction (SPE) was coupled with liquid chromatography-ion mobility-mass spectrometry (LC-IM-MS) to characterize the peptides secreted from these cells. MS data demonstrate that ENK comprises a substantial part of the adrenal peptidome and reveals differences between peptidergic content and release. Overall, this work provides unprecedented kinetic insight into unique signaling profiles of catecholamines and opioid neuropeptides while suggesting that these two neurochemical classes may modulate different populations of postsynaptic cells or targets to drive downstream effects.

P1.16: Enhancing durability and sensitivity of carbon fiber microelectrodes for prolonged in vivo dopamine monitoring (Kwon)

Presenter name: Haeun Kwon, Hanyang University

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Fast scan cyclic voltammetry (FSCV) coupled with 7-10 μm diameter carbon fiber microelectrodes (CFME) offers outstanding sensitivity for catecholamine families, enabling high temporal and spatial resolution. While this technique has proven valuable in studying rapid changes in neurotransmitter concentration and their correlation with animal behavior, the conventional 7 μm CFME faces challenges in longitudinal studies due to its fragility and short lifetime caused by repetitive voltage application. In addressing this limitation, our study

explores using 30 µm CFME to enhance electrode durability. Our 1 µM dopamine detection experiment reveals that untreated 30 µm electrodes exhibit superior sensitivity to dopamine compared to their 7 µm counterparts. However, the in vivo experiment measured in striatum indicates a substantial decline in performance, primarily attributed to severe brain cell damage incurred during the insertion of the 30 µm bare electrode. To mitigate this, we employ a homemade electrochemical etching system to transform the 30 µm carbon fiber into a conical shape with a 100-110 µm length. In vitro tests on the cone-shaped 30 µm CFMEs demonstrate comparable properties to bare 30 µm electrodes, with the in vivo experiment showing an impressive 3.0 times improvement in dopamine detection based on normalized current with surface area. Furthermore, the etched 30 µm electrodes exhibit a 4.7-fold increase in lifetime compared to the conventional 7 µm CFME under long-term repetitive voltage, showcasing enhanced electrode durability. The observed outcomes suggest that the 30 µm etched CFME, modified into a cone shape through etching, led to a reduction in brain cell damage during the insertion process. This interpretation is supported by the extent of immune response observed, which varies based on the type of electrode. Immunofluorescent staining conducted after a 12-day insertion period revealed that 30 µm bare CFME exhibited a higher presence of activated microglia compared to both 30 µm etched CFME and 7 µm CFME. Our study signifies a significant advancement in the field, offering improved stability and persistence for long-term dopamine detection in chronic disease models and large animals. The conical shape of the 30 µm electrodes mitigates brain cell damage and enhances dopamine detection capability, ensuring a prolonged electrode life for repetitive voltage applications compared to 30 µm bare electrodes. This research has the potential to revolutionize the landscape of neurochemical monitoring, paving the way for more robust and enduring electrode technologies in chronic disease studies and large animal experiments.

P1.17: Examining the effect of xylazine on dopamine dynamics (Dezha-Bolteada)

Presenter name: Crystal Dezha-Bolteada, UNC Chapel Hill

Authors: Crystal Dezha-Bolteada, UNC Chapel Hill; Madigan Bedard, UNC Chapel Hill; Jackson Murray, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill; Zoe Mcelligott, University of North Carolina; Crystal Dezha-Bolteada, UNC Chapel Hill

Xylazine is a veterinary sedative drug principally thought to target the alpha2-adrenergic receptor, that has been found in the unregulated drug supply with increasing frequency in cities across the U.S. While xylazine is a drug commonly used in veterinary practices or lab settings, it is not approved for human use. However, it has recently been found to be mixed with other substances like fentanyl in the street drug supply, which could make the combination even deadlier. Because this is a relatively recent trend, there is little to no research on how xylazine affects the brain systems that are involved in addiction. Other drugs like clonidine, which is an alpha2-adrenergic agonist, are known to reduce dopamine levels in the brain. Our preliminary data demonstrate that xylazine has “off target” agonist properties at the kappa opioid receptor, and is sensitive to naloxone precipitated withdrawal. Because kappa opioid receptors reduce dopamine release presynaptically, we hypothesize that xylazine will reduce dopamine levels in the brain, particularly in regions like the nucleus accumbens and the ventral tegmental area. To test this hypothesis we conducted ex vivo fast scan cyclic voltammetry (FSCV) in mouse brain slices and subjected the slices to a drug wash of xylazine and then took recordings of dopamine

levels to observe changes. Next, I will probe if kappa opioid receptor antagonists can prevent the effects of xylazine. To complement these data, we have investigated early immediate gene activation following xylazine in relevant brain circuits. Following withdrawal, we observe sex dependent increases in cFos activation across multiple nodes, where we see locus coeruleus activation by xylazine, fentanyl, and in combination in both sexes (p<0.0001), while differences are observed in the bed nucleus of the stria terminalis and central nucleus of the amygdala only in females (p<0.01). These changes in dopamine levels can let us know if xylazine is affecting the brain in similar ways to other sedatives. Ultimately the findings will expand our knowledge about xylazine so we can better understand how it affects structures and functions in the brain.

P1.18: Glutamate delta-1 receptors influence tonic and synaptic excitatory signaling in the bed nucleus of the stria terminalis (Conley)

Presenter name: Sara Conley, UNC at Chapel Hill

Authors: Sara Conley, University of North Carolina at Chapel Hill; Sarah Sizer, University of North Carolina at Chapel Hill; Madigan Bedard, UNC Chapel Hill; Kathleen Grant, Oregon Health & Science University; Zoe Mcelligott, University of North Carolina

While much work has been done on various aspects of the glutamatergic system and the perturbations induced by stress and drug exposure, one area that remains unexplored is the role of delta glutamate receptors (GluD1 and GluD2). Rather than function as canonical ligand-gated ionotropic receptors, the GluD family is known to act as effectors for Gq-coupled receptors to mediate slow excitatory transmission as well as induction of long-term depression (LTD) through the internalization of calcium-permeable AMPARs (CP-AMPARs). Recently, GluD1 has also been shown to be constitutively active in the dorsal raphe, providing a tonic excitatory current that is blocked by 1-naphthylacetyl spermine trihydrochloride (NASPM). In addition, several genome-wide association studies have linked GluD1 to alcohol use disorder, depression, and schizophrenia.The bed nucleus of the stria terminalis (BNST) is a forebrain nucleus reciprocally connected to many important stress and reward-related structures, positioning it as a critical nexus through which the negative affective symptoms common to many psychiatric- and substance use-disorders are mediated. Studies have shown alterations in BNST plasticity and function following stress and drug and alcohol exposure. We report that the anterolateral (al) BNST expresses a NASPM-sensitive, GluD1-mediated tonic excitatory conductance which influences cell excitability. Further, this is conserved across mice and rhesus macaques. Lastly, we show preliminary evidence suggesting that acute withdrawal from chronic intermittent ethanol vapor (CIE) decreases both CP-AMPAR synaptic current and attenuates the GluD1 tonic current, suggesting that alcohol exposure causes a loss of excitability in alBNST neurons through multiple converging mechanisms.

P1.19: Sleep disturbances are associated with mesolimbic dopamine dysfunction and cue-induced drug seeking during abstinence from cocaine (Cohen)

Presenter name: Sophie Cohen, Drexel University

Authors: Sophie Cohen, Drexel University; I. Pamela Alonso, Drexel University; Volodar Migovich, Drexel University; Rodrigo Espana, Drexel University

Individuals who struggle with cocaine use disorder display sleep dysregulation which is often marked by persistent reductions in rapid eye movement (REM) sleep and significant REM sleep fragmentation. We previously demonstrated that sleep/wake state influences dopamine transporter (DAT) function in the nucleus accumbens (NAc) such that dopamine uptake rate is faster during sleep yet slower during wakefulness and that sleep/wake state influences the effects of cocaine ex vivo. Further, we have shown that intermittent access (IntA) to cocaine, but not short access (ShA) to cocaine increases dopamine uptake rate and DAT sensitivity to cocaine. Given that the mesolimbic dopamine system is implicated in sleep/wake behavior and in the effects of cocaine, we examined to what extent REM sleep impairments observed during abstinence contribute to increased cue-induced cocaine seeking and dopamine transmission dysfunction following abstinence from IntA to cocaine. We monitored sleep/wake behavior prior to and following ShA or IntA cocaine self-administration throughout a 7- or 28-day abstinence period to examine sleep disruptions using EEG/EMG recordings and dopamine adaptations using ex vivo FSCV. To determine if restoring sleep following IntA to cocaine is sufficient to reduce cocaine seeking and aberrant dopamine adaptations, we implemented a behavioral sleep restoration procedure to reverse dysregulated sleep architecture. During sleep restoration periods, rats were kept awake in their active period (dark phase) to consolidate sleep during their inactive period (light phase), thereby improving REM sleep quality. Cue-induced cocaine seeking was measured on the first and last day of abstinence, and dopamine transmission was measured 24 hours after the final seeking test. Preliminary results suggest disrupted sleep architecture following IntA, but not ShA, self-administration that is accompanied by increases in drug seeking and aberrant dopamine transmission. Further, sleep restoration prevented these aberrant cocaine-associated effects. Together, these preliminary findings suggest that manipulation of sleep may serve as a novel therapeutic to prevent alterations in dopamine transmission that contribute to drug seeking.

P1.20: Sustained rescue of social deficits in a mouse model for ASD (Llorach)

Presenter name: Pierre Llorach, UNC at Chapel Hill

Authors: Pierre Llorach, University of North Carolina at Chapel Hill; Sarah Mcdaniel, University of North Carolina at Chapel Hill; Caroline Hertweck, University of North Carolina at Chapel Hill; Anish Kodali, University of North Carolina at Chapel Hill; Jessica Walsh, University of North Carolina at Chapel Hill

Dysfunction of the serotonin (5-HT) system has long been associated with sociability deficits in psychiatric and neurodevelopmental disorders, notably autism spectrum disorder (ASD). Therapeutics targeting the 5-HT system such as serotonin reuptake inhibitors (SSRIs) are often ineffective in treating social impairments. Accordingly, development of better therapeutics is critical. One of the most frequently mutated genes in ASD is Arid1b, a subunit of the neuron-specific nBAF chromatin remodeling complex (mammalian SWI/SNF complex). Mice with heterozygous Arid1b deletion selectively in 5-HT neurons (Sert-Cre+/-:Arid1b+/-) display impaired social behavior, providing a mouse model for ASD. Previous pre-clinical studies have shown that a 5-HT1b receptor (5-HT1bR) agonist or MDMA acutely enhances sociability in control mice and reverses social deficits present in multiple mouse models for ASD. Our preliminary data confirm that one dose of MDMA acutely enhances sociability in control mice, and rescues social deficits in Sert-Cre+/-:Arid1b+/- mice. Strikingly, we find a sustained reversal

of social deficits in Sert-Cre+/-:Arid1b+/-mice lasting a full week after a second dose of MDMA, an effect absent in control mice. Interestingly, a two-dose regimen of the 5-HT1bR agonist is not sufficient to induce the sustained rescue. Additionally, a 5-HT1bR antagonist does not block MDMA's effect. This begs the question, what is the molecular locus of this long-lasting prosocial effect? Several lines of evidence support the notion that 5-HT's actions in the nucleus accumbens (NAc) are critical for social behavior. Additionally, recent work showed that medial prefrontal cortex (mPFC) activity is necessary for MDMA's prosocial effects. We find that optogenetic activation of mPFC-to-NAc is sufficient to mimic the pharmacological phenotype. Additionally, we find sustained electrophysiological changes in the mPFC-to-NAc circuit in a region specific manner. By studying the neural mechanisms underlying sociability, we aim to shed light on a critically important element of the human experience, suggesting a novel approach to effective treatments for this devastating component of many neuropsychiatric disorders.

P1.21: A role for medial septum glutamate neurons in reward-seeking: strategy switching and nucleus accumbens dopamine (Kesner)

Presenter name: Andrew Kesner, NIAAA

Authors: Andrew Kesner, NIAAA; Nina Westcott, NIAAA; Stephanie Ramos-Maciel, NIAAA

The septum was first region discovered by Olds and colleagues to support electrical intracranial self-stimulation in the rat. Controversial studies in the 1970s showed humans will similarly press a button to earn intra-septal electrical stimulation. Despite this evidence, further interest in the septum, in particular the medial septum (MS), as a locus for reward related behaviors remained limited. We previously found that mice will lever press to earn optogenetic stimulation of the MS, and in particular MS glutamate neurons (MS-GLUn), and MS-GLUn in turn project to the VTA to influence dopamine (DA) release in the nucleus accumbens (NAc) (Kesner et al., 2021). Little else is known about the role of MS-GLU neurons during natural reward-seeking behaviors. To address this knowledge gap, we recorded MS-GLUn population activity using GCaMP7F and fiber photometry techniques while mice performed various operant and Pavlovian reward-seeking behaviors. We found that MS-GLUn indeed respond differentially to reward-related stimuli (e.g., active vs inactive lever presses, reward consumption, and reward predictive cues). We next modulated MS-GLUn activity using a chemogenetic approach (Gi and Gq DREADDs, or mCherry control), and found that enhancing MS-GLUn excitability increased the rate that mice incorporated new information to obtain goals, i.e., strategy switching. Since we previously found optogenetic stimulation of MS-GLUn can increase NAc-DA, we next hypothesized that the effect on strategy switching behavior from chemogenetic modulation of MS-GLUn may be driven by resultant changes in NAc-DA during these tasks. We recorded NAc-DA via fiber photometry of dLight1.3b during the same operant and Pavlovian strategy switching behaviors while MS-GLUn were chemogenetically manipulated. We observed differences in NAc-DA during these tasks that were dependent on chemogenetic modulation of MS-GLUn that appears to correspond to NAc-DA responses to new reward related cues/stimuli. These findings are an important step in understanding the role of this understudied population of neurons in an understudied brain region related to reward and motivational processes, and could lead to novel therapeutic interventions for treating psychiatric disorders related to maladaptation in motivated behaviors.

P1.22: Prelimbic cortex ensemble mechanisms in suppressing cue-evoked food seeking following environmental enrichment (Peters)

Presenter name: Kate Peters, University of Sussex

Authors: Kate Peters, University of Sussex; Romarua Agbude, University of Sussex; Olga Tsaponina, University of Sussex; Emily Woods, University of Sussex; Zuzana Pedan, University of Sussex; Eisuke Koya, University of Sussex

Exposure to food cues provokes food cravings and food seeking in humans and animals. To date, little is known about the brain mechanisms that suppress the reactivity of food cues and promote resilience. Brief episodes of cognitive and physical stimulation through games and exercise reduce food cravings in humans. In mice, we model this through environmental enrichment (EE) using spacious cages with toys, objects to interact with and tunnels, running wheels and varied nesting materials. Here, we examined how brief (24 hr) EE modulates cue-evoked sucrose seeking and prelimbic cortex (PL) neuronal ensemble activity in mice. We found EE attenuated cue-evoked sucrose seeking, sucrose consumption, and anxiety like behaviour. Electrophysiology experiments show EE selectively enhances PL interneuron excitability without affecting pyramidal cell excitability. In addition, we examined cue-evoked Fos in two key projections using retrograde tracing – prelimbic to nucleus acumbens (NAc) and prelimbic to paraventricular nucleus of the thalamus (PVT) neurons. We found decreased Fos in PL to PVT projection neurons and in local PL interneurons but not in PL to NAc projections. Finally, we performed fibre photometry recordings of GCaMP-expressing PL pyramidal cells using a CamKII-GCaMP during conditioning and food seeking. PL pyramidal cells show increased activity during food seeking when the cue is present across training. EE generally elevates PL pyramidal activity but abolishes the cue specificity of these neurons that we observe in standard housed controls. Our findings suggest that EE suppresses cue reactivity by modulating the baseline excitability properties of PL interneurons and the cue-evoked activity patterns of PL pyramidal cells and interneurons.

P1.23: Reciprocal regulation of dopamine and serotonin release in healthy and Parkinsonian mouse striatum (Qiao)

Presenter name: Qinbo Qiao, University of Oxford

Authors: Qinbo Qiao, University of Oxford; Stephanie Cragg, University of Oxford

Mammalian midbrain dopamine (DA)-releasing neurons project extensively to the striatum, where they play critical roles in motivation, action selection and reinforcement learning. Striatal DA axons are thought to be key sites where striatal neuromodulators can shape DA output. The striatum receives a sparse innervation from serotonin (5-HT) neurons, with a 30-fold lower varicosity density than seen for DA. Striatal 5-HT has been suggested to have complementary opposing outcomes to DA on some striatal functions but also to directly regulate DA release which might act to support (or counteract) their opposing functions. Evidence is also accumulating that the 5-HT system is dysregulated in the pathophysiology of Parkinson's disease (PD). Correspondingly, changes to 5-HT and/or DA release in PD might contribute further to circuit dysregulation through changes to reciprocal regulation. We explored a reciprocal regulation of DA and 5-HT release in mouse striatal slices, using fast-scan

voltammetry (FCV) at carbon-fibre microelectrodes (CFMs) to detect predominantly DA, and imaging of a new generation fluorescent G-protein-coupled receptor (GPCR)-activation-based 5-HT sensor, GRAB5-HT3.0 (Deng et al., 2023) during electrical stimulation. We first characterised GRAB5-HT3.0 responses to a range of stimulus trains: evoked signals had different kinetics to those detected with FCV that are predominantly due to DA. Evoked presumed 5-HT signals detected with GRAB5-HT3.0 sensor were reduced by 5-HT1B/1D receptor agonists, corroborating 5-HT as the monoamine detected. We explored control of striatal 5-HT release by D1 and D2 DA receptors, and the reciprocal control of DA release by the 5-HT4 receptor (5-HT4R). Evoked striatal 5-HT release was inhibited by D1 and D2 receptors agonists – CY208243 and Quinpirole. The release of DA was reduced by 5-HT4R agonist RS67333, which also promoted the activity-dependence of DA release. We found that the modulation of DA release by 5-HT4Rs was abolished in the presence of a competitive nicotinic acetylcholine receptor (nAChR) antagonist DHβE, indicating an indirect mechanism of regulation of DA involving a 5HT4-R-mediated change to ACh release from striatal cholinergic interneurons. Finally, we tested whether there was a change to the 5-HT4R regulation of DA release in a mouse model of early PD. We found a reduction in the 5-HT4R-mediated inhibition of DA in 18-month-old PD mice restricted to dorsal striatum, indicating a diminished interaction between 5-HT and DA localised to the region most affected in PD. In summary, these data indicate that striatal DA and 5-HT reciprocally inhibit their release, partly involving ACh, that might facilitate their opposing functions. Some loss to this interactive inhibition in a mouse model of PD suggests that dysregulation of DA-5-HT-ACh dynamics in disease might contribute to dysfunction, such as in movement control.

P1.24: Sex-dependent impact of high fat foods during adolescence on brain circuits of action control (Naneix)

Presenter name: Fabien Naneix, University of Aberdeen

Authors: Solenne Rougeux, University of Aberdeen; Robert West, University of Aberdeen; Fabien Naneix, University of Aberdeen

Obesity is now one of the most important health issues in modern society but its long-term impact on brain circuits and function is still not fully characterised. In recent years, the incidence of obesity has especially grown in children and teens. Early life periods appear to be crucial vulnerability windows for the deleterious impact of unbalanced diets on the regulation of food-seeking later in life as related brain circuits are still under development.Using rodent models, we previously showed that chronic consumption of obesogenic diet from adolescence promotes nonflexible habitual control of food-seeking in males. However, it remains unknown: i) if similar effects are observed in females, ii) if these effects are dependent of the levels of fatty acids in diet, and iii) how long-lasting changes in the control of food-seeking are related to alterations in the functioning of related brain circuits.Male and female adolescent C57Bl/6J mice were given 5 weeks (post-natal day 28-63) of free and continuous access to a combination of control chow pellets and high-fat (HFD; 45% kcal from fat) or very high-fat pellets (vHFD; 60% kcal from fat). Control animals were only given standard diet (SD, chow; 9% kcal from fat). At adulthood, all mice were switched to SD and trained to perform an action (lever press) to obtain palatable food rewards. Our initial results demonstrate a complex sex-dependent and fat content-dependent impact of adolescent diet on the ability to correctly control food-seeking

behaviour according to changes in the value of the food (outcome devaluation) or changes in action-outcome relationships (reversal learning and contingency degradation).Dopamine signalling plays a central role in the balance between goal-directed and habitual behavioural control. Using in vivo fibre photometry recordings and specific fluorescent sensors, we are currently investigating how adolescent diet-related behavioural changes are related to differential dopaminergic activity within corticostriatal circuits.

P1.25: Aversion Encoding in the Nucleus Accumbens (Cote)

Presenter name: Bridgitte Cote, Marquette University

Authors: Bridgitte Cote, Marquette University; Daniel Wheeler, Biomedical Sciences, Marquette University; Elaine Grafelman, Marquette University; Lisa Vlach, Marquette University; Matthew Hearing, Marquette University; Robert Wheeler, Marquette University; John Mantsch, Medical College of Wisconsin

More than 90% of the neurons in the nucleus accumbens (NAc) are GABAergic medium spiny neurons (MSNs) that express either D1 or D2 dopamine receptors (approximately 5% express both). These NAc neuronal subtypes have been thought to exert opposing influences on behavior, as D1 MSN activity has been associated with reward-related behavior, while D2 MSN activity has been associated with aversion. However, more recent work suggests that this view may be overly simplistic. Stimulated D2 MSN activity has been found to increase reward-related behaviors, and D1 and D2 MSNs have been found to respond similarly to rewarding and aversive stimuli. To investigate how innately aversive experiences are encoded by NAc MSNs, we measured calcium activity using fiber photometry. Adora2 Cre+ mice were injected with the calcium sensor, GCaMP6f in the NAc core and received a chronically implanted optic fiber. After allowing sufficient time for viral expression, mice were then exposed to an aversive 90dB white noise, and a mild 72dB white noise (7dB above ambient noise). White noise was presented in a pseudorandom schedule with ITIs ranging from 50 to 90 seconds while in vivo calcium activity was recorded. Results indicate that both D1 and D2 MSN calcium activity significantly increases at the onset of the aversive white noise (f(1,4)=54.9; p<0.01) while the less aversive noise elicits smaller response in both D1 and D2 MSNs MSNs (f(1,4)=54.9; p<0.05). This suggests that D1 and D2 MSNs may not differentially encode aversive stimuli. Ongoing experiments are comparing these findings with in vivo D1 and D2 MSN activity during a negative reinforcement task. This will test if this encoding pattern changes when the stimulus is controllable and will capture how D1 and D2 MSNs encode aversion-induced motivation. Additionally, previous work from our lab has found that aversive stimuli (including 90db white noise) cause significant reductions in NAc dopamine. To understand the relationship between this dopamine signal and the MSN response to aversive stimuli, ongoing experiments are using patch-clamp slice electrophysiology to measure how high and low concentrations of dopamine influence intrinsic excitability of NAc D1 and D2 MSNs. Together, this work will further our understanding of the mechanism by which aversive experiences are encoded in the nucleus accumbens.

P1.26: Aversive white noise reduces nucleus accumbens core dopamine signaling and promotes both cocaine intake and escape behavior. (Grafelman)

Presenter name: Elaine Grafelman, Marquette University

Authors: Bridgitte Cote, Marquette University; Daniel Wheeler, Biomedical Sciences, Marquette University; Elaine Grafelman, Marquette University; Lisa Vlach, Marquette University; Matthew Hearing, Marquette University; Robert Wheeler, Marquette University; John Mantsch, Medical College of Wisconsin

Adverse life events are a primary cause of relapse to drug use in humans, and acute stressors can trigger drug seeking in rodents. In previous studies we observed that aversive stimuli can enhance ongoing cocaine self-administration. We have recently seen that intense (90dB), but not mild (55dB), white noise increases lever pressing for cocaine. This intense white noise also reduces dopamine in the nucleus accumbens (NAc) core and we hypothesize that the aversion-induced reduction in dopamine promotes cocaine seeking. To investigate whether that aversion signal is broadly associated with other aversively motivated behaviors, we tested the relationship between aversive white noise, negatively reinforced behavior, and NAc dopamine. Female and male Sprague Dawley rats (n= 13 females, n = 13 males) underwent surgery in which they received an infusion of AAV5-hSyn-dLight into the NAc core and implantation of an optic fiber to the same location. Following recovery, rats were trained in daily sessions to press levers for the delivery of a sucrose pellet. After stable responding was achieved on both levers at variable interval schedule (ranging from 30 to 150 seconds), rats were transitioned to negative reinforcement. In this phase, a white noise (either intense (90dB) or mild (55dB)) was intermittently presented and responses on one lever (active lever, counterbalanced) terminated the white noise while responses on the inactive lever had no consequence. If no response was made, the white noise timed out after 60s. The next trial started 6s after a timeout, or 11s after an active lever response. Intense white noise (but not mild white noise) significantly reduced dopamine signaling on the first day of negative reinforcement training ($F(1,20) = 9.21$, $p=0.007$). Consistent with this, animals exposed to intense white noise maintained responding on the lever that terminated the noise while rats exposed to mild white noise did not ($F(8,192) = 5.00$, p <0.0001). Interestingly, following several days of training both mild and intense WN reduced dopamine significantly at onset ($F(1,18)=39.09$, p<0.00001), and ongoing studies are investigating this transition. Additionally, ongoing experiments are testing the influence of chronic stress on dopamine signaling. Specifically, we are testing the effect of chronic variable stress on dopamine release in response to intense white noise and measuring its effect on negatively reinforced behavior.

P1.27: Blue-light evoked photoactivation in mApple-based sensors (Taniguchi)

Presenter name: James Taniguchi, Fresco Institute for Parkinson's and Movement Disorders

Authors: James Taniguchi, Neuroscience Institute and Fresco Institute for Parkinson's and Movement Disorders; Riccardo Melani, Neuroscience Institute and Fresco Institute for Parkinson's and Movement Disorders; Lynne Chantranupong, Howard Hughes Medical Institute, Harvard Medical School; Michelle Wen, Howard Hughes Medical Institute, Harvard Medical School; Ali Mohebi, Department of Neurology, University of California, San Francisco; Joshua Berke, Department of Neurology, University of California, San Francisco; Bernardo Sabatini, Howard Hughes Medical Institute, Harvard Medical School; Nicolas Tritsch, New York University; James Taniguchi, Neuroscience Institute and Fresco Institute for Parkinson's and Movement Disorders

Presynaptic modulation is a ubiquitous mechanism through which neural circuits control the amount of chemical transmitter that axons release per incoming action potential. Striatum-projecting midbrain dopamine (DA) neurons are no exception; many transmitters present in the striatum directly act on DA axons to facilitate or depress vesicular release of DA. Acetylcholine is widely believed to modulate the release of dopamine in the striatum of mammals. Experiments in brain slices clearly show that synchronous activation of striatal cholinergic interneurons is sufficient to drive dopamine release via axo-axonal stimulation of nicotinic acetylcholine receptors. The evidence for this mechanism in vivo has been less forthcoming. A recent paper provided some of the most compelling evidence to date that optogenetic activation of channelrhodopsin-expressing striatal cholinergic interneurons drives DA release in the NAc of awake behaving rats, as measured with the red-shifted DA sensor RdLight1. However, one concern with these experiments is that mApple-based fluorescent sensors – including RdLight1 and the GRAB-rDA3 series – may exhibit photoactivation (also known as 'photoswitching' or 'photoconversion'), a process whereby mApple's red fluorescence changes in the presence of blue light. This phenomenon is one of the main downsides of the R-GECO family of red Ca2+ indicators, which also use mApple and grow brighter independently of Ca2+ for hundreds of milliseconds following brief flashes of blue light, limiting their use with optogenetics.Here, we show that blue light alone alters the fluorescent properties of RdLight1 in a manner that may be misconstrued as phasic dopamine release, and that this artefactual photoactivation can account for the effects attributed to cholinergic interneurons. Our findings indicate that measurements of dopamine using the red-shifted fluorescent sensor RdLight1 should be interpreted with caution when combined with optogenetics. In light of this and other publications that did not observe large acetylcholine-evoked dopamine transients in vivo, the conditions under which such release occurs in behaving animals remain unknown.

P1.28: Kynurenic acid formation and synthesis inhibition is Zeitgeber-dependent in rats: Implications for sleep and behavior (Wright)

Presenter name: Courtney Wright, USC School of Medicine

Authors: Courtney Wright, University of South Carolina School of Medicine; Silas Buck, Maryland Psychiatric Research Center; Annalisa Baratta, Maryland Psychiatric Research Center; Carly Fabian, Maryland Psychiatric Research Center; Ana Pocivavsek, University of South Carolina School of Medicine

Neurochemical imbalances, such as alterations in tryptophan metabolism, are commonly observed in individuals with neuropsychiatric disorders. Elevated kynurenic acid (KYNA), a tryptophan metabolite of the kynurenine pathway and endogenous antagonist of glutamatergic and cholinergic receptors is hypothesized to contribute to cognitive dysfunction and sleep disturbances in these patients. Inhibition of the main KYNA synthesizing enzyme in the brain, kynurenine aminotransferase II (KAT II), is being pursued as a therapeutic strategy in translational studies. We presently employed in vivo microdialysis and fluorometric high-performance liquid chromatography to monitor extracellular levels of the small molecule KYNA in the rodent brain. We explore the hypothesis that de novo KYNA formation and synthesis inhibition is Zeitgeber-dependent, such that circadian time impacts kynurenine pathway metabolism to KYNA. At the beginning of the light or dark phase, Zeitgeber time (ZT) 0 or ZT 12 respectively, male or female Wistar rats (N=6-10) received an injection of vehicle, kynurenine

("kynurenine challenge," to stimulate de novo KYNA production; 100 mg/kg, i.p.), PF-04859989 (KAT-II inhibitor, to reduce KYNA levels; 30 mg/kg, s.c.), or PF-04859989 and kynurenine. We collected 30-min fractions of microdialysate in the dorsal hippocampus for 6 hours to evaluate de novo KYNA production and KAT II inhibition. In separate animals, ex vivo enzyme activity assays for KAT isoforms I and II were conducted in brain tissue collected at ZT3 or ZT15 (N=3-4). In separate animals, we compared sleep behavior with EEG/EMG polysomnography obtained in kynurenine-challenged animals at ZT0 (Rentschler et al. J Sleep Res 2023) or ZT12 presently (N=9-10). In a similar treatment paradigm, we investigated hippocampal-dependent memory consolidation with the novel objection recognition task (N=12-15). Our microdialysis results demonstrate that PF-04859989 reduces basal KYNA levels in male rats (P<0.01). Kynurenine challenge at ZT12, compared to ZT0, resulted in increased de novo KYNA synthesis (P<0.0001) and greater inhibition by PF-94859989 in males. In vitro, KAT II enzyme activity and the ability of PF-04859989 to inhibit KYNA formation were significantly greater in ZT15 tissue compared to ZT3 tissue (P<0.05). ZT0 kynurenine challenge reduced rapid eye movement (REM) sleep during the immediate light phase, whereas ZT12 kynurenine challenge resulted in a delayed increase in REM sleep duration (P<0.05). Both ZT0 and ZT12 kynurenine challenge impaired learning in males and females. Taken together, our results demonstrate the importance of considering Zeitgeber-dependent dynamics of kynurenine pathway metabolism and KYNA formation. Monitoring molecules in the brain across the light and dark phases provides a rich understanding of the circadian dimensions of neuromodulators and may prove essential to the future development of efficacious therapeutic strategies targeting KYNA formation.

P1.29: High-resolution neurochemical characterization of the paraventricular nucleus of the thalamus (Ramos)

Presenter name: Brianna Ramos, University of Michigan

Authors: Brianna Ramos, University of Michigan; Shelly Flagel, University of Michigan; Robert Kennedy, University of Michigan; Brianna Ramos, University of Michigan

Psychiatric disorders are associated with neurochemical imbalances in the brain that promote maladaptive behaviors. Environmental stimuli can guide individuals toward valuable resources, but they can also attain excessive control over behavior, leading to maladaptive outcomes. In this study, we use an animal model to better understand individual differences in response to environmental stimuli and the underlying neurochemical correlates. Specifically, we use a Pavlovian conditioned approach (PavCA) paradigm to identify sign-trackers (ST), intermediate responders (IN), and goal-trackers (GT). Both STs and GTs ascribe predictive value to reward cues, but only sign-trackers attribute additional incentive value. The paraventricular nucleus of the thalamus (PVT) has emerged as a key neural node that acts to encode the value of reward cues and, in turn, guide motivated behavior. It is well-placed to do so as it integrates input pertaining to the internal state of the body as well as cognitive and emotional states. Of particular interest is the role of the posterior PVT (pPVT) within the subcortical hypothalamic-thalamic-striatal motivation circuit. Our research aims to characterize the neurotransmitters, their metabolites, and energy molecule concentrations that contribute to the role of the pPVT in encoding the value of reward cues and eliciting behavior. While the PVT is known to be neurochemically heterogeneous, it has not yet been well characterized, likely because of its neuroanatomical location and difficulty in detecting neurochemical signals with high resolution

due to various physicochemical properties and low concentration. Here we employ a liquid chromatography mass spectrometry (LC-MS) method that utilizes benzoyl chloride (BzCl) labeling to account for these difficulties. Specifically, we combined a neurochemical profiling approach with microdialysis sampling in outbred rats to characterize a panel of 24 neurochemicals in the PVT. We successfully detected dopamine, norepinephrine, serotonin, histamine, glutamate, GABA, acetylcholine, choline, adenosine, DOPAC, 3MT, HVA, normetanephrine, taurine, serine, aspartate, glycine, glutamine, epinephrine, cysteine, VMA, DOPEG, MOPEG, and glucose. Glutamine was found at high concentrations (31000 nM) at baseline conditions, more than a 10-fold increase relative to the other dialysate concentrations. The next tier of abundant analytes included glucose (2700 nM), taurine (1700 nM), choline (1500 nM), and GABA (160 nM), respectively. In addition, we have begun to examine the neurochemical profile of the pPVT following Pavlovian conditioning approach (PavCA) training and subsequent characterization of rats as STs, INs, or GTs. Preliminary data suggest that, relative to other behavioral phenotypes, STs exhibit an increase in the concentration of multiple neurochemicals from pre-PavCA to post-PavCA. This pattern was most apparent with glutamine, aspartate, and VMA concentrations. Ongoing studies will determine if sex affects the neurochemical signature of the pPVT. This research may identify neurochemical markers that predispose an animal to form of associative learning that promotes maladaptive behavior and is may therefore be critical to the development of novel therapeutic targets for the treatment of psychiatric disorders.

P1.30: The iron-chelating agent deferoxamine grafted on polyethylene glycol could ameliorate the clearance of iron from the extracellular space using intracerebral microdialysis in rats (de Deurwaerdere)

Presenter name: Philippe de Deurwaerdere, Université de Bordeaux

Authors: Philippe De Deurwaerdere, Université de Bordeaux; Nicolas Boissart, University of Bordeaux; Zora Pelloquin-Mvogo, University of Bordeaux; Noel Pinaud, University of Bordeaux; Yannick Crémillieux, University of Bordeaux; Juan Salazar-Ariza, University of Lyon; Olivier Tillement, University of Lyon; François Lux, University of Lyon; Philippe De Deurwaerdere, Université de Bordeaux

Intracerebral microdialysis can be used for extracting metals from the extracellular space including iron. The efficacy could be ameliorated by adding in the artificial cerebrospinal fluid (aCSF) chelating agents that do not cross the dialysis probe membrane. Such a strategy could be interesting in neurodegenerative diseases in which the accumulation of iron in some tissues is suspected to alter neuronal integrity (Parkinson's disease) and to avoid the direct contact of the chelating agents with the tissue. The efficacy of such an approach deserves characterization. Here we report the effect of deferoxamine grafted on molecules of polyethylene glycol (PEG-DFO; PM: 43kDa) on the extracellular levels of iron using two implanted microdialysis probes in the striatum of isoflurane (1.5% in mask)-anesthetized rats. We also measured dopamine and its metabolite DOPAC in one out the two sets of experiments. The probes (PAES, 4 mm length, 20 kDa size pore, 500µm diameter, Carnegie medicin, Phymep, France) were implanted at 1.5/2mm distance in the left striatum of male sprague dawley rats. We did preliminary in vitro studies to evaluate the stability of iron in solution with bathophenanthroline for the detection, to determine the concentration of PEG-DFO (10µM) that

cleared more iron in solution than normal aCSF, and to fix the flow rate for PEG-DFO probe for an optimal clearance. Thus, microdialysates were collected in tubes containing 30µL of bathophenanthroline (10 µM) for subsequent analysis of iron dialysate content using a spectrophotometer (535nm). The collection of samples (20 min) started two hours after probe implantation. The flow rate was fixed at 1 or 3 µL/min depending on the cannula, and the experiment. The detection of DOPAC and dopamine in microdialysate was achieved using an HPLC system coupled to coulometric detection [5014 analytical cell (175 mV) connected to a Coulochem 2 detector].In the first in vivo experiment, we found that basal levels of iron were closed to the limit of detection (approximately 0.6 µM). The application of PEG-DFO (10µM, 3 µl/min) in one probe did not alter the concentration of iron in the distal probe. It did not alter the concentration of dopamine or DOPAC. The application of 6-hydroxydopamine (6-OHDA, 200 µM, 20 minutes) in one probe enhanced the extracellular levels of iron, but it did not alter the iron concentration at the distal probe. PEG-DFO masked the excess of iron in the probe induced by 6-OHDA. In a second experiment, to address a distal influence of PEG-DFO at distal site, PEG-DFO (10 µM) or the normal aCSF was applied from the beginning of the perfusion in one cannula. 6-OHDA was applied during 20 minutes in the second cannula 4 hours after the beginning of the perfusion. 6-OHDA enhanced extracellular levels of iron, but this effect had a similar magnitude whether or not PEG-DFO was present in the other probe.The results suggest that the application of chelating agents confined in the microdialysis probe is efficacious locally, but it should be improved to have larger chelating influence at distal sites.

P1.31: Multi-organ-on-chip device for modeling opioid reinforcement and withdrawal, and the negative affective component of pain: a therapeutic screening tool (Shu)

Presenter name: Zhan Shu, University of California, Los Angeles

Authors: Zhan Shu, University of California, Los Angeles; Danial Khorsandi, Terasaki Institute for Biomedical Innovation; Ze Zhong Wang, University of Texas, Austin; Dmitriy Ruckodanov, University of California, Los Angeles; Vadim Jucaud, Terasaki Institute for Biomedical Innovation; Satoru Kawakita, Terasaki Institute for Biomedical Innovation; Alexander Laperle, Cedars Sinai Los Angeles; Nureddin Ashammakhi, Michigan State University; Harold Monbouquette, University of California, Los Angeles; Mehmet Dokmeci, Terasaki Institute for Biomedical Innovation; Ali Khademhosseini, Terasaki Institute for Biomedical Innovation; Clive Svendsen, Cedars Sinai Los Angeles; Stephanie Seidlits, University of Texas, Austin; Nigel Maidment, University of California, Los Angeles

We are developing a human iPSC-derived model of a key component of addictive circuitry – the dopaminergic and GABAergic neurons of the midbrain, recognized as responsible for mediating the reinforcing properties of many classes of abused drugs. Our goal is to produce a multi-tissue microphysiological system (MPS) that incorporates neurons, microglia, blood-brain-barrier (BBB), and liver metabolism on-a-chip components, which will be used to investigate the plasticity of dopamine neurons in response to repeated opioid exposure and withdrawal, using dopamine release as the primary output measure. Plasticity at the molecular level is being assessed by single-nuclei RNA sequencing (snRNAseq) and metabolomics analyses, through which we hope to identify novel targets for therapeutic interventions in treating opioid use disorder and the affective component of pain. These output measures will then be used to screen drug libraries for favorable activity.We have optimized conditions for 3-dimensional

cultures of dopaminergic and GABAergic neurons in hyaluronic acid-based hydrogels and have conducted extensive pharmacological characterization of the cultures using dopamine release as a primary output measure. We observed reproducible, concentration-dependent, morphine-induced dopamine release from multiple independent iPSC lines that were blocked by naloxone. Fentanyl was approximately 100-fold more potent than morphine, consistent with in vivo data, and was blocked by the mu selective antagonist, CTOP. Furthermore, exposure to GABA receptor antagonists significantly attenuated morphine's stimulatory effect on dopamine release, as previously demonstrated in vivo. Also similar to in vivo animal data, exposure to the kappa opioid receptor agonist, U50,488, significantly attenuated dopamine efflux, an effect that was blocked by the kappa antagonist, JDTic. These neuronal cultures are being transferred to newly designed PMMA chips that incorporate a BBB channel having immortalized human brain microvascular endothelial cells (hBMECs) aimed to create a blood-brain-barrier (BBB) which was evaluated with integrated carbon-based screen-printed electrodes for transendothelial electrical resistance (TEER) measurements and a silicon wafer-based electrode for online detection of dopamine. Parallel experiments are perfecting a liver module for incorporation into this MPS to model opioid metabolism.

P1.32: A Neurochemical Study of Dual inhibitors of DAT and sigma receptors as Potential Therapeutic Options for Psychostimulant Use Disorder (Tanda)

Presenter name: Gianluigi Tanda, National Institute on Drug Abuse

Authors: Melinda Hersey, NIDA; Maddalena Mereu, NIDA-IRP; Claire Jones, NIDA-IRP; Mattingly Bartole, NIDA; Andy Chen, NIDA-IRP; Takato Hiranita, NIDA-IRP; Jiangjing Cao, NIDA-IRP; Lauren Chun, NIDA-IRP; Jessica Lopez, NIDA-IRP; Jonathan Katz, NIDA-IRP; Amy Hauck Newman, NIDA; Gianluigi Tanda, National Institute on Drug Abuse, Intramural Research Program

Psychostimulant use disorders (PSUD) are prevalent, however, no FDA-approved medications have been made available for treatment. Potential therapeutic options currently being explored include atypical dopamine (DA) uptake inhibitors (DUIs), which produce behavioral and neurochemical effects inconsistent with those elicited by typical abused psychostimulants. Additionally, targeting of the sigma receptor is also of interest as a therapeutic option alone or in combination with DAT inhibition. Previous studies have shown that dual inhibitors of the dopamine transporter (DAT) and sigma receptors significantly reduce the behavioral/reinforcing effects of cocaine, which have been associated with stimulation of extracellular dopamine (DA) levels resulting from DAT inhibition. In this work, we employ microdialysis and fast scan cyclic voltammetry (FSCV) procedures to probe nucleus accumbens shell (NAS) DA dynamics in naïve male Sprague Dawley rats. Using microdialysis, we found that administration of rimcazole (3, 10 mg/kg; i.p.) or its structural analog SH 3-24 (1, 3 mg/kg; i.p.), which are compounds that inhibit DAT and sigma receptors, did not alter extracellular DA efflux in the NAS when administered alone. Additionally, we show that pretreatments with rimcazole (3, 10 mg/kg; i.p.) or its structural analog SH 3-24 (1, 3 mg/kg; i.p.) significantly reduced extracellular NAS DA levels stimulated by increasing doses of cocaine (0.1, 0.3, 1.0 mg/kg; i.v.). Using the same experimental conditions, in FSCV tests we found that rimcazole pretreatments attenuated cocaine-induced stimulation of evoked NAS DA release but produced no additional effect on DA clearance rate, even though rimcazole appeared to attenuate the rate of DA clearance when injected alone. Under the same conditions, JJC8-091, a modafinil analog and dual inhibitor of

DAT and sigma receptors, similarly attenuated cocaine-induced stimulation of evoked NAS DA release but produced no additional effect on DA clearance rate. In contrast, JJC8-088 (3, 10 mg/kg, i.p.), another modafinil analog with cocaine-like actions at DAT and less significant actions at sigma receptors, produced divergent effects from JJC8-091. Administration of JJC8-088 enhanced cocaine-induced increases in evoked DA release suggesting an additive effect of these compounds. Our results provide the neurochemical groundwork for understanding the actions of dual inhibitors of DAT and sigma receptors on DA dynamics that likely mediate the neurochemical and behavioral effects of psychostimulants like cocaine.This work was supported by the National Institute on Drug Abuse, Intramural Research Program, NIH

P1.33: Developing multiplexed tools to study neuromodulation of neurotransmission (Surendran)

Presenter name: Dayana Surendran, University of Virginia

Authors: Dayana Surendran, University of Virginia

Fast-scan cyclic voltammetry (FSCV) has been used for the detection of electroactive molecules for 40 years. Genetically-encoded biosensors have recently been developed to allow for sensitive, cell-type-specific, and non-invasive glutamate measurements with high temporal and spatial resolution. While both are rapid and sensitive, a limitation for FSCV is that it only detects electroactive analytes and a limitation for genetically-encoded sensors is that they photobleach with continuous light exposure. These techniques can be used in combination to detect multiple neurotransmitters because one uses fluorescence for detection and the other electrochemistry, and hence will not interfere with each other. A1 subtype adenosine receptors are highly expressed both pre-and postsynaptically in the hippocampal CA1 region. Adenosine, released upon a variety of physiological and pathological stimuli from neuronal and non-neuronal sources, has transient suppressive effects on synaptic transmission in hippocampal CA1. Activation of presynaptic A1 receptors is known to modulate the release of the major excitatory neurotransmitter glutamate. However, the range and effects of adenosine modulation of glutamate are not well understood. The diversity of glutamatergic neurons and their interplay with neurons that release the neuromodulator adenosine necessitates tools for specific, multiplexed detection of these neurochemicals. We developed multiplex tools by employing FSCV combined with the AAV-driven fluorescent biosensor, iGluSnFR to detect adenosine release and measure its downstream effects on glutamate neurotransmission in the hippocampal CA1 region. Using this multiplexed approach, we simultaneously measured stimulated adenosine release (using FSCV) and its effect on glutamate neurotransmission (using fluorescence imaging) in CA1 region. These real-time, multiplexed measurements will provide a much better understanding of the spatial and temporal scales of adenosine neuromodulation of glutamate release in the hippocampal CA1, which is useful in the future to develop adenosine-based therapies for disease. While our focus is on biological applications of adenosine here, the techniques will also enable future measurements of real-time changes in other neurochemicals and their interactions.

P1.34: Multiplexing Fast-Scan Cyclic Voltammetry and Fluorescence Microscopy for Simultaneous Measurement of Dopamine and Glutamate (Donarski)

Presenter name: Eric Donarski, University of Virginia

Authors: Eric Donarski, University of Virginia; Kailash Shrestha, University of Virginia; Dayana Surendran, University of Virginia; B. Jill Venton, University of Virginia

Fast-scan cyclic voltammetry (FSCV) has routinely been used for accurate subsecond detection of neurotransmitters for decades. Utilizing carbon-fiber microelectrodes, FSCV leverages the electrochemical properties of various neurotransmitters, such as dopamine, to interrogate the dynamics of neurotransmission. However, while many neurotransmitters in the brain are electroactive, others, such as glutamate, are non-electroactive and require additional supplementary techniques other than FSCV to identify and understand their transmission. Due to their electroactivity, many neurotransmitters are often studied separately, which can obscure biological relevance of the interplay between multiple neurotransmitters. We leveraged carbon-fiber microelectrode FSCV supplemented by fluorescence microscopy to simultaneously study neurotransmitters, regardless of their electroactivity. To do so, we used FSCV to detect dopamine and a viral vector-mediated fluorescent iGluSnFR sensor to detect glutamate in mouse brain slices. By combining these techniques, we examined the common interplay between dopamine and glutamate in various brain regions. In this work, the goal is to compare two brain regions, separately, that are expected to contain interactions between dopamine and glutamate: the Prefrontal Cortex (PFC) and the Nucleus Accumbens (NAc). Within the PFC, dopamine modulates glutamate transmission dependent on D1 and D2 receptor activity, while glutamate helps to regulate striatal dopamine function. Disruption of these interactions and systems in the PFC often leads to neuropsychiatric disease, making the region a prime target for both electrochemical and clinical inquiry. In the NAc, dopamine-glutamate neurons projected from the ventral tegmental area (VTA) have been shown to affect substance use disorder neurochemistry. As these neurotransmitters are coreleased by the same neuron in the NAc, it is necessary to study them simultaneously to understand how relative concentrations of both can change the overall signal transduction pathway. By observing the transmission of these two neurotransmitters simultaneously, we will further clarify the interactions between dopamine and glutamate, and their effects, in two separate brain regions.

P1.35: Characterization and validation of an ex vivo ischemia model for neurochemical analysis (Caldwell)

Presenter name: Kaejaren Caldwell, University of Cincinnati

Authors: Kaejaren Caldwell, University of Cincinnati; Ashley Ross, University of Cincinnati; Kaejaren Caldwell, University of Cincinnati

Disruption of the ability to exchange glucose and oxygen in the brain leads to one of the most common forms of brain damage, ischemic stroke. The increases in inflammation, glutamatergic excitotoxicity, and oxidative stress compound to the detrimental outcomes of this disease. Work on understanding various important biomarkers during stroke is useful for the development of therapeutics; however, there remains a lack of understanding of the neuropathological impact of ischemic events. Guanosine is a signaling molecule and is an emerging biomarker of interest for neuroprotection. Many have shown the ameliorating effects of exogenous guanosine treatment in ischemic stroke yet the molecular mechanism of how endogenous guanosine aids in recovery of stroke events has not been elucidated. Previously, our lab has shown an increase in rapid, endogenous guanosine signaling during severe ischemic events using fast-scan cyclic

voltammetry (FSCV). Despite this finding, an understanding of how this subsecond signaling changes as a function of ischemic severity is not well understood, therefore it is advantageous to interrogate the brain's immediate neuroprotective response during varying severities of injury. In this work, we use an optical oxygen sensor to characterize a tunable ex vivo oxygen-glucose deprivation (OGD) model (normoxia, mild, and severe). In doing so, we have cultivated a standard for ex vivo slice ischemic studies that better correlate to the varying ischemic severity models that exist for in vivo analysis. Immunohistochemistry and 2,3,5-Triphenyltetrazolium chloride (TTC), and ELISA assays were used to help further correlate the changes observed as a function of OGD model to the changes measured in guanosine signaling as a function of ischemic severity. Overall, this work provides the first method to specifically control ischemic severity ex vivo with correlations to how these changes influence neural signaling.

P1.36: Adolescent ethanol exposure blunts brain Arc and HMGB1 responsivity to an acute ethanol challenge in adulthood (Macht)

Presenter name: Victoria Macht, University of North Carolina at Chapel Hill

Authors: Victoria Macht, University of North Carolina at Chapel Hill; Sarrah Ankeny, University of North Carolina at Chapel Hill; Aldrin Mosqueda, University of North Carolina at Chapel Hill; Aaron Wei, University of North Carolina at Chapel Hill; Ryan Vetreno, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill; Fulton Crews, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill

Adolescent drinking increases risk for adult alcohol use disorder (AUD) in humans. Similarly, models of human adolescent binge drinking (adolescent intermittent ethanol; AIE) increase adult ethanol drinking in rodents, increase behavioral tolerance to acute ethanol, and shift ethanol-induced neuronal activation to reward mediation networks. To expand upon these findings, we used a rat model of human adolescent binge drinking (AIE; 5 g/kg/day ethanol or water, i.g., 2-day on/2-day off cycle from postnatal day (PND) 25-54) to assess whether a history of developmental ethanol disrupted neuronal, immune, and endocrine responsivity to an acute ethanol challenge (4 g/kg) in adulthood (PND 105-110) in males. We assessed hippocampal and amygdalar neuronal plasticity (Arc), innate immune (HMGB1) immunoreactivity (IR), and plasma endocrine (corticosterone), immune (HMGB1, CRP) and liver enzymatic (ALT) reactivity. Results indicate an adult ethanol challenge acutely decreases Arc+IR in the dentate gyrus (DG) of the hippocampus and the basolateral amygdala (BLA) while increasing Arc+IR in the central amygdala (CeA). AIE-exposed rats fail to show acute ethanol-induced suppression of Arc+IR in the DG and BLA while still exhibiting a robust Arc+IR induction in the CeA, suggesting brain regional differences to AIE-induced neuronal plasticity. Adult ethanol challenge reduces HMGB1+IR in the DG in controls, indicative of acute ethanol-induced neuronal extracellular release. AIE-treated animals fail to exhibit acute ethanol-induced changes in the DG while exhibiting an increase in HMGB1+IR in the BLA. Similarly, plasma HMGB1 is increased after acute ethanol in control but not AIE rats. Adult ethanol challenge also increased corticosterone levels in both control and AIE animals, indicating that the endocrine response to acute ethanol does not exhibit the same physiological adaptations to AIE as HMGB1. Neither AIE nor the acute ethanol challenge impacted CRP or ALT levels. Collectively, these results indicate that AIE persistently disrupts brain hippocampal and amygdalar neuronal plasticity and both brain and plasma HMGB1-related innate immune response to later ethanol exposure, providing further

evidence that adolescent ethanol exposure produces protracted consequences on the physiological response to ethanol in adulthood.Supported by the Neurobiology of Adolescent Binge Drinking in Adulthood (U24 AA020024, U01 AA020023), the Bowles Center for Alcohol Studies (P60 AA011605), K01 AA025713 (RPV), and K99 AA030089 (VAM).

P1.37: Dopamine D3 and D4 receptor modulation of electrically stimulated dopamine release in rat brain slices: implications for treatment of schizophrenia (Tenibiaje)

Presenter name: Mokolapo Tenibiaje, University of Leicester

Authors: Mokolapo Tenibiaje, University of Leicester; Andrew Young, University of Leicester; Mokolapo Tenibiaje, University of Leicester

Schizophrenia - a debilitating mental illness affecting ~ 0.5% of the population, yet many patients do not respond well to antipsychotic treatment, emphasising a need for novel drug therapies. Dysregulation of dopamine signalling in the mesolimbic pathway, projecting to nucleus accumbens (NAc) may contribute to the expression of schizophrenia symptoms, particularly positive symptoms, and drugs targeting dopamine D2 receptors are the mainstay of current antipsychotic treatment. However, recent interest has been directed towards other receptors belonging to the D2-like dopamine receptor subfamily, including D3 (D3R) and D4 (D4R) receptors. Behavioural studies have suggested that D4R agonists (Sood et al, 2011; J. Psychopharm 25, 792) and D3R antagonists (unpublished) may exhibit an antipsychotic-like profile in rat models of schizophrenia. D3R have a relatively high density in limbic brain areas, including NAc, and may play a pivotal role in the modulation of dopamine release, and changes in both D3R and D4R density in schizophrenia have been reported. Therefore, drugs affecting D3R and D4R signalling may provide potential novel antipsychotic drugs. However, little is known about the function of D3R or D4R in modulating dopamine release.These experiments aim to study the action of the D3R agonist PD128907 and the D4R agonist A412997, on electrically-stimulated dopamine release in NAc in brain slices taken from normal rats and from rats pretreated with phencyclidine (PCP), modelling schizophrenia. Brain slices (400μM) containing NAc were taken from male and female juvenile Wistar rats and fast-scan cyclic voltammetry was used to measure stimulated dopamine release from NAc at two different stimulation frequencies, mimicking tonic and phasic firing. Stimulations were given at 3 min intervals: after four baseline stimulations, drugs were applied for the next four stimulations (12 min), then a further six stimulations (18 min) were carried out after the end of drug application. PD128907 (D3R agonist) caused a dose-dependent decrease in stimulated dopamine release with high frequency stimulation but less so with low frequency stimulation. Both effects were reduced in slices taken from PCP pretreated animals. A412997 (D4R agonist) also showed a dose-dependent decrease in stimulated dopamine release, which was similar with high and low frequency stimulation. In slices from PCP pretreated animals, A412997 did not decrease stimulated dopamine release evoked by high frequency stimulation, but with low frequency stimulation the attenuation caused by A412997 remained intact. These findings demonstrate that both D3R and D4R modulate dopamine release NAc at both high and low frequencies, but that the different stimulus frequencies may be differentially susceptible to PCP pretreatment, providing insights how these receptors may be potential targets for future treatments in schizophrenia.

P1.38: Diurnal variation in effects of cholinergic interneuron modulation on Pavlovian conditioned responding and outcome devaluation (Gallinger)

Presenter name: Isabel Gallinger, Wake Forest School of Medicine

Authors: Isabel Gallinger, Wake Forest School of Medicine; Amelia G. Bonsib, Wake Forest School of Medicine; Lacey Sexton, Wake Forest School of Medicine; Taylor Stowe, University of Pittsburgh; Mark Ferris, Wake Forest School of Medicine; Isabel Gallinger, Wake Forest School of Medicine

Substance use disorder (SUD) is characterized by chronic maladaptive patterns of drug consumption. Only a fraction of people who use drugs develop SUD, and understanding determinants of this variation is critical to improving treatment outcomes. Heterogeneity in endogenous neurobehavioral responses to natural rewards may contribute to SUD vulnerability. Propensity to attribute incentive salience to reward-related cues is thought to correspond with individual differences in substance use behavior. The Pavlovian Conditioned Approach (PCA) task is a preclinical model for incentive salience. When presented with Pavlovian-conditioned cues, animals tend to develop a preference for the location of the cue or reward referred to respectively as sign-tracking (ST) or goal-tracking (GT). ST is associated with increased drug self-administration and represents a potential biomarker for vulnerability to SUD. ST is thought to be resistant to reward devaluation, a model of habit-like behavior. We have previously shown higher rates of ST in the dark portion of the 24-hour light cycle in male rats, consistent with evidence for diurnal rhythms in substance use behavior. Some clinical data suggests habit formation may vary diurnally as well, but to our knowledge outcome devaluation has not been measured across times of day. Induction of ST behavior is known to be dependent on phasic release of dopamine (DA) in the nucleus accumbens core (NAc), a regional neurotransmitter system canonically implicated in SUD. Our group has demonstrated greater relative phasic DA firing during the dark cycle, an effect which is mediated by a diurnal reduction in tonic cholinergic interneuron (CIN) inputs. In the present study, we further explore these findings by manipulating NAc CIN firing in vivo during PCA and outcome devaluation. To test the hypothesis that altering accumbal acetylcholine (ACh) release would alter induction of ST in a diurnal manner, we used designer receptors exclusively activated by designer drugs (DREADDs) to manipulate CIN firing across times of day. We surgically infused Cre-dependent DREADDs into the NAc of male Long-Evans rats expressing Cre-recombinase under control of the cholinergic acetyltransferase promoter. Rats were assigned to run during their light cycle and receive an inhibitory DREADD (N=4) or run during their dark cycle and receive an excitatory DREADD (N=4). Rats were habituated to an operant chamber and trained to receive sugar pellets from a food hopper. PCA sessions consisted of 30 trials in which an 8-second visual cue was followed by a sugar pellet delivery repeating at random intervals for a total of 30 minutes. Subjects received an injection of clozapine n-oxide dihydrochloride (CNO) 20 minutes before completing their first 10 sessions to activate the DREADDs followed by 7 sessions with only a saline injection. Finally, they completed two additional sessions after exposure to an outcome devaluation protocol. We used a within-subjects design to compare responding with and without DREADD activation. Consistent with prior work, ST was decreased in the dark cycle when the DREADDs were activated, but not altered in the light cycle. Furthermore, ST behavior was insensitive to

outcome devaluation. Additional studies will use pharmacology to examine the relevant receptor subtypes.

P2.1: Cholinergic Signaling in the Ventral and Dorsal Subiculum Differentially Participates in Avoidance Response to Threats (Wang)

Presenter name: Shaohua Wang, NIEHS

Authors: Shaohua Wang, NIEHS; Korey Stevanovic, NIEHS; Jesse Cushman, NIEHS; Jerrel Yakel, NIEHS

The subiculum plays a pivotal role in facilitating information exchange between the hippocampus and brain regions implicated in threat learning. Various studies suggest that both the dorsal subiculum (dSub) and the ventral subiculum (vSub) contribute to threat learning. However, it remains unclear whether these two subregions process threat information independently, or in coordination. Both dSub and vSub circuits are highly modulated by acetylcholine (ACh) signaling. Our previous investigations have revealed that vSub ACh levels increase in response to threatening stimulus such as foot-shock and conditioned sound cue. Yet, the precise contribution of ACh in processing this threat information and how ACh signaling in the dSub and vSub coordinates it remain elusive. To better understand the function of ACh signaling in threat response, we designed a series of behavioral tasks and systematically compared the dynamic change of the d/vSub ACh signaling at various stages of threat processing. In this study, we expressed a genetically encoded ACh sensor (GRABACh3.0) in both dSub and vSub and used a fiber photometry system to monitor the dynamic change of simultaneous, real-time ACh levels at multi-sites. Our findings revealed that both dSub and vSub ACh levels immediately increased in response to different types of threatening stimuli, including foot-shock, conditioned sound presentation, hand-looming, and tail-picking, coinciding with the mice exhibiting escaping behavior. However, after the initial encounter with threats, the ACh level increased only in the vSub (but not dSub) as mice habituated to the stimuli and began exploration. Secondly, in the light-dark shuttle box test and elevated plus maze tests, we observed a significant rise in the ACh level in the vSub (but not dSub) when the mice transitioned from the more dangerous to safer environments. Notably, all ACh level changes occurred prior to the initiation of movements. Taken together, these results support our hypothesis that ACh signaling in the dSub and vSub differentially participate in threat processing, while ACh in vSub underlies the induction of avoidance response to threat. Further analysis employing pharmacology and chemogenetic manipulation on ACh signaling will be performed to confirm this hypothesis. By elucidating the intricate function of ACh signaling in the vSub and dSub, our study provides valuable insights into the neural mechanisms that govern behavioral responses to threatening stimuli.

P2.2: Real-time co-detection of dopamine and glutamate in rat striatum. (Sombers)

Presenter name: Leslie Sombers, North Carolina State University

Authors: Leslie Sombers, North Carolina State University; Gregory Mccarty, North Carolina State University; Laney Kimble, North Carolina State University; Jenna Berger, North Carolina State University; Alexandra Forderhase, North Carolina State University; John Meitzen, North Carolina State University

The nucleus accumbens (NAC) integrates multiple inputs, including glutamatergic (GLUT) and dopaminergic (DA) afferents, which ultimately affect the predominant neurons of the NAC, the medium spiny neurons. The release and removal of GLUT from the synapse occur within milliseconds, and fast-scan cyclic voltammetry (FSCV) provides analyte-specific, quantitative information on this timescale. But unlike electroactive species, GLUT does not readily undergo redox reactions within the electrolysis window of water, rendering direct electrochemical detection impossible in the physiological environment. We have developed a biosensing strategy whereby carbon-fiber microelectrodes are modified with a chitosan matrix containing GLUT oxidase (GlutOx) to allow for the indirect voltammetric detection of GLUT with naturally engineered selectivity. GlutOx catalytically generates hydrogen peroxide (H2O2), a readily detectable electroactive reporter molecule, selectively in the presence of GLUT. Importantly, FSCV can report on the simultaneous presence of multiple species at a single micron-scale recording site, enabling precise correlation of distinct neurochemical signals with one another, as well as with respect to time. DA is readily electroactive, and it generates a voltammogram unique from that of H2O2. As such, DA and GLUT transients can be simultaneously detected at the same striatal recording sites. We are using this strategy to comparatively characterize GLUT and DA transients in rat striatal tissue slices and in intact rats, and we have discovered unique neurochemical kinetics inherent to each of these preparations. The data demonstrate key differences in the extracellular lifetime of these signaling molecules and suggest that each can effectively modulate the other. This is important because it is entirely possible – even likely – that the co-activation of different neurochemical receptors enables integration of separate intracellular signaling pathways to generate a range of postsynaptic responses. Overall, this unique approach to biosensing transforms the molecular monitoring landscape from a ‘one molecule at a time’ perspective to one focused on how separate molecules work cooperatively to shape circuit output and – ultimately - to drive striatal function.

P2.3: Real-time, voltammetric co-detection of serotonin and glucose at carbon-fiber microbiosensors (Turner)

Presenter name: Kalynn Turner, North Carolina State University

Authors: Kalynn Turner, North Carolina State University; Jenna Berger, North Carolina State University; Gracie Mcnair, North Carolina State University; Gregory Mccarty, North Carolina State University; Leslie Sombers, North Carolina State University

Glucose is the major fuel source for the brain. As such, glucose availability and metabolism are inextricably linked to neurotransmission and, thus, to circuit function. Serotonin (5-HT) plays an important role in the human gut-brain axis and is widely studied because it has been implicated in diverse neuropsychiatric disorders including mood disorders (anxiety and depression), schizophrenia, substance abuse, and Parkinson’s disease. Historically, 5-HT has been recorded in brain tissue using electrochemical techniques and glucose has been studied using microdialysis sampling techniques, but these methods have very different spatial and temporal resolution, making direct correlation of these signals difficult. Due to the challenges in the co-detection of these analytes, it remains unknown how local glucose availability modulates 5-HT transmission at discrete release sites. Glucose is not inherently electroactive; however, glucose oxidase generates electroactive hydrogen peroxide selectively in the presence of glucose. In this work, carbon-fiber microelectrodes were modified with a chitosan matrix containing glucose

oxidase to allow for the simultaneous voltammetric detection of 5-HT and glucose in rat brain tissue. This approach is completely different from the typical biosensing paradigm which utilizes amperometry coupled with biosensors that are specifically designed to exclusively evaluate only one analyte at a time. By contrast, our voltammetric strategy exploits the unique chemical signature from each analyte to allow for identification of both electroactive components in a complex mixture. The voltammetric biosensors have been characterized for stability, selectivity, and sensitivity to glucose, 5-HT, and a range of potential interferents including dopamine, norepinephrine, and 5-hydroxyindole acetic acid. The biosensors have been utilized for quantitative co-detection of evoked 5-HT and glucose transients in both male and female rat brain tissue. The ability to simultaneously record rapid fluctuations of glucose and 5-HT at a single location promises to inform improved therapeutic strategies for a wide range of disorders in which both 5-HT transmission and glucose metabolism is altered providing a new perspective that links neurotransmission with metabolic activity.

P2.4: Electrochemical recording of Acetylcholine Release by Exocytosis (Cans)

Presenter name: Ann-Sofie Cans, Chalmers University of Technology

Authors: Ann-Sofie Cans, Chalmers University of Technology; Yuanmo Wang, Chalmers University of Technology; Ajay Pradhan, The Sahlgrenska Academy at the University of Gothenburg; Pankaj Gupta, Chalmers Univeristy of Technology; Jörg Hanrieder, The Sahlgrenska Academy at the University of Gothenburg; Henrik Zetterberg, The Sahlgrenska Academy at the University of Gothenburg; Ann-Sofie Cans, Chalmers University of Technology

Acetylcholine, a critical neurotransmitter involved in many brain functions, lacks methodology to directly and timely monitor quantal exocytosis release of acetylcholine at the presynapse. In this work, we introduce an ultrafast enzyme-based amperometric biosensor for detection of acetylcholine. The sensor is fabricated based on a previous invention in our lab to significantly improve temporal resolution of biosensors. Here carbon fiber microelectrodes are modified using noble metal particles and the enzymes acetylcholine esterase and choline oxidase are co-immobilized and creating an ultrathin layer at the sensor surface. The enzymatic sequential catalytic reaction of acetylcholine producing hydrogen peroxide serve as the electroactive detection for acetylcholine. By placing the biosensor in close contact to neurites of differentiated human cholinergic cells, sub-millisecond recording of exocytotic fusion pore regulation of acetylcholine was recorded. The resulting data revealed a diversity of exocytosis current spike kinetics and dynamics, indicating complex mechanisms for the transient exocytosis fusion regulating acetylcholine release. Categorizing the spikes in terms of lifetime and shape showed a significant difference in relative quantal release. For absolute quantification, electroanalysis of synthetic vesicles pre-loaded with different concentrations of acetylcholine served to create a calibration curve and was used to determine the absolute number of acetylcholine molecules involved in quantal release. This study presents a significant advancement in acetylcholine detection and paves the way for further advancements in research regarding fusion pore regulation of acetylcholine and mechanistic studies of exocytosis involving acetylcholine at single cell level.

P2.5: Selectively pyrolyzed parylene-N with Nanoscribe two-photon laser for MEMS development (Zhao)

Presenter name: He Zhao, University of Virginia

Authors: He Zhao, University of Virginia; He Zhao, University of Virginia

Polyimide is a commercially accessible polymer to achieve laser-induced-graphene (LIG). However, polyimide can only be used on silicon wafer by spin coating, which limits the cylinder electrode fabrication. We synthesized laser-induced-graphite (LIGT) in a unique way with parylene (poly(p-xylene)), a benzene-rich polymer. Parylene is chemically inertness, flexible, optically transparency, and a good insulator for electronics. Parylene-N (PN), one of parylene derivatives, was deposited on Nb wires via chemical vapor deposition (CVD), and this method ensured uniform and thin coating. PN was treated on a microhotplate (HP-PN) by increasing temperature to 340 °C and lasting for 10 min. Microhotplate treatment in air enhanced light adsorption of PN from 0 to 69.5 %, shrunk PN coating thickness to the nanoscale, and added oxygen atoms to the PN structure. We used a Nanoscribe two-photon laser, which is usually utilized for 3D printing, to laser induce graphene (LIGT-PN) by controlling the laser. Raman spectroscopy confirmed graphitic features and rich defect sites. Modified electrodes with pyrolyzed parylene were used for electrochemical detection of low-potential analytes with fast-scan cyclic voltammetry (FSCV). LIGT-PN had higher detection sensitivities than carbon fiber microelectrodes (CFMEs) and anti-fouling properties because of high surface roughness and rich defect sites. Increased oxygen functional groups also promoted adsorption of analytes to the electrode surface via electrostatic force. Because of LIGT-PN biocompatibility and long-term stability, modified electrodes are suitable for in vivo testing to real-time monitor released neurochemicals. With the feasibility of laser-induced-graphite from PN for electrochemical detection, we are proceeding to design a four-channel microelectromechanical systems (MEMS) device for dopamine detection. Laser-induced graphene from parylene offers the unique opportunity to customize the shape of an electrode by forming electrodes precisely from an insulating material.

P2.6: Estrous-cycle dependent regulation of central catecholamine signaling in response to food and drug reward (Bhimani)

Presenter name: Rohan Bhimani, University at Buffalo

Authors: Rohan Bhimani, University at Buffalo; Jinwoo Park, University at Buffalo

The central catecholamine systems, dopamine and norepinephrine, play a critical role in encoding the valence of environmental stimuli to promote engagement in behaviors that potentiate an organism's survival. Furthermore, both systems in limbic brain areas such as the ventral striatum and bed nucleus of the stria terminals (BNST) are major targets of stimulant drugs. Canonically, limbic dopamine transmission is generally considered to increase in response to appetitive or rewarding stimuli whereas norepinephrine signaling is enhanced in the presence of aversive or noxious stimuli. However, it remains to be elucidated whether sex differences, especially at different stages of the estrous cycle, distinctly modulate such responses. In this study we (i) identified estrous cycle-dependent changes in catecholamine regulation via their transporters and autoreceptors in the ventral striatum and BNST of anesthetized rats and (ii) determined how natural (sucrose) and stimulant (methamphetamine) reward impacts norepinephrine and dopamine transmission in the ventral striatum and BNST,

respectively, in male and female rats using in vivo fast-scan cyclic voltammetry. Our results demonstrate opposing limbic catecholamine regulation throughout different stages of the estrous cycle wherein dopamine responses are heightened during the estrus stages of the cycle. In contrast, norepinephrine levels are impacted the greatest during the diestrus/proestrus stages, suggesting a critical role of plasma estrogens. These findings offer new insights into the role of estrous cycle stage on how the brain encodes environmental stimuli and provides a new framework for therapeutically targeting the central catecholamine systems in health and disease ranging from drug use disorders to obesity.

P2.7: Exploring the neurochemical actions of dopamine transporter inhibitors, psychostimulants, and drugs of abuse on dopamine dynamics in the nucleus accumbens of mice (Hersey)

Presenter name: Melinda Hersey, NIDA

Authors: Melinda Hersey, NIDA; Mattingly Bartole, NIDA; Amy Hauck Newman, NIDA; Gianluigi Tanda, National Institute on Drug Abuse, Intramural Research Program

Psychostimulants like cocaine and methylphenidate are readily abused/misused and affected individuals can develop psychostimulant use disorder (PSUD) which currently has no FDA-approved pharmacological treatments. It is essential to understand the dopaminergic neurochemistry underlying PSUD in order to develop efficacious therapeutic options. Many psychostimulants produce robust increases in nucleus accumbens dopamine following an acute dose via actions at the dopamine transporter (DAT) which are largely thought to account for their misuse and dependence. There is evidence that some of these agents may exhibit sex-dependent differences in their effects on DAT and dopamine (DA) dynamics and this is especially well documented for cocaine. In this work, we employ fast scan cyclic voltammetry (FSCV) to probe DA dynamics in the nucleus accumbens shell (NAS) of C57BL/6 male and female mice after administration of DAT targeting drugs like WIN 35,428, psychostimulants like cocaine and methylphenidate, and expanding to other drugs of abuse/misuse. We previously found that cocaine (3 and 10 mg/kg; i.p.) slowed DA clearance in both male and female mice but produced more robust increases in evoked NAS DA in female mice. WIN 35,428 (0.1 and 0.5 mg/kg; i.p.) produced a robust increase in NAS DA as well as slowed DA clearance in both male and female mice. While not statistically significant, we did observe some subtle sex differences in the dopaminergic response to the high dose of WIN 35,428. Methylphenidate (3 and 10 mg/kg; i.p.) also produced a robust increase in NAS DA as well as slowed DA clearance in both male and female mice. No statistically significant sex differences in DA dynamics were observed following methylphenidate administration. Current studies are aimed at expanding to include other psychostimulants and drugs of abuse and probing DA dynamics. In conclusion, we have begun to tease out how sex differences may alter the neurochemical effects of DAT targeting and highlight how this may help focus research progressing toward effective treatment options for PSUD.This work was supported by the National Institute on Drug Abuse, Intramural Research Program, NIH.

P2.8: Chasing the enkephalins: simultaneous co-detection of met-enkephalin and dopamine release in rat striatum (Berger)

Presenter name: Jenna Berger, North Carolina State University

Authors: Jenna Berger, North Carolina State University; Jovica Todorov, North Carolina State University; Kalynn Turner, North Carolina State University; Gregory Mccarty, North Carolina State University; Leslie Sombers, North Carolina State University

Many studies have investigated striatal dopamine (DA) transmission with respect to reward-related learning and goal-directed behavior. Atypical opioid peptide activity in the striatum has also been heavily linked to several aspects of drug abuse and addiction; however, these neuropeptides have proven difficult to detect in situ because they exist at low concentrations and are readily broken down by endogenous protease activity. As such, many fundamental questions regarding opioid peptide transmission remain unanswered, including the timescale of release, the physiological conditions required to elicit neuropeptide release, and how these kinetics directly compare with those of more classical small-molecule neurotransmitters, like DA. In this work, fast-scan cyclic voltammetry (FSCV) is coupled with carbon-fiber microelectrodes for simultaneous monitoring of DA and M-ENK at single recording sites in male and female rat striatal brain slices. An inhibitory DREADD was used to minimize mesolimbic DA release enabling co-detection of both analytes. The unique voltammetric waveform was specifically designed to incorporate three distinct scan rates in each sweep. This strategy minimizes sensitivity to DA, maximizes sensitivity to M-ENK, and mitigates electrode fouling. A three-second hold at 1.3V oxidizes and cleans the carbon surface with each scan, because the electro-oxidation of M-ENK can readily foul the sensor. DA and M-ENK release were locally evoked and recorded at single recording sites in striatal slices to characterize how extracellular kinetics depend on the stimulation frequency and duration. The data demonstrate that a longer stimulation amplifies release of both the neurotransmitter (DA) and the neuropeptide (M-ENK). Interestingly, M-ENK dynamics display a unique biphasic release profile with a significant latency to peak extracellular concentrations that occurs ~30 seconds after stimulation, suggesting a diffusion distance of ~75 µm which is roughly 3x larger than that of DA (~22 µm). Finally, a series of independent experiments combined solid phase extraction with liquid-chromatography mass spectrometry (LC-MS) to validate the electrochemical signal of M-ENK by using the m/z ratio and ion mobility. In summary, these findings provide direct evidence to support widely held assumptions regarding neuropeptide release, and they demonstrate how the simultaneous release of these neurotransmitters can affect distinct cell populations in striatum to ultimately shape circuit output.

P2.9: Complex contingency learning induces enzymatic and transporter plasticity at accumbal dopamine terminals (Nolan)

Presenter name: Suzanne Nolan, Vanderbilt University

Authors: Suzanne Nolan, Vanderbilt University; Michelle Kwon, Vanderbilt University; Stephen Harper, Vanderbilt University; Kirsty Erickson, Vanderbilt University; Zahra Farahbkhsh, Vanderbilt University; Hannah Chen, Vanderbilt University; Soren Emerson, Vanderbilt University; Alex Brown, Vanderbilt University; Hannah Branthwaite, Vanderbilt University; Lillian Brady, University of Alabama at Birmingham; Jordan Yorgason, Brigham Young University; Erin Calipari, Vanderbilt University; Cody Siciliano, Vanderbilt University

Dopamine release dynamics in the nucleus accumbens (NAc) core are integral to reinforcement learning. A large body of work has detailed the stimuli that evoke NAc dopamine release in vivo,

and how release patterns evolve over the course of reinforcement learning. At the level of the cell body, learning-induced changes in excitability and upstream inputs to dopamine neurons have been explored in detail, and it is thought that dopamine efferents in the NAc act as a global broadcast system, passively transmitting somatic signals to the downstream striatum. However, despite a multitude of regulatory factors present in the canonical striatal microcircuit, surprisingly little is known as to whether presynaptic dopamine release sites undergo enduring intrinsic plasticity during learning. Here, we characterized learning-dependent presynaptic plasticity following complex contingency learning using ex vivo fast-scan cyclic voltammetry (FSCV) in the NAc core of mice. We first show that the magnitude of phasic evoked release is increased following the acquisition of a complex contingency learning task, concomitant with augmented maximal rate of dopamine reuptake. Additionally, we verified these effects with calorically-yoked contingency-free controls and saw no effect on either dopamine reuptake or phasic-evoked release in these animals. Further, release magnitude mapped onto individual differences in learning rate, showing a positive association between the rate of learning and the magnitude of phasic evoked release, suggesting that intrinsic plasticity at dopamine terminals is requisite for optimal learning. Using liquid chromatography-mass spectrometry and western blot, we show that these functional changes in release and reuptake are concurrent with reduced tyrosine hydroxylase efficiency and increased dopamine transporter expression. Finally, we test whether knockdown of transporter expression in vivo can fundamentally alter learning. Overall, the results suggest that diametric transporter and enzymatic plasticity allow for optimal learning by shifting dopamine release mechanisms towards reliance on reuptake and repackaging and away from vesicular replenishment via de novo synthesis. This work demonstrates that intrinsic plasticity at axonal release sites is fundamentally linked to reward learning and indicates that the presynaptic microenvironment is a dynamic, experience-dependent modulator of dopamine signaling rather than a passive relay as previously suggested.

P2.10: Studying dopamine dynamics in drosophila melanogaster brain using FSCV and GRABDA sensor (Deshpande)

Presenter name: Aaditya Deshpande, University of Virginia

Authors: Aaditya Deshpande, University of Virginia; B. Jill Venton, University of Virginia

Fast Scan Cyclic Voltammetry (FSCV) is an electrochemical technique capable of detecting electroactive neurotransmitters with sub second temporal resolution. It provides quantitative data of the change in neurotransmitter levels. However, resolution between similarly structed neurotransmitters is not high enough and the spatial resolution is measured only at the site of electrode implantation. Previous studies describing its usage in drosophila melanogaster brain (ex-vivo and in-vivo) have been published by our group. GPCR-based-activation (GRAB) sensors are genetically encoded sensors that express fluorescence in specific brain regions of drosophila upon binding with its specific neurotransmitter. Its advantage is its specificity and measurements in real time at cellular levels at a sub second level while its limitation includes data output with non-calibrated concentrations. We are working to combine both techniques to help us measure dopamine in multiple regions with high spatial and temporal resolution, ultimately leading to neural circuit mapping.Dopamine is a well-known neurotransmitter involved in the reward system. The Mushroom Body region in the fly brain is a major region

containing the projections of the dopaminergic neurons. Particularly, γ lobe is the major region comprising of these neurons. In the present work the dopamine GRAB sensor was expressed in drosophila in the mushroom body region by making stable fly-lines. The dopaminergic regions were stimulated chemically using acetylcholine and the change in fluorescence and current were measured using a wide-field fluorescence microscope and FSCV. For the FSCV measurement a carbon fiber microelectrode with 7μm diameter was inserted in the γ lobe of the MB region. This study compares dopamine dynamics in different compartments of the γ lobe during stimulations.

P2.11: The Synaptic Vesicular Protein Synaptogyrin-3 Alters Dopamine Transmission (Curry)

Presenter name: Alyson Curry, Wake Forest University School of Medicine

Authors: Alyson Curry, Wake Forest University School of Medicine; Gracie Peck, Wake Forest University School of Medicine; Sara Jones, Wake Forest University School of Medicine

Synaptogyrin-3 (Syngr3) is a relatively understudied synaptic vesicle protein that has a role in dopamine exocytosis and reuptake through binding to microtubule-associated tau and the dopamine transporter (DAT). As cocaine works predominantly through alterations in DAT function, post-mortem samples from the ventral tegmental area of individuals with cocaine use disorder (CUD) were analyzed and found to have substantially reduced levels of Syngr3 mRNA. Similar changes were also found in a rat model of CUD, where Syngr3 levels negatively correlated with cocaine-taking behaviors. Viral overexpression of syngr3 in the dopaminergic neurons of the ventral tegmental area led to a reduction in cocaine self-administration but had no effect on acquisition of self-administration behavior. Characterization of dopamine transmission kinetics through fast-scan cyclic voltammetry revealed variation in dopamine release and reuptake both at basal conditions and upon exposure to cocaine. Thus, Syngr3 had a protective role against cocaine-induced dysfunction which could potentially prevent escalation of cocaine intake. In order to examine the effect of reduced Syngr3 on dopamine dynamics, we again used fast-scan cyclic voltammetry to probe changes in release and reuptake.

P2.12: In vivo real-time measurements of neurotransmitters in mice brain by electrochemical methods (Xu)

Presenter name: Peibo Xu, University of Illinois at Urbana-Champaign

Authors: Peibo Xu, University of Illinois at Urbana-Champaign; Hazirah Muhamad Rapidi, University of Illinois; Sidrah Ahmed, University of Illinois; Daniel Abel, University of Illinois; Roy Zhou, University of Illinois; Kiersten Garcia, University of Illinois; Ran Chen, University of Illinois; Nicholas Iwai, University of Illinois; Mei Shen, University of Illinois at Urbana-Champaign

Neurotransmitters play important roles in plenty of brain functions such as learning, memory, and emotion. Abnormal levels of some neurotransmitters are also reported to be related to neurological disorders. For example, decreased levels of acetylcholine (ACh) is found in Alzheimer's' diseases patients. Recent years, there have been studies to develop devices trying to monitor neurotransmitters levels, among all the devices, electrochemical probes can provide

high temporal resolution with limited invasive damage, making it an ideal choice for in vivo measurements of neurotransmitters. In vivo electrochemical detection of neurotransmitters can be achieved by brain implantation surgery to insert carbon fiber microelectrodes. Then fast scan cyclic voltammetry (FSCV) is applied for the detection of neurotransmitters at high temporal resolution. Our lab also successfully achieved in vivo detection of dopamine (DA) in the mice brain.However, previous electrochemical studies mainly focused on DA as it is redox-active. Modification of electrode surface is necessary for other non-redox-active neurotransmitters, such as ACh, which cannot be detected directly on electrode surface. Our lab has been working on developing electrochemical probes targeting those non-redox-active neurotransmitters.In this poster, I would like to present a novel electrochemical probe for ACh we recently reported, whichis an ion-selective electrode with deposition of ionophore layer targeting ACh on electrode surface. A glassy carbon electrode was firstly deposited with poly(3,4-ethylenedioxythiophene (PEDOT) layer electrochemically, then an ionophore-doped poly(vinyl) chloride (PVC) membrane was deposited by drop-casting. This electrode showed great ACh detection using cyclic voltammetry. Though it was achieved on large electrode, we have been trying to apply this strategy on carbon fiber microelectrode. We have got promising preliminary results. The electrodeposition of PEDOT is consistently successful on carbon fiber microelectrode. The PVC-ionophore layer, which is hard to be uniformly deposited on microelectrodes, also showed good deposition on some electrodes by immersing the electrode in the PVC-ionophore solution. As a result, we saw ACh detection via cyclic voltammetry for some modified carbon fiber microelectrodes. We are trying to reproduce this result and apply this electrode for FSCV detection. The final goal is to develop an electrochemical probe capable of simultaneously detection of multiple neurotransmitters. Once the platform is completed, it has the potential to be applied to different kinds of real-world problems related to brain neurotransmitter level. For example, one of our future projects we are planning to work on is to study the potential relationship between neurotransmitter levels and diseases.

P2.13: Co-detection of dopamine and octopamine for detecting neurotransmitters in the Drosophila Melanogaster (Park)

Presenter name: Cheonho Park, University of Virginia

Authors: Cheonho Park, University of Virginia; Aaditya Deshpande, University of Virginia; Jeffrey Copeland, Department of Biology, Eastern Mennonite University; B. Jill Venton, University of Virginia

The fruit fly, Drosophila melanogaster, is an excellent simple genetic model for studying the fundamental principles underlying brain development, behavioral events, and neuropathological diseases. This model has great promise for applying its findings to more complex organisms, including humans. Nonetheless, real-time measurement of neurotransmitters in Drosophila provides a significant challenge due to its microscale size, emphasizing the importance of improved analytical approaches to unravel the regulatory dynamics of neurotransmitters within the Drosophila brain architecture. The purpose of this study is to characterize dopamine and octopamine release in adult flies using fast-scan cyclic voltammetry (FSCV). FSCV is a real-time neurotransmitter measuring electrochemical technique with a micron-scale carbon-fiber electrode. Octopamine regulates arousal, aggression, and appetite, while dopamine is involved in motor control, learning, memory, and

reward processing. However, it is challenging to detect dopamine and octopamine because octopamine has a complex FSCV voltammogram with a delayed secondary peak that overlaps with the oxidation potential of dopamine. In this study, we used the Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm to distinguish between the collected dopamine and octopamine mixture voltammograms from flow-injection analysis. In summary, research shows that it is feasible to quantify dopamine in a Drosophila melanogaster's brain with a mixture response. It might be expanded to an in-vivo study in the future employing a neurological disease model or behavioral studies related to dopamine and octopamine.

P2.14: How voluntary exercise affects striatal dopamine release in female mice: Influence of estrous state, nAChRs, and BDNF (Feeley)

Presenter name: Ryan Feeley, NYU Grossman School of Medicine

Authors: Ryan Feeley, NYU Grossman School of Medicine; Jyoti Patel, NYU Grossman School of Medicine; Megan Fernandes, NYU Grossman School of Medicine; Vanessa Khachaturyan, NYU Grossman School of Medicine; Adam Mar, NYU Grossman School of Medicine; Begoña Gamallo-Lana, NYU Grossman School of Medicine; Margaret Rice, NYU Grossman School of Medicine

Dopamine (DA) is an important modulator of movement, and loss of DA plays a pivotal role in the neurodegenerative motor disorder, Parkinson's disease (PD). Exercise has been shown to mitigate the symptoms of PD and has been used as an adjunct to DA replacement therapy. Previously, using fast-scan cyclic voltammetry (FSCV), we found that 30 days of voluntary running-wheel-exercise led to a 30-40% increase in evoked extracellular DA concentration ([DA]o) in striatal slices from young male mice compared to matched controls housed with fixed wheels. Evoked increases in [DA]o in dorsal striatum (dStr), in nucleus accumbens core (NAcC) and NAc shell (NAcS) in runners vs. controls persisted in the presence of a nicotinic acetylcholine receptor (nAChR) antagonist, DHβE, showing a cell-autonomous effect on DA axons. These increases were also dependent on brain-derived neurotrophic factor (BDNF), as no increases were seen in slices from runner mice that were heterozygous for BDNF deletion (BDNF+/-). Consistent with a role for BDNF regulation of DA release, application of LM22-A4, an agonist for TrkB receptors that mediate BDNF signaling, increases evoked [DA]o in all striatal subregions in slices. Here, we tested the hypothesis that voluntary exercise also boosts evoked DA release in the striatum of young (15-20 week old) wild type (WT) female mice in a BDNF-dependent manner. As in young males, LM22-A4 (1 μM) increased single-pulse evoked [DA]o in the dStr and NAcC (16-20%), albeit to a lesser extent than seen in males, and with no increase in NAcS. Surprisingly, young females showed no obvious change in evoked [DA]o in any striatal subregion after voluntary exercise – except when nAChRs were antagonized using DHβE (1 μM). Under these conditions, evoked [DA]o was 70-90% higher in female runners vs. controls, which is similar to the difference seen in young male runners in DHβE. Given that estrogen has been shown to increase nAChR sensitivity, this implies a possible regulatory role for estrogen and the estrous cycle in these studies. Among other factors, the estrus stage of the estrous cycle is associated with elevation of brain BDNF expression. Indeed, using Western blots to assess striatal BDNF expression, we found a significant increase in BDNF in the dStr of runners vs. controls, similar to the pattern seen in males. However, in all females, whether runners or controls, dStr BDNF levels were also significantly higher during estrus than in non-estrus states.

Our results thus far support a DA-enhancing effect of exercise on striatal DA release in females, but that estrous cycle influence on striatal BDNF levels and possibly nAChR-dependent regulation of axonal DA release add interesting complexity to the process.

P2.15: Introducing variability into the inter-pulse interval of electrical brain stimulation in the MFB and mPFC alters dopaminergic release (Hamilton)

Presenter name: Andrea Hamilton, University of Arizona

Authors: Andrea Hamilton, University of Arizona; Nathan Weintraub, University of Arizona; Abhilasha Vishwanath, University of Arizona; Stephen Cowen, University of Arizona; Micahel Heien, University of Arizona; Andrea Hamilton, University of Arizona

Dopamine release in the striatum is crucial to drive reward-guided learning, movement, and decision making. Electrical brain stimulation has been utilized in the past to understand neural connectivity and behavioral outputs. Deep brain stimulation, a form of electrical stimulation, has been recently popularized as a treatment for tremors in Parkinson's patients. These forms of electrical stimulation typically use fixed frequencies and fixed inter-pulse intervals; however, previous research shows that neurons and neural circuits are more responsive to variable inter-pulse intervals. In this study, we seek to investigate the effect of altering the inter-pulse interval on the downstream dopaminergic release in the nucleus accumbens (NAc). This study focuses on two stimulation regions: the medial forebrain bundle (MFB) and the medial prefrontal cortex (mPFC). Stimulating the MFB directly activates dopaminergic axons that innervate the NAc. On the other hand, stimulating the mPFC activates a multi-synaptic circuit that ultimately releases dopamine in the NAc. We hypothesized that a more variable inter-pulse interval would result in higher dopamine release in the NAc. Methods: A carbon-fiber microelectrode was implanted into the NAc of anesthetized, male, Sprague-Dawley rats to monitor extracellular dopamine concentration using fast-scan cyclic voltammetry (FSCV). To apply the stimulation, a bipolar stimulating electrode was lowered into the MFB or mPFC. Stimulation patterns were generated with differing degrees of inter-pulse variability and constrained to have an average frequency of 10 Hz or 20 Hz. Local variance was used to quantify the inter-pulse interval variability of each stimulation pattern. Results: Data indicate that increasing the frequency of stimulation in the MFB increases dopamine release; however, an increase of stimulation frequency in the mPFC did not cause a significant increase of dopamine. Increasing the variability of the inter-pulse interval caused opposite trends in the MFB and mPFC. When stimulation patterns with higher variability are applied to the MFB, there is an increase in subsequent dopamine release, and dopaminergic release is tightly correlated to changes in stimulation frequency. Conversely, increasing the variability of the inter-pulse interval in the mPFC evoked less dopamine than a fixed frequency stimulation. To isolate the effects of local variance itself, stimulation patterns were generated with a four-pulse, repeating unit where those four pulses have a specific local variance. In this case, an increased variability of the inter-pulse interval evoked less release during both MFB and mPFC stimulation. These results indicate that MFB stimulation is more driven by frequency, and the subsequent dopamine release is highly responsive to frequency changes of the stimulation patterns.

P2.16: A platform for simultaneous long-term monitoring of phasic and tonic dopamine signaling (Weintraub)

Presenter name: Nathan Weintraub, University of Arizona

Authors: Nathan Weintraub, University of Arizona; James Siegenthaler, University of Arizona; Micahel Heien, University of Arizona; Nathan Weintraub, University of Arizona

Long-term dysfunction in dopamine signaling is associated with many neurological disorders, such as Parkinson's disease, depression, and addiction. Dopamine neurotransmission has two signaling modalities that occur over different timescales, making concurrent monitoring technically challenging. Phasic signaling is a rapid burst fire, occurring on the order of milliseconds to seconds, in response to salient stimuli, while tonic signaling is a continuous, steady-state release establishing the baseline level of dopamine activity and which changes over the course of minutes to hours. To be able to be able to monitor these dynamics, a technique requires sufficient temporal, spatial, and chemical resolution to capture both modalities of dopamine release Another challenge for long-term electrochemical measurements is a loss of sensitivity due to biofouling of the electrodes, which degrades voltammetric performance, obscuring dopamine detection. The aim of this work is to develop a method to simultaneously monitor phasic and tonic neurotransmitter signaling stably over weeks to months, allowing for more powerful measurements to capture complex interactions and neurotransmitter dynamics. Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique which monitors phasic dopamine release through oxidation and reduction of dopamine at the electrode surface. Fast-scan controlled adsorption voltammetry (FSCAV) is a complimentary technique which allows for quantification of tonic levels of extracellular dopamine using the same experimental design. To perform simultaneous FSCV-FSCAV, we developed a single board potentiostat capable of outputting multiple unique waveforms, new custom-built inhouse software, and a novel multichannel headstage based on a three-electrode configuration using a common Ag/AgCl reference, platinum counter, and multiple individually addressable working electrodes. The three-electrode design mitigates changes in the electrode signal due to biofouling occurring at the electrode surface, preserving dopamine sensitivity over long-term recording. This platform lays the foundation for future research into complex dynamics of dopamine release and how dopamine signaling correlates with behavioral and physiological responses, while reducing the number of animal subjects required for future studies, contributing to more ethical and efficient research practices.

P2.17: Kappa opioid receptors on dopamine terminals in the nucleus accumbens regulate anxiety-like behavior and dopaminergic signaling in response to stress and alcohol. (Holleran)

Presenter name: Katherine Holleran, Wake Forest School of Medicine

Authors: Katherine Holleran, Wake Forest School of Medicine; Sara Jones, Wake Forest University School of Medicine; Katherine Holleran, Wake Forest School of Medicine

A hallmark symptom of alcohol use disorder is negative affect, which can precipitate relapse, particularly following stress exposure. It is believed that this negative affect may be engendered, in part, by a reduction in dopaminergic signaling, or hypodopaminergia. We and others have found that kappa opioid receptor (KOR) function is augmented following chronic alcohol exposure, and contributes to hypodopaminergia within the nucleus accumbens (NAc) and negative affect. We also found that stress augments extracellular NAc levels of the KOR ligand

dynorphin. Here, we examined how KORs on dopamine neurons impact anxiety-like behavior and dopamine signaling through viral knockdown of KORs in ventral tegmental area (VTA; KOR-KDVTA DA) and NAc-projecting (KOR-KDVTAàNAc DA) dopamine neurons in KOR floxed mice. KOR-KDVTA DA mice had a profound decrease in anxiety-like behavior compared to control mice in the marble burying task, as well as nearly complete insensitivity to the dopamine-decreasing effects of the KOR agonist U50,488 in the NAc using ex vivo fast scan cyclic voltammetry. We found that the extent of insensitivity to KOR agonist application in slice robustly inversely correlated with anxiety-like behavior, indicating that KORs within the NAc terminals regulate this behavior. KOR-KDVTAàNAc DA mice had an intermediary phenotype, with reduced anxiety-like behavior and sensitivity to U50,488 compared to controls. There was no difference between KOR-KDVTA DA, KOR-KDVTAàNAc DA, or control mice in baseline stimulated dopamine release or reuptake. Mice were exposed to the PTSD-like stress paradigm, mouse single prolonged stress (mSPS), consisting of exposure to 4 multimodal stressors. While there was no difference between groups in baseline stimulated dopamine release, at low stimulation intensities, KOR-KDVTA DA and KOR-KDVTAàNAc DA mice had augmented release compared to controls. When comparing stress-exposed an naïve control animals, mSPS exposure resulted in significantly reduced release at low stimulation intensities, indicating that DA-targeted KOR deletion was able to overcome this stress-induced deficit. Surprisingly, bath application of 80mM ethanol reduced release at high stimulation intensities in control animals, and this effect was not mitigated through targeted KOR knockdown. KOR-KDVTA DA mice retained near complete insensitivity to U50,488 following mSPS exposure and did not differ from stress-naïve KOR-KDVTA mice. Control mice exposed to mSPS showed a trend towards increased U50,488 sensitivity compared to stress-naïve controls. However, KOR-KDVTAàNAc DA mice exposed to mSPS displayed a profound sensitization to the dopamine-decreasing effect of U50,488 following mSPS exposure, such that KOR-KDVTAàNAc DA and control mice had similar U50,488 sensitivity following mSPS. These data indicate that KORs on dopamine neurons in the VTA and dopamine terminals in the NAc mediate anxiety-like behavior and altered dopamine signaling in both stress-naïve and mSPS exposed mice.

P2.18: Understanding the differential effects of fouling mechanisms on working and reference electrodes in Fast-Scan Cyclic Voltammetry for neurotransmitter detection (Jang)

Presenter name: Jaehyun Jang, Hanyang University

Authors: Jaehyun Jang, Hanyang University

Fast-scan cyclic voltammetry (FSCV) is a crucial tool in neurochemistry and neuroscience research, allowing for the real-time detection of neurotransmitters. However, the accuracy and sensitivity of FSCV may be diminished by electrode fouling, which refers to the accumulation of unwanted substances on the electrode surfaces. This accumulation alters the electrochemical characteristics of the electrodes and affects the accuracy of the measurements. The focus of our study is to examine the accumulation of biomolecules, known as biofouling, and the deposition of chemical species, known as chemical fouling, on the carbon fiber micro-electrode (CFME) used as the working electrode and the Ag/AgCl reference electrode in FSCV setups.Both biofouling and chemical fouling had a significant effect on sensitivity, causing peak voltage shifts in the FSCV signal. Significantly, these effects were more prominent in the CFME

in comparison to the reference electrode. While previous studies have reported peak voltage shifts in FSCV signals resulting from the fouling of Ag/AgCl electrodes post-implantation in the brain, our investigation further revealed an increase in sulfide ion concentrations on the Ag/AgCl electrode's surface through energy-dispersive spectroscopy (EDS) after implantation.In order to confirm this discovery, an intentional addition of sulfide ions was made to the buffer solution. This resulted in a drop in the open circuit potential of the Ag/AgCl electrodes and subsequent shifts in peak voltage in the FSCV voltammograms. Our investigation offers important insights into the complex causes of electrode fouling and its subsequent effects on FSCV measurements. These findings have important implications for improving the design of FSCV experiments, which can lead to the development of new ways to increase the accuracy and reliability of in vivo FSCV measurements. Our study adds vital insight to the optimization of FSCV techniques, which will result in more robust and precise findings in neuroscience research as researchers probe into the intricacies of neurochemical processes.

P2.19: Oral Fentanyl Self-Administration Increases Excitability and Excitatory Synaptic Inputs of Noradrenergic Neurons in the Nucleus of the Solitary Tract (Downs)

Presenter name: Anthony Downs, University of North Carolina at Chapel Hill

Authors: Anthony Downs, University of North Carolina at Chapel Hill; Gracianne Kmiec, University of North Carolina at Chapel Hill; Zoe Mcelligott, University of North Carolina

Fentanyl is a highly potent synthetic opioid agonist that has become a key driver of the deadly opioid crisis affecting the United States. However, relatively little is known about specific neural circuitry that is modified by fentanyl use and how those circuits impact the development of opioid use disorder. While many previous studies on fentanyl and other opioids have focused on intravenous administration, other routes of administration remain relatively understudied. This is particularly important given the increased incidence of oral consumption of illicit fentanyl. Here, we used a home-cage fentanyl drinking paradigm that we recently developed in our lab to study how long-term fentanyl consumption impacts the physiology of noradrenergic neurons in the nucleus of the solitary tract (A2 neurons). Previous work from our lab and others has implicated these neurons in withdrawal responses to opioids. Mice consumed escalating amounts of fentanyl across the 4 weeks of the paradigm and female mice consumed more fentanyl than males. We found that chronic oral fentanyl consumption results in increased excitatory inputs to A2 neurons and alters the stoichiometry of AMPA receptor subunits in these cells. We used the calcium-permeable AMPA receptor antagonist NASPM to show that fentanyl increases the insertion of calcium-permeable AMPA receptors into glutamatergic synapses. A2 neurons also become more tonically active and are more excitable following fentanyl consumption. We further investigated molecular changes that may underlie increased excitability following fentanyl administration. This work points to changes in A2 neuron plasticity that may be important for the somatic and affective signs of fentanyl withdrawal and later the development of opioid use disorder.

P2.20: Intermittent access promotes dynamic and sex-specific changes to the E/I balance and excitability of glutamatergic projections to the nucleus accumbens (Sizer)

Presenter name: Sarah Sizer, University of North Carolina at Chapel Hill

Authors: Sarah Sizer, University of North Carolina at Chapel Hill; Sara Conley, University of North Carolina at Chapel Hill; Jackson Murray, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill; Zoe Mcelligott, University of North Carolina

Heavy drinking diminishes the euphoric effects of alcohol and dysregulates neural circuits controlling reward-seeking behavior, driving increased consumption and the progression of alcohol use disorder (AUD). However, the neural mechanisms governing maladaptive escalations in alcohol consumption remain unclear. Therefore, the goal of this project was to understand whether chronic ethanol consumption dysregulates the excitatory/inhibitory (E/I) balance and the neuronal excitability of glutamatergic neurons in the basolateral amygdala (BLA), prelimbic cortex (PL), and infralimbic cortex (IL) that project to the nucleus accumbens (NAc). We bilaterally injected retrograde AAV (AAVrg-hSyn-HI-eGFP-Cre.WPRE.SV40) into the NAc of male and female C57BL/6 mice. Following 7 weeks of intermittent access (IA) to ethanol or water and one two-hour session immediately before sacrifice, we used whole-cell patch clamp electrophysiology and recorded the E/I ratios (EPSC frequency/IPSC frequency) and the excitability of fluorescent neurons in each region. We found that although IA reduces both the EPSC and IPSC frequency in male BLA-NAc projections, there were no changes in the E/I ratio or excitability of these neurons. Female BLA-NAc projections express increases in IPSC amplitude with no changes in the E/I ratio, and a significant decrease in neuronal excitability compared to controls. Together, these results suggest that IA produces sexually dimorphic adaptations to BLA projections without altering the E/I ratios in either sex. We also found that IA does not change the E/I ratios of male PL-NAc projections but significantly increases the excitability of these neurons. In contrast, IA decreases the E/I ratio of female PL-NAc projections without altering the neuronal excitability. Finally, IA does not change the excitability of female IL-NAc projections and increases the E/I ratio through decreases in IPSC frequency, while causing no changes in either the E/I ratio or excitability in male IL-NAc projections. PL-NAc and IL-NAc E/I and synaptic drive ratios positively correlate with the time elapsed since alcohol drinking in IA females, suggesting that IA-induced plasticity may be transient and dependent on the presence of alcohol in the system. Collectively, our data suggest that IA produces sex-dependent, region-specific, and dynamic alterations to the E/I balance and the excitability of BLA-, PL-, and IL-NAc projections.

P2.21: Cholinergic regulation of theta oscillations in amygdala underlying fear behaviors. (Higley)

Presenter name: Samantha Higley, University of South Carolina

Authors: Samantha Higley, University of South Carolina; Joshua Bratsch-Prince, University of South Carolina; Carly Halcomb, University of South Carolina; James Warren, University of South Carolina; Aaron Jasnow, University of South Carolina; David Mott, University of South Carolina

Areas of the brain involved in emotion, such as the basolateral amygdala (BL) and medial prefrontal cortex (mPFC), rely on synchronized oscillations in the theta band (4-12 Hz) to entrain local pyramidal neurons (PYRs) and synchronize activity across brain regions for proper long-range communication and information processing. Aberrant synchronization of these regions contributes to emotional disturbances that underlie fear-related disorders. Despite the established role of theta oscillations in mPFC and BL in emotion processing, the mechanisms

through which oscillations are generated in the BL and how they are synchronized with mPFC are poorly understood. Critical to the function of these regions is acetylcholine (ACh), which promotes emotional learning and theta oscillations in BL and mPFC. Importantly, deficits in cholinergic signaling are associated with several disorders that are linked with emotional disturbances. Moreover, in all mammals, including humans, the BL receives by far the most robust cholinergic innervation of any target of the cholinergic basal forebrain. Despite this dense cholinergic innervation and critical importance in emotional memory, surprisingly little is known about the mechanisms through which ACh modulates BL circuits to regulate emotional memory. Here, we explored the circuit mechanism by which ACh contributes to fear behaviors. We found that chemogenetic inhibition of basal forebrain cholinergic projections to the BL did not affect freezing during conditioning but significantly disrupted fear memory. To explore this finding further, we examined the effect of optogenetically released ACh in mouse brain slices. Released ACh had a bidirectional effect on BL local field potential oscillations. A prolonged increase in theta oscillatory activity followed an initial suppression in response to ACh release. At the single cell level, ACh induced an initial hyperpolarization in BL PYRs, which was followed by a depolarization and an increase in excitability that was coupled to theta frequency membrane potential oscillations (MPO). This MPO was driven by GABAA receptors, which synchronized neighboring PYRs. Underlying the theta MPO were large, rhythmic, compound IPSCs that were driven by CCK interneurons but not PV or SOM interneurons. Cholinergic activation of these CCK interneurons and the rhythmic IPSCs in PYRs was blocked by the M3 antagonist 4-DAMP, indicating that the cholinergic oscillation depended upon M3 receptors on CCK INs. Similarly, in vivo, M3 receptor inhibition in the BL had no effect on freezing during training but significantly impaired fear memory. These studies outline at the circuit level how synaptic ACh modulates BL oscillations and suggest a critical role for M3 receptors on CCK interneurons in the induction of theta oscillations and fear memory. Supported by R01MH104638 and R01MH131808.

P2.22: Insulin targets striatal cholinergic neurons for acetylcholine and dopamine dependent nutrient sensing (Patel)

Presenter name: Jyoti Patel, NYU Grossman School of Medicine

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Following ingestion, the metabolic peptide insulin crosses the blood-brain barrier from the periphery and acts as a satiety signal in the hypothalamus to curtail further feeding. However, receptors for insulin are also found in other brain regions including the striatum. Our past studies showed that insulin amplifies evoked dopamine (DA) release in ex vivo striatal slices from male rats through a local mechanism involving enhanced activity of striatal cholinergic interneurons (ChIs) and activation of nicotinic acetylcholine receptors (nAChRs) on DA axons. Here we examined the actions and consequences of insulin in the striatum of male and female mice. Through analysis of RNA-seq data in DropViz (http://dropviz.org), we found that although mRNA for insulin receptors (InsRs) is present in striatal projection neurons, interneurons, and

astrocytes, the highest expression of InsR-mRNA is in ChIs. Patch-clamp recordings from non-labeled or tdTomato-labeled ChIs show that physiological concentrations of insulin (30 nM) increase action potential firing in ChIs during a series of 3 sec depolarizing current injections in males, thereby mirroring our previous observations in male rats. In females, however, the effect of insulin was variable. Strikingly, in both sexes the effect of insulin on increasing ChI excitability was lost in mice that lack forebrain choline acetylcholinesterase (ChAT-KO mice), and thus lack the ability to synthesize acetylcholine (ACh). As a result, insulin-induced amplification of evoked DA release monitored using fast-scan cyclic voltammetry, is also lost in male and female ChAT-KOs. To determine the behavioral consequences of insulin's actions on striatal ChIs and the interplay between ACh and DA in ingestive reward signaling, we examined nutrient sensing in ChAT-KO mice and their littermate controls. Mice were given 1 hr free access to a solution containing either 6% glucose (nutritious) or equally sweet 0.25% saccharin (non-nutritious) on alternating days in their home cage. As predicted, we found that consumption of glucose escalated over 3 to 4 pairs of sessions in control males, whereas escalation of saccharin intake was less pronounced. In ChAT-KO mice, however, there was no significant preference for glucose versus saccharin over the same time period, although total consumption of fluids was unaltered. Similar trends were seen in females, albeit not as robust. Together, these physiological, electrochemical, and behavioral data show how insulin signaling via InsRs on ChIs enhances ChI excitability to produce an ACh-dependent boost in striatal DA transmission across species. This local mechanism plays an important role in nutrient sensing and supports our hypothesis that insulin acts as a nutrient reward signal. Studies powered to discern sex differences and the impact of the estrous cycle in females are on-going.

P2.23: Monitoring noradrenergic activity in the LC and BNST during opioid withdrawal (Nowlan)

Presenter name: Alexandra Nowlan, UNC at Chapel Hill

Authors: Alexandra Nowlan, University of North Carolina at Chapel Hill; Zoe McElligott, University of North Carolina

The opioid epidemic continues to inflict staggering economic and societal costs as the leading cause of accidental death in the United States. Opioid use disorder is a chronically relapsing condition where individuals who have developed a physical dependence will continue to misuse opioids to avoid the distress of withdrawal. The severity of withdrawal, characterized by acute physical symptoms (shaking, diarrhea, vomiting, etc.) alongside prolonged emotional distress, correlates to poor compliance with OUD treatment. Although the neurobiological underpinnings of withdrawal are not entirely clear, prior research indicates that norepinephrine (NE) signaling within the locus coeruleus (LC) and the bed nucleus of the stria terminalis (BNST) is affected by and may contribute to chronic opioid use and withdrawal. To explore the neural mechanisms involved in withdrawal in vivo, we used a repeated naloxone-precipitated morphine withdrawal paradigm in mice. We performed fiber photometry to monitor NE activity in the LC and BNST of freely moving animals experiencing withdrawal. We hypothesized that hyperarousal of LC-NE neurons and/or elevated NE release in the BNST may be linked to specific withdrawal behaviors, like tremors or swallowing. Our observations revealed heightened activity in LC-NE neurons and greater NE release in both the LC and BNST that aligns with escape jumps, which are generally considered to be indicative of withdrawal intensity.

Furthermore, we suspected that withdrawal severity and/or hyperarousal of the NE system may be linked with the anxiogenic effects observed in acute or protracted withdrawal. Our preliminary data demonstrate a female-specific attenuation of acoustic startle-evoked activity in LC-NE neurons 24 hours after withdrawal, although the behavioral startle response remains consistent across treatment groups. One possible interpretation is that after withdrawal, less neuromodulatory input is needed to elicit an equivalent behavioral response to the startle stimulus. Future experiments will attempt to resolve potential influences on noradrenergic tone and establish a causal link between the effect of withdrawal-induced hyperarousal of the LC-NE neurons and anxiety-like behaviors.In summary, our study suggests a link between heightened LC-NE activity and specific withdrawal behaviors and provides insights into how this connection might influence the affective aspect of withdrawal beyond its physical symptoms.

P2.24: Investigating the mt-Keima reporter as an in vivo mitophagy readout following USP30 inhibition (Sorensen)

Presenter name: Nicole Fadahunsi, H. Lundbeck A/S

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Mutations in Parkin and PINK1 represent the most common genetic causes of recessive Parkinson's Disease. These mutations disrupt the PINK1/Parkin pathway, a regulator of mitophagy – the process responsible for removing damaged mitochondria. Disruption of mitophagy results in accumulation of dysfunctional mitochondria, contributing to the cellular damage that occurs in Parkinson's Disease. USP30 is a potential disease-modifying target for the treatment of Parkinson's Disease. By removing ubiquitin modifications on mitochondrial proteins (including Parkin) USP30 halts mitophagy. Inhibition of USP30 therefore has the potential to prevent cellular damage, by facilitating removal of damaged mitochondria.We investigated the effect of pharmacological USP30 inhibition on mitophagy. Using mass spectrometry, we confirm that USP30 inhibition increases ubiquitinylation levels of the mitochondrial membrane proteins TOM20 and VDAC1 in mouse brains. To further explore effects on mitophagy, we utilised a pH-sensitive fluorescent reporter mitochondria-tagged Keima (mt-Keima) in ex vivo preparations and in vivo models. We examined the effect of USP30 inhibition on basal mitophagy in young (2- months-old) and aged (15-months- old) cohorts of transgenic mt-Keima mice. Additionally, we performed fiber photometry experiments in mt-Keima transgenic and C57BL/6J mice pre-injected with AAV.hSyn.mtKeima into medial prefrontal cortex (mPFC) after 7 days of consecutive treatment with a USP30 inhibitor (USP30i). Finally, we explored LPS as a stressor, and investigated the ability of USP30i to increase mitophagy in the brains of LPS treated mice.We found no differences in basal mitophagy in the dentate gyrus of young and aged mt-Keima mice treated with USP30i or vehicle. Similarly, we observed no effect of USP30i in mPFC in fiber photometry studies with transgenic mt-Keima mice, or mice that received an intracranial injection of the mt-Keima reporter. We observed no effect of USP30i on mitophagy in brains of LPS treated mice. To conclude, our results suggest that the mt-Keima biosensor does not provide a sufficient assay window for testing the effect of

USP30i in vivo. Further work will be required to define an optimal in vivo mitophagy model and appropriate mitochondrial stressors to induce an observable effect with the mt-Keima biosensor.

P2.25: Partial or Complete Loss of Norepinephrine Differentially Alters Contextual Fear and Catecholamine Release Dynamics in Hippocampal CA1 (Wilson)

Presenter name: Leslie Wilson, NIEHS

Authors: Leslie Wilson, NIEHS; Nicholas Plummer, Neurobiology Laboratory, NIEHS; Irina Evsyukova, Neurobiology Laboratory, NIEHS; Daniela Patino, Neurobiology Laboratory, NIEHS; Casey Stewart, Neurobiology Laboratory, NIEHS; Kathleen Smith, Neurobiology Laboratory, NIEHS; Kathryn Konrad, Social and Scientific Systems, Inc., a DLH Holdings Corp Company; Sydney Fry, Neurobiology Laboratory, NIEHS; Alex Deal, Wake Forest University School of Medicine; Sambit Panda, Neurobiology Laboratory, NIEHS; Natale Sciolino, Department of Physiology and Neurobiology, Department of Biomedical Engineering; Jesse Cushman, NIEHS; Patricia Jensen, Neurobiology Laboratory, NIEHS

Background: Contextual fear learning is heavily dependent on the hippocampus. Despite evidence that catecholamines contribute to contextual encoding and memory retrieval, the precise temporal dynamics of their release in the hippocampus during behavior is unknown. In addition, new animal models are required to probe the effects of altered catecholamine synthesis on release dynamics and contextual learning.Methods: We generated 2 new mouse models of altered locus coeruleus–norepinephrine (NE) synthesis and utilized them together with GRAB-NE and GRAB-DA sensors and in vivo fiber photometry to investigate NE and dopamine (DA) release dynamics in the dorsal hippocampal CA1 during contextual fear conditioning.ResultsAversive foot shock increased both NE and DA release in the dorsal CA1, while freezing behavior associated with recall of fear memory was accompanied by decreased release. Moreover, we found that freezing at the recent time point was sensitive to both partial and complete loss of locus coeruleus–NE synthesis throughout prenatal and postnatal development, similar to previous observations of mice with global loss of NE synthesis beginning postnatally. In contrast, freezing at the remote time point was compromised only by complete loss of locus coeruleus–NE synthesis beginning prenatally.Conclusions: Overall, these findings provide novel insights into the role of NE in contextual fear and the precise temporal dynamics of both NE and DA during freezing behavior and highlight complex relationships between genotype, sex, and NE signaling.

P2.26: Dopamine dynamics in rat nucleus accumbens during alcohol drinking following adolescent social isolation (Deal)

Presenter name: Alex Deal, Wake Forest University School of Medicine

Authors: Alex Deal, Wake Forest University School of Medicine; Conner Wallace, Wake Forest University School of Medicine; Katherine Holleran, Wake Forest University School of Medicine; Sara Jones, Wake Forest University School of Medicine

Chronic social isolation during adolescence alters behavior associated with drugs of abuse, increasing the risk for substance use disorder development in humans and rodents later in life.

In addition to behavioral changes, adolescent social isolation (aSI) exposure has been shown to result in changes to the dopaminergic response to drug exposure in multiple brain regions of rodents . Using an emerging technique, fiber photometry, we are able to repeatedly measure real-time dopamine release in freely moving rats with high temporal and spatial resolution. In the present study, we used fiber photometry to determine the effects of alcohol drinking on dopamine dynamics in the shell and core subregions of the nucleus accumbens in aSI rodents compared to group housed (GH) controls. Due to sex differences in the anxiety-like behaviors resulting from the aSI paradigm, male rats were used in this study. aSI exposure began on postnatal day (PD) 28 and continued into adulthood. GH rats remained together from adolescence into adulthood and then individually housed following surgery. Social isolation in adulthood (after PD 60) does not result in an anxiety-like phenotype. After reaching adulthood, aSI and GH rats were unilaterally injected in either the nucleus accumbens shell or core with a photosensor, dLight 1.2, and implanted with a fiber optic cannula into the same region. Dopamine responses were recorded in these rats during sessions where noncontingent alcohol and water bottles were accessible. Analysis of the data collected during these sessions provides insight into the effects of aSI on dopamine dynamics such as signal amplitude, frequency, width, uptake, and ramping related to approach and consumption of alcohol and water as well as spontaneous dopamine activity between drinking bouts. These data will deepen our understanding of the changes in accumbal core and shell dopamine response to alcohol drinking behaviors following chronic early life stress. Future experiments will include aSI and GH adult rats exposed to chronic intermittent ethanol vapor to induce alcohol dependence and the incorporation of chemogenetics to examine changes in a receptor- and circuit-specific manner.

P2.27: Grid1 and Gria1 mRNA expression patterns in the bed nucleus of the stria terminalis (Manjunath)

Presenter name: Madhura Manjunath, UNC at Chapel Hill

Authors: Madhura Manjunath, University of North Carolina at Chapel Hill; Sara Conley, University of North Carolina at Chapel Hill; Zoe Mcelligott, University of North Carolina

Glutamate delta-1 receptor (GluD1), a cation channel with sequence homology to other glutamate receptors, remains one of the least understood of the GluR family. Although it does not bind to glutamate, and knowledge on the endogenous ligand of GluD1 is still being researched, it has been shown to be involved in mediating anxiety-like, depression-like, and social behaviors. Previous studies on GluD2 have established its role in a mechanism that induces long-term depression (LTD) in synapses within the cerebellum through the internalization of GluA1-containing calcium-permeable AMPA receptors (CP-AMPARs), and other studies have reported that GluD1 also mediates LTD in the hippocampus similarly through interacting with metabotropic glutamate receptors. Furthermore, abnormal GluA1 expression was previously noted in GluD1 knockouts.A nexus between various stress and reward circuitry, the bed nucleus of the stria terminalis (BNST) has been shown by previous preclinical research to regulate anxiety-like and fear-learning behaviors, ethanol-seeking behavior, and drug-seeking reinstatement behaviors. While GluD1 presence in the BNST was noted in a survey of GluD receptor distribution throughout the brain, there remains to be an in-depth study of GluD1 expression specifically within the BNST, as well as how expression patterns of GluA1-containing

CP-AMPARs are changed in the presence or absence of GluD1 in the BNST. We examine these topics through a series of fluorescence in-situ hybridization (FISH) experiments analyzing GluD1 and GluA1 mRNA expression in the BNST of naive C57B6/J, wildtype, GluD1 heterozygous, and GluD1 knockout mice, creating a broad overview of expression patterns in the BNST. Specifically, we analyze differences in expression of GluD1 and GluA1 mRNA, co-expressing cells, and any trends in expression of either mRNA in co-expressing cells in the BNST between genotypes, sexes, and BNST subregions.In the future, we aim to follow up on our experiments by looking at the effects of alcohol exposure on these expression patterns. In our previous studies, we found that alpha-1 adrenergic receptor-induced LTD is occluded in the anterolateral BNST after chronic alcohol exposure and chronic restraint stress (CRS), and the CRS may induce LTD via the internalization of GluA1-containing CP-AMPARs. Additionally, the GluD1 gene in humans has been associated with alcohol dependence in genetic association studies. With GluD1 also known to be involved in synaptic plasticity changes, our questions extend to how these mechanisms may deviate from that of naive mice by an alcohol exposure paradigm, investigated by additional FISH analyses and electrophysiology experiments. Taken together, this data can provide future directions for further research on receptor-targeted therapies and interventions for alcohol use disorder.

P2.28: Head-to-head comparison of fast-scan cyclic voltammetry and dLight 1.3b (Sandberg)

Presenter name: Stefan Sandberg, University of Washington

Authors: Stefan Sandberg, University of Washington; David Daberkow, Eastern Washington University; Paul Phillips, University of Washington

Recent developments of genetically expressed sensors have afforded great advances in the detection selectivity of neurochemical monitoring. One such genetically expressed sensor, dLight 1.3b (dLight), has focused on detecting dopamine with great promise and utility. To date, a benchmarking against an established technique has not been demonstrated. To this end, we affixed an optical fiber (for dLight) to a carbon fiber electrode (for fast-scan cyclic voltammetry, FSCV), which allows for side by side in-situ comparison. Electrical stimulation of the medial forebrain bundle in urethane (1.5 g/kg, i.p.) anesthetized Sprague-Dawley rats was used to elicit dopamine release in the nucleus accumbens. dLight has significantly faster response kinetics and greater signal to noise ratio, in particular with smaller dopamine release events elicited by brief, low frequency, and/or low current stimulations. Further analysis of signals did not demonstrate a lag in the onset of the signal due to low affinity of the genetic sensor, suggesting that dLight sensors have the ability to measure low levels of dopamine. Raclopride (RAC) administration (20 mg/kg, i.p.) resulted in significant increase in electrically evoked dopamine amplitudes with FSCV, but not with dLight, whereas area under the curve was significantly increased post RAC for both techniques. Preliminary data suggests that dLight measured, lidocaine sensitive (2%, 0.25 µL/min, 5 uL, intra ventral tegmental area) spontaneous transients in the anesthetized preparation, something that has only been observed with FSCV post amphetamine administration. Moreover, the amplitudes and counts of spontaneous transients were significantly increased post RAC. In addition, we observed that dLight signals saturate at high dopamine release events caused by 60 Hz stimulations longer than 30 pulses, which was not seen with FSCV, hence indicating a smaller dynamic range of the dLight sensor. Taken

together, we find that the dLight sensor has significantly better sensor response time kinetics, and sensitivity compared to FSCV. Signal saturation at the dLight sensor was observed at 60 Hz stimulations over 30 pulses, arguably not physiologically relevant. The surprisingly high sensitivity of the dLight sensor could be due the expression level of the dLight sensor and/or the location of expression with respect to the synaptic cleft, which has been mathematically modeled to have neurotransmitter concentrations in the millimolar range. FSCV is not without its advantages, as the carbon fiber electrode is smaller compared the optical fiber (7 µm vs 400 µm) which limits the spatial resolution and could cause more tissue disruption, although this was not apparent in the kinetics of the signals. However, the smaller size does allow for measuring from a smaller sample volume. The larger sample volume of the dLight sensor could be underlying the larger signal to noise ratio and higher sensitivity, as well.

P2.29: Real-time dopamine responses to volitional ethanol drinking differ between the nucleus accumbens and basolateral amygdala (Wallace)

Presenter name: Conner Wallace, Wake Forest University School of Medicine

Authors: Conner Wallace, Wake Forest University School of Medicine; Katherine Holleran, Wake Forest University School of Medicine; Clare Slinkard, Wake Forest University School of Medicine; Christopher Lapish, Indiana University School of Medicine; Sara Jones, Wake Forest University School of Medicine

Dopamine neurotransmission in the nucleus accumbens (NAc) is integral to associative learning and drives cue reactivity, value prediction, and motivation. In the basolateral amygdala (BLA), dopamine plays different roles, gating and relaying information about a given stimulus. However, it is not clear how BLA dopamine contributes to reward processing during EtOH consumption. The purpose of this study was to determine differences in NAc versus BLA dopamine responses (monitored via the photosensor dLight1.2 and fiber photometry) in male and female mice freely drinking alcohol (ethanol; EtOH). C57BL/6J mice completed a modified drinking in the dark paradigm, during which a bottle with 15% EtOH replaced home cage water for 2 hours/day, 5 days/week over 6 weeks. Mice were acclimated to drinking in operant boxes and all intake was subsequently monitored via lickometers for time locking to photometry data. Fiber photometry recordings were taken during 1-hour sessions with 15% EtOH freely available in the operant boxes. Immediately prior to the first lick in each bout, dopamine in the NAc displayed a ramp-like elevation. In contrast, ramp-like responses in the BLA were less defined, smaller, and occurred in closer temporal proximity to bout initiation. In the NAc, dLight signals decreased quickly after licking started. In contrast, after dopamine peaked in the BLA in response to drinking, the signal returned to baseline at a slow and constant rate, and signals were sustained after drinking cessation. In conclusion, dopamine in the BLA showed reduced ramping prior to EtOH drinking and a greater amount of time to return to baseline once drinking was initiated. Our current findings suggest that dopamine responds differently during EtOH drinking in each region, which likely serves different functions. For example, it is possible that dopamine ‘ramping’ in the NAc could drive anticipation and increase the likelihood that an animal will start drinking, while BLA dopamine may help to determine the salience of EtOH in a given context.

P2.30: Estrous-dependent effect of stress on reward learning and dopamine release (Wanat)

Presenter name: Matt Wanat, University of Texas at San Antonio

Authors: Matt Wanat, University of Texas at San Antonio; Morgan Johnston, University of Texas at San Antonio; Matt Wanat, University of Texas at San Antonio

Human studies demonstrate that stress alters reward processing and dampens the ability to update cue-driven actions in reward-based tasks. Importantly, sex differences in stress responsivity have been linked to alterations in cognitive processing. Therefore, to mitigate the maladaptive consequences of stress, we must first identify how stress impacts neural circuits controlling appetitive cue-driven actions in both males and females. To address this, rats were exposed to stress prior to training on an associative learning task with food rewards. Stress produced a persistent increase in conditioned responding when administered to males as well as females in metestrus/diestrus. In contrast, females experiencing stress during proestrus/estrus exhibited a persistent reduction in conditioned responding. Furthermore, stress produced subsequent extinction learning impairments only in metestrus/diestrus females. Our prior research using voltammetry recordings illustrate that stress-enhanced conditioned responding in males was accompanied by elevated reward-evoked dopamine release in the ventral lateral striatum (VLS). By using fiber photometry recordings with GRABDA, we find that the stress-mediated suppression of conditioned responding in proestrus/estrus females is accompanied by a decrease in cue-evoked dopamine release in the VLS. Collectively, our results indicate that the net effect of stress on cue-driven behavior and VLS dopamine release is determined by sex and the estrous cycle.

P2.31: Pairing microdialysis with droplet microfluidics and direct mass spectrometry for multiplexed, high temporal resolution neurochemical monitoring (Bain)

Presenter name: Ian Bain, University of Michigan

Authors: Ian Bain, University of Michigan; Robert Kennedy, University of Michigan

In order to fully profile neurotransmission, measurement techniques must be sensitive, selective, have high temporal resolution, and ideally able to measure multiple neurotransmitters simultaneously. Microdialysis is an essential in vivo sampling technique, that, when paired with LCMS, achieves sensitivity, selectivity, and multiplexing for a large number of compounds. However, the temporal resolution of this technique is limited by the need to collect a few microliters of dialysate before sufficiently sensitive analysis can be performed. In this work, we combat this shortcoming by separating dialysate flow into droplets using microfluidics and analyzing by direct mass spectrometry, allowing for low second temporal resolution. Using this strategy, a sufficiently sensitive assay for the simultaneous measurement of 13 neurotransmitters has been developed and applied in awake, behaving, animals. The developed technique will allow for deep neurotransmitter profiling and association with animal behavior, opening up the path to a more thorough understanding of the brain and of neurological disorders and illnesses.

P2.32: Time-course concentration of ethanol and its metabolites in rat brain after ethanol self-administration (Lee)

Presenter name: Tse-Ang Lee, University of Texas at Austin

Authors: Tse-Ang Lee, The university of Texas at Austin; Hongjoo Lee, The University of Texas at Austin; Rueben Gonzales, The University of Texas at Austin; Heba Ajmald, The University of Texas at Austin; Regina Mangieri, The University of Texas at Austin; Tanya Hutter, The University of Texas at Austin; Tse-Ang Lee, The University of Texas at Austin

Understanding the neurobiological consequences of alcohol exposure, particularly the dynamics of ethanol metabolism, is essential for advancing neuroscience, neuropharmacology, and the development of interventions targeting alcohol-related disorders. Despite this significance, little is known about the time course of ethanol and its metabolites in living systems, especially in the brain. To bridge this gap, our study used a microdialysis probe to simultaneously sample ethanol, acetaldehyde, and acetate in the extracellular fluid of the striatum of Long-Evans rats. The probe featured inlet and outlet fused silica tubing inserted into a regenerated cellulose membrane with a MWCO of 13 kDa. The working distance of the probe was set to 3 mm by coating epoxy on unused membrane portions.Rats were trained to self-administer ethanol by providing 15% ethanol solutions three days a week for 24 hours for 3-5 weeks. Once intake stabilized, the rats were then switched to a 30 min limited access session to mimic the condition that would occur during microdialysis sampling. Rats were prepared by surgically implanting a guide cannula above the striatal brain region, with both the tether bolt, securing the microdialysis probe, and the guide cannula fixed to the skull using stainless steel screws and dental cement. Following surgery, the rat was given a seven-day recovery period. During the experiment, rats were granted access to ethanol solutions of 15% concentration in tap water for 30 minutes. Dialysate samples, collected at 10-minute intervals over a 2.5-hour duration, were measured using gas chromatography with a flame ionization detector for ethanol, acetaldehyde, and acetate concentrations. Ethanol consumption during a 30 min session was 0.3 – 1.4 g/kg among 7 experiment subjects. Peak ethanol concentrations in the dialysate samples (0.3-9.3 mM) were dependent on the dose consumed. Acetaldehyde, approximately 100 times lower than ethanol, was detected in dialysates from all rats after consumption of ethanol, except for one rat. Baseline acetate concentration spiked from a baseline concentration of 0.04-0.08 mM to 0.1-0.24 mM. Notably, in a subject with low ethanol consumption (0.29 g/kg), the concentration of acetaldehyde remained within the limit of detection throughout the experiment. However, the acetate concentration was clearly increased after ethanol consumption in this subject, which is comparable to that of other rats with higher ethanol consumption. For all analytes, the area under the curve for all analytes was directly related to the dose of ethanol consumed. In conclusion, our study demonstrated the simultaneous assessment of ethanol and its metabolites in brains of behaving rats, providing insights into their temporal dynamics in the rat brain and establishing a foundation for further exploration in the field of alcohol metabolism research.

P2.33: Analysis Of Endomorphin Analogues As Candidate Drugs For Pain Relief Using Microdialysis Sampling Coupled with Liquid Chromatography-Tandem Mass Spectrometry (Ogbu)

Presenter name: Chidiebere Ogbu, University of Arizona

Authors: Chidiebere Ogbu, University of Arizona; Chenxi Liu, University of Arizona; Mitchell Bartlett, University of Arizona; Lajos Szabó, University of Arizona; Robin Polt, University of Arizona; Michael Heien, University of Arizona; Chidiebere Ogbu, University of Arizona

Neuropeptide-based drugs have garnered widespread attention due to their high efficacy and good selectivity. However, they are susceptible to enzymatic degradation, which leads to low blood-brain barrier (BBB) penetration and a short half-life. Previous studies have shown that glycosylation of neuropeptides increases their blood-brain barrier penetration and stability. Endomorphins are endogenous opioid peptides that have potential as analgesics and as a morphine substitute due to their selectivity and affinity for the μ-opioid receptor. Introducing a lactam bridge and glycosylation of the native endomorphin increases BBB penetration, making it a potent antinociceptive opioid agonist. Here, we analyze two endomorphin analogues of glycosylated and non-glycosylated endomorphin using microdialysis sampling with liquid chromatography-tandem mass spectrometry. These analogues of endomorphin have demonstrated comparable or superior antinociceptive efficacy to morphine while reducing multiple adverse effects, including abuse potential, tolerance, respiratory depression, and inflammatory glial responses. Preliminary results for the glycosylated endomorphin analog show a maximum plasma drug concentration of 8.3 ± 0.6 μM, an AUC0-t of 121.4 ± 5.3 μM*minutes, a maximum dialysate drug concentration of 4.3 ± 0.4 μM and an AUC0-t of 68.3 ± 38.8 μM*minutes (mean ± SEM, n = 2 rats). These cyclic glycosylated neuropeptides have the potential as analgesic and as a treatment for opioid use disorder.

P2.34: Deep brain stimulation of ventral tegmental area modulates dopamine surge in rat nucleus accumbens following acute fentanyl administration (Vettleson-Trutza)

Presenter name: Sara Vettleson-Trutza, Mayo Clinic

Authors: Sara Vettleson-Trutza, Mayo Clinic; Kristen Scheitler, Mayo Clinic; Juan M. Rojas-Cabrera, Mayo Clinic; Abhinav Goyal, Mayo Clinic; Anupama Gupta, Mayo Clinic; Yoonbae Oh, Mayo Clinic; Kendall H. Lee, Mayo Clinic; Hojin Shin, Mayo Clinic

Background: Substance use disorder constitutes a public health emergency in the United States. One of the leading culprits of drug overdose is fentanyl, a synthetic opioid that is utilized medically for general anesthesia or in the clinical management of terminal cancer pain. Addiction is a pathology of the reward circuitry of the brain; dopaminergic surges in response to drug administration confer drug-seeking behavior and promote neuroplastic alterations to the reward circuit anatomy. We have previously demonstrated a dopaminergic surge in the rat nucleus accumbens (NAcc) following acute administration of fentanyl. We sought to investigate whether ventral tegmental area (VTA) deep brain stimulation (DBS) could block this NAcc dopaminergic surge following acute fentanyl administration. Methods: The Mayo Clinic Neural Engineering Laboratories and Division of Engineering developed a next-generation platform technology for in vivo neurochemical recordings in real time called the Multifunctional Apparatus for Voltammetry, Electrophysiology and Neuromodulation (MAVEN). Male Sprague-Dawley rats were anesthetized. Optimized for the detection of dopamine, multiple cyclic square wave voltammetry (MCSWV) was applied to the carbon fiber electrode in the rat nucleus accumbens. Recordings were obtained from a carbon fiber microelectrode implanted in the

NAcc at baseline and following acute fentanyl administration of 50 ug/kg. High frequency stimulation was applied at 130 Hz, 200 micro-sec and 0.2 mA before and during acute fentanyl administration. Both stimulation and electrochemical recordings were performed using MAVEN. Results: M-CSWV recordings demonstrated a surge in dopamine release after acute fentanyl exposure in rodents with a 333.5% change from baseline. In response to acute fentanyl exposure, rat respiration rate spiked to an average of 120 breaths per minute within the first minute of exposure and rapidly dropped to 63 breaths per minute by minute six of exposure, until eventual death. Acute fentanyl administration did not result in a surge of dopamine while applying stimulation. Conclusions: High frequency stimulation of rodent VTA prevents a surge in tonic dopamine levels in NAcc following acute exposure to fentanyl.

P2.35: Multiplexing FSCV and iGluSnFR3.0 sensors reveals adenosine transiently inhibits stimulated dopamine and glutamate release via A1 receptors (Shrestha)

Presenter name: Kailash Shrestha, University of Virginia

Authors: Kailash Shrestha, University of Virginia

Different parts of the brain are connected through intricate wiring of neurons and communicate via a variety of neurotransmitters at synapses. Neurotransmitters such as adenosine and glutamate are well-known neuromodulators that alter the release of neurotransmitters. Simultaneous measurements of different neurotransmitters are challenging but crucial to understanding interactions in the brain. Genetically-encoded sensors have enhanced spatial resolution, but there are still limited colors, while electrochemistry provides high time resolution but for limited electroactive analytes. Here, we multiplexed fast-scan cyclic voltammetry (FSCV) and genetically encoded fluorescence sensors to simultaneously measure adenosine, dopamine, and glutamate to investigate the spatial and temporal profiles of adenosine neuromodulation in caudate-putamen and nucleus accumbens. A genetically encoded glutamate sensor (iGluSnFR3) was expressed in the caudate putamen. Carbon-fiber microelectrode (CFME) was implanted near the expressed cells in a brain slice to monitor electrically-stimulated dopamine release using adenosine waveform (-0.4V, +1.45V, 400V/s scan rate). Exogenous adenosine was applied locally to the slice, lasting for 20 s, resulting in a transient inhibitory effect on both electrically-stimulated dopamine and glutamate release. These inhibitions were observed only within a 250 µm distance, showing regional inhibition effects. Dopamine and glutamate recovered after 5 minutes. Furthermore, the A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) blocked the adenosine inhibition of both glutamate and dopamine release, indicating adenosine has a global transient inhibition effect on dopamine and glutamate release via A1 receptor modulation. This study shows that multiplexing FSCV and fluorescence sensors (iGluSnFR3) allows simultaneous monitoring of multiple neurotransmitters and reveals an overall inhibition by adenosine in the caudate putamen area.A similar approach will be used to study the neuromodulatory role of adenosine in the nucleus accumbent (NAc), which is a major part of the mesolimbic system. It's associated with adaptive and motivated behaviors such as rewards, aversion, and drug abuse via dopamine and glutamate release. The changes in glutamate and dopamine release will be monitored through changes in fluorescence and FSCV respectively. We were able to demonstrate neuromodulation role of adenosine on other neurotransmitters through multiplexing two different techniques. This is the first experiment to simultaneously record

electroactive (adenosine and dopamine) and non-electroactive (glutamate) neurochemical release in real-time, with a high spatial resolution by integrating fluorescence and electrochemistry techniques.

P2.36: A voltammetric analysis of dopaminergic transients during global and focal ischemia (Witt)

Presenter name: Colby Witt, University of Cincinnati

Authors: Colby Witt, University of Cincinnati; Ashley Ross, University of Cincinnati; Colby Witt, University of Cincinnati

Strokes (ischemic and hemorrhagic) are the 5th leading cause of death in United States thus leading to a national health burden. Volumes of work have gone into understanding the behavior of neurochemicals during global ischemic events; however, there is a large shift within the neuroscience community to study these events at the site of injury (focal ischemia). Focal ischemia is of particular interest because of its confounding effects in localized regions. Over the past 5 years, the Ross lab has worked on crafting microfluidic platforms to study localized and sustained ischemic events in tissue. In this work, these technologies were used to compare standard tissue interrogation methods for analysis (i.e. perfusion chamber for ex vivo work) to unravel how dopamine (DA) dysregulation is handled in the brain. This work focuses on the CA1 region of the hippocampus due to the high levels of glutamate and DA that accumulate in the extracellular space during ischemic events. Herein, signaling patterns of dopamine were investigated under both ischemic conditions using fast-scan cyclic voltammetry (FSCV). Transient DA was monitored in hippocampal slices for 45 minutes and the amount released, extracellular duration, and event frequency were analyzed. Overall, this work goes to establish baseline signaling profiles for DA in healthy CA1 regions of hippocampi while also uncovering the nuanced changes in DA response to global vs focal ischemia.

P2.37: High-throughput, minimally invasive intranasal cocaine self-administration in head-restrained mice (Erickson)

Presenter name: Kirsty Erickson, Vanderbilt University

Authors: Kirsty Erickson, Vanderbilt University; Keaton Song, Department of Pharmacology, Vanderbilt University; Justin Kim, Department of Pharmacology, Vanderbilt University; Hannah Branthwaite, Department of Pharmacology, Vanderbilt University; Eyal Kimchi, Department of Neurology, Northwestern University Feinberg School of Medicine; Cody Siciliano, Vanderbilt University

Intravenous cocaine self-administration has long been considered the gold-standard for modeling cocaine abuse in non-human animals, and has been the mostly widely used addiction model across drug classes. Historically, intravenous cocaine self-administration has also been an invaluable tool for investigating the neurobiological basis of motivated behavior. However, in recent years the utility of this model has declined dramatically. This is largely due to 1) the technique is notoriously difficult, as significant surgical expertise is required for jugular vein catheterizations, 2) incompatibility with modern neurotechnoliges due to the necessity of tethering the subject to a fluid delivery system, and 3) difficulty is establishing this approach in

mice, which typically show dramatically lower response rates than rats. An additional shortcoming of intravenous cocaine self-administration models is that, with the exception of opioid co-injection, intravenous cocaine use in humans is exceedingly rare, as the intranasal route is preferred almost ubiquitously among both recreational and heavy users. Given that cocaine is the only major narcotic that does not engender escalation to intravenous use in heavy users, we reasoned that providing the means for intranasal cocaine consumption might circumvent both the technical and conceptual issues raised above. Here we have developed an intranasal cocaine self-administration procedure, which is to our knowledge the first documented example of volitional insufflation of any compound in non-human animals. To achieve this, a specialized microfluidic delivery system is used to deliver nanoliter droplets of cocaine dissolved in saline directly in front of the nostril of a head-restrained mouse. Delivery is contingent upon operant responding and once delivered can easily be insufflated by the subject. The procedure is minimally invasive, requiring only a head-post without catheterization or cannulation, utilizing typical operandum which allows direct transfer of tasks from freely-moving assays, and engenders very high levels of cocaine intake in mice. Here, we parameterize this model in C57BL/6J mice, characterize full dose-response and demand curves, showing sustained, high-effort, dose-dependent responding, as well as stable consumption patterns across days and prolonged sessions. Our results show robust acquisition and maintenance of self-administration behavior, far surpassing performance of intravenous models. This procedure does not require any indwelling surgical implants, and, thus, facilitates a more accessible and efficient means of studying drug self-administration in mice and permits assessment of longitudinal mechanisms of drug-induced plasticity; moreover, it enhances the translational relevance of preclinical to human conditions, given that cocaine is primarily used intranasally even amongst patients with severe cocaine use disorder.

P2.38: Exploring Neurochemical Profiles in Selectively Bred Rat Lines: Implications for Emotional Traits and Cocaine Response (Popov)

Presenter name: Pavlo Popov, University of Michigan

Authors: Pavlo Popov, University of Michigan; Robert Kennedy, University of Michigan; Pavlo Popov, University of Michigan

Through a selective breeding approach, our collaborator at the University of Michigan has established distinct rat lines displaying various temperamental phenotypes representing broad emotional traits. Specifically, low responder rats (bLRs) exhibit aversion to novelty and passive coping, while high responder rats (bHRs) demonstrate exploratory behavior and active coping. However, the relationship between individual emotional differences and brain metabolome variations remains largely unexplored. To address this gap, our laboratory conducted a microdialysis-based in vivo study involving 12 bLRs, 12 bHRs, and 3 control subjects. Microdialysis samples were collected both before and after intraperitoneal cocaine administration to assess changes in neurochemical metabolome profiles. Using untargeted LC tandem mass spectrometry (LC-MS/MS), we analyzed alterations in known neurochemicals and unidentified features. Our findings reveal potential metabolomic differences between these behavioral phenotypes before and after cocaine exposure, across sexes and with repeated administrations, specifically in the ventral and dorsal striatal regions of the bLR and bHR rat brains.

P2.39: Epigenetic Silencing of Dorsal Raphe Serotonergic Neurons Following Adolescent Intermittent Ethanol is Reversible by Glycerrrhizin Administration (De Castro)

Presenter name: Sagan De Castro, University of North Carolina

Authors: Sagan De Castro, University of North Carolina; Fulton Crews, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill; Ryan Vetreno, Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill

Human adolescent binge drinking leads to lifelong social and emotional difficulties, including increased risk for anxiety and affective disorders and substance use disorders. Adolescent intermittent ethanol (AIE; 5.0 g/kg EtOH, i.g., 2-day on/2-day off from postnatal day [P]25 to P54), a rodent model of human adolescent binge drinking, produces an array of anxiety-like behaviors that persist into adulthood in the absence of further alcohol consumption. Serotonin (5-HT) is established as a mediator of social and emotional processes, and we previously reported a reduction of serotonergic neurons (i.e., 5-HT+IR) in the raphe nucleus that persists into adulthood. We previously reported that AIE causes a reversible loss of cholinergic neurons through an epigenetic repressive mechanism mediated by neuroimmune activation via high mobility group box 1 (HMGB1). We therefore hypothesized that the AIE-induced loss of serotonergic neurons is mediated by epigenetic silencing of serotonergic genes, and that this silencing would be reversible through an HMGB1-specific anti-inflammatory treatment. Immunohistochemical assessment of TPH2+IR and 5-HT+IR in the adult dorsal raphe nucleus (DRN) following AIE revealed reductions of both TPH2 and 5-HT relative to age-matched CONs. Loss of 5-HT in the DRN is similar to that seen following injection of the inflammagen lipopolysaccharide, which is consistent with a shared inflammatory mechanism. Chromatin immunoprecipitation assessment of the epigenetic repressive marker H3K9me2 revealed increased occupancy at the serotonin transporter (Sert) and the 5-HT synthesizing enzyme tryptophan hydroxylase 2 (Tph2) in the adult DRN of AIE-treated animals. Administration of glycyrrhizin, a HMBG1-specific anti-inflammatory agent, to adult rats post-AIE reversed the loss of TPH2+IR and 5HT+IR in the DRN. Further, AIE reduced serotonin receptor expression (5-HT1A+IR) across multiple DRN projection sites (e.g. Prefrontal Cortex & Dorsomedial Periaqueductal Grey) involved in social & anxiety-like behaviors that are disrupted following AIE. This combined with the reduction of serotonergic neurons in the DRN following AIE, creates a phenotype of impaired 5-HT signaling. This supports our working hypothesis of an overall reduction in 5-HT signaling mediating diverse behavioral pathology after AIE. Future work will assess the efficacy of glycyrrhizin in preventing adverse behavioral outcomes thought to be mediated by serotonergic dysfunction following AIE, such as heightened anxiety-like behaviors and social deficits.

P2.40: Circuit-specific modulation of dopamine release and downstream signaling biomarkers in the mesolimbic system by Low-intensity Focused Ultrasound (Olaitan)

Presenter name: Greatness Olaitan, University of Virginia

Authors: Greatness Olaitan, Univeristy of Virginia; Dayana Surendran, University of Virginia; B. Jill Venton, University of Virginia; Greatness Olaitan, Univeristy of Virginia

Poster Abstracts

Synchronous oscillations of cortical neurons have been implicated in cognitive function, influencing both inhibitory and excitatory downstream neurotransmission and signaling. The dysregulation of dopamine in the nucleus accumbens is associated with the disruption of temporal coupling in prefrontal cortex oscillations. Previous studies have demonstrated the impact of Low-Intensity Focused Ultrasound (LIFU) on the downstream inhibition of dopamine neurotransmission in the accumbens core. In this study, we tested three different frequency couplings (Theta-Gamma, Gamma only, and Delta-Beta) of LIFU sonication on the prelimbic cortex (PLC) and measured dopamine levels in the nucleus accumbens core (NAc core) using fast-scan cyclic voltammetry. Dopamine in the nucleus accumbens increased by 25% for 30 minutes after three successive applications of 80 seconds of 5:50 Hz (Theta-Gamma) LIFU sonication on the PLC. The same effect was observed with LIFU sonication at 50 Hz (Gamma only), while three successions of 2:24 Hz (Delta-Beta) LIFU sonications inhibited dopamine by 30% for 1 hour. Anatomical controls applying LIFU to the primary somatosensory cortex did not change NAc core dopamine, and LIFU to the prelimbic region did not affect dopamine release in the caudate or NAc shell. Immunoblotting showed an increased expression of cFOS, pERK1/2, glutamate biomarker (Ser845-AMPAGluN1), and dopamine biomarker (DARP-32-thr34) in the PLC and NAc core with excitatory LIFU parameters, and vice versa. Conversely, the expression of the GABA receptor (GABAA α1) in both the PLC and NAc core was contrarily reduced by parameters that excited dopamine and glutamate release, and vice versa. Histochemical staining also confirmed the results from the western blotting experiments, and histological evaluations showed no evidence of cell damage or death with any of the parameters. These studies demonstrate that frequency modulation of LIFU parameters could be used in future studies for the circuit-specific treatment of different phases of psychiatric diseases, including substance use disorder.